

# Effect of EPS on biofilm structure and function as revealed by an individual-based model of biofilm growth

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**Abstract** We have simulated a nitrifying biofilm with one ammonia and one nitrite oxidising species in order to elucidate the effect of various extracellular polymeric substance (EPS) production scenarios on biofilm structure and function. The individual-based model (IbM) BacSim simulates diffusion of all substrates on a two-dimensional lattice. Each bacterium is individually simulated as a sphere of given size in a continuous, three-dimensional space. EPS production kinetics was described by a growth rate dependent and an independent term (Luedeking-Piret equation). The structure of the biofilm was dramatically influenced by EPS production or capsule formation. EPS production decreased growth of producers and stimulated growth of non-producers because of the energy cost involved. For the same reason, EPS accumulation can fall as its rate of production increases. The patchiness and roughness of the biofilm decreased and the porosity increased due to EPS production. EPS density was maximal in the middle of the vertical profile. Introduction of binding forces between like cells increased clustering.

**Keywords** Biofilm; EPS; extracellular polymeric substances; individual-based modelling; nitrification; simulation

## Introduction

This simulation study aims to elucidate the effect of different extracellular polymeric substance (EPS) production scenarios on bacterial growth, as well as on biofilm structure and function. We have used a nitrifying biofilm with one ammonia and one nitrite oxidising species as our model system, because it provides an interesting example of a food chain and nitrification is an important process in nature, sewage treatment plants, and agriculture.

Biofilm models have undergone an evolution of increasing complexity (Noguera *et al.*, 1999). In the 1990s sophisticated two- and three-dimensional biofilm models incorporating the whole range of transport processes and biofilm growth and detachment were developed. They follow a bottom-up approach since the biofilm structure is an emergent property of these models rather than the model input.

IbM is also a bottom-up approach as it attempts to model a population or community by describing the actions and properties of the individuals comprising the population or community. These properties emerge as a result of the actions and interactions of the cells with each other and the environment rather than being a model input (DeAngelis and Gross, 1992). IbM allows individual variability and treats organisms, in our case bacterial cells, as the units. This has important consequences regarding biomass spreading. When such a unit moves or is pushed aside, its biomass, fixed and variable properties (its genome, state of differentiation, etc.), and its cell-number (one) are displaced co-ordinately.

All bottom-up models can address questions about self-organisation and the relationship of microscopic and macroscopic properties. IbM is particularly suited to address questions about the effects of individual variability and rare events.

## The model

BacSim (Kreft *et al.*, 1998) was extended in order to include EPS production and capsule

formation, as well as binding forces between cells. A Java port by Ginger Booth (Yale University) can now also be run over the web (<http://www.eeb.yale.edu/ginger/bacillus>). It simulates diffusion and growth steps alternately, since these two processes can be separated on the basis of their time scale (Picioreanu *et al.*, 1999).

Each bacterium is individually simulated as a sphere of variable size in a continuous, three-dimensional space. Using a 3D space, the movements of the simulated bacteria can have the same degree of freedom as in reality. The substrate uptake rates were described by a Monod equation with substrate limitation for both the electron donor and acceptor (oxygen), as well as a substrate inhibition term for the electron donor substrate ammonia or nitrite, respectively.

EPS production kinetics was modelled by a growth rate dependent and an independent term (Luedeking-Piret equation), with rates and yields given by Robinson *et al.* (1984). Low production rates were set to 10% of the high production rates of *Pseudomonas aeruginosa*. The density of EPS was estimated as 75 g C/l from typical values of sludge floc composition (Per Nielsen, University of Ålborg, personal communication). Capsule formation was simulated by keeping the produced EPS volume in a sphere surrounding the producing cell. EPS production was simulated by excreting the produced EPS in cell-sized blobs into the environment.

The spreading of the growing biomass was simulated by maintenance of a minimum distance between neighbouring cells. The weighted vector sum of all overlap radii  $r_0$  with neighbouring cells was calculated, and the position of the cell was shifted in the opposite direction. If there was an overlap between cells, the magnitude of this overlap was used as a weight in order to tolerate slight overlaps and place a heavier penalty on stronger overlaps. In order to model short-range, weak attractive forces between sticky cells, the overlap between cells was not minimised but optimised in the following way. The attractive force between cells was assumed to be (a) weak compared with the repellent force between overlapping cells, (b) slowly rising with distance, and (c) fading out in the further distance. If a neighbour was close (within 4 cell radii), the distance between the cell and the neighbour was weighted with  $0.01r_0 / (1 + r_0)^3$ . We believe this models the effect of binding forces between cell wall structures or pili.

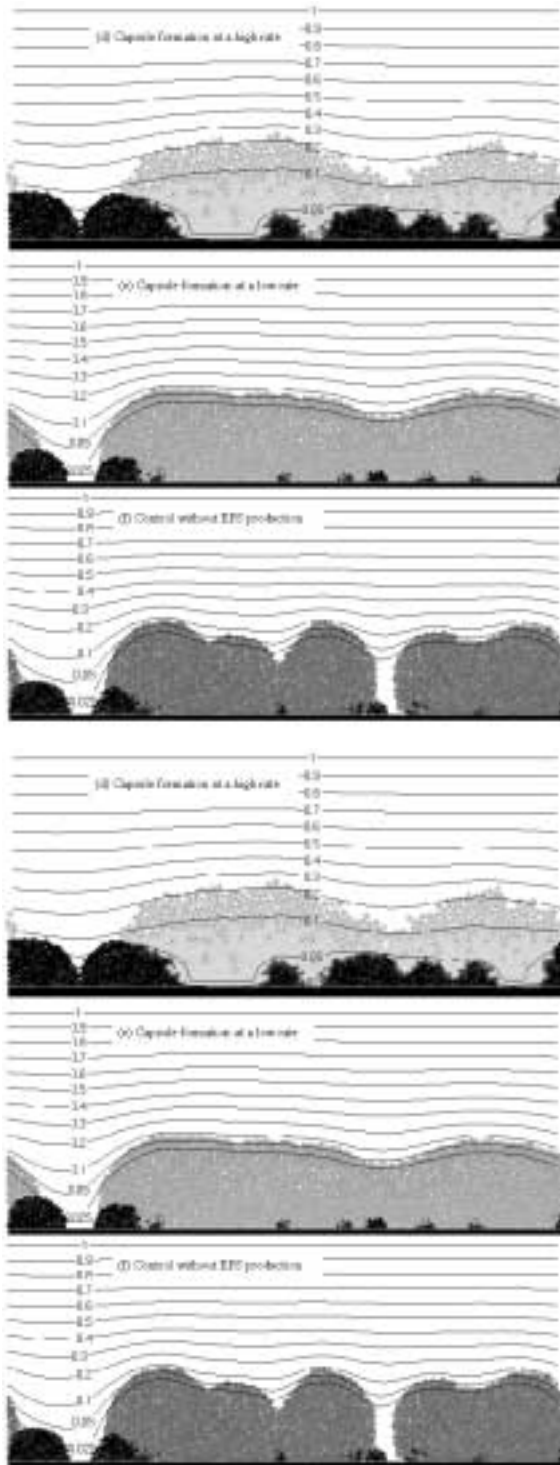
The concentrations of substrates and products (oxygen, ammonia, nitrite, and nitrate) result from diffusion and reaction. The continuous diffusion-reaction equation was solved as described (Picioreanu *et al.* 1998a).

The system, 200  $\mu\text{m}$  wide by 2  $\mu\text{m}$  deep by 200  $\mu\text{m}$  high, was inoculated with 10 bacteria of each species, placing them at randomly chosen locations along the surface of the substratum at time zero. Substrate concentrations in the bulk liquid were: ammonia, 4 mM; nitrite, 6 mM, nitrate, 3 mM, and oxygen, 31  $\mu\text{M}$  (1 mg/l). The hydrodynamic boundary layer was assumed to be 40  $\mu\text{m}$  thick. The temperature was set to 30°C, and the pH to 7. Diffusion coefficients, kinetic parameters and growth yields were taken from Picioreanu *et al.* (1997). Cell sizes of *Nitrosomonas europaea* and *Nitrobacter winogradskyi* were from Bergy *et al.* (1974). Cell density and minimal cell volume were as in Kreft *et al.* (1998).

The computational domain had periodic boundaries, apart from the non-periodic vertical dimension. The periodic boundary of the third dimension (depth), which is only modelled for the bacteria and not for the substrates, assured a uniform distribution of biomass in this dimension. Therefore, substrate concentration must also be uniform and it was not necessary to compute diffusion in this dimension. A comprehensive description, apart from the EPS extension, is part of another manuscript which is currently in preparation.

## Results and discussion

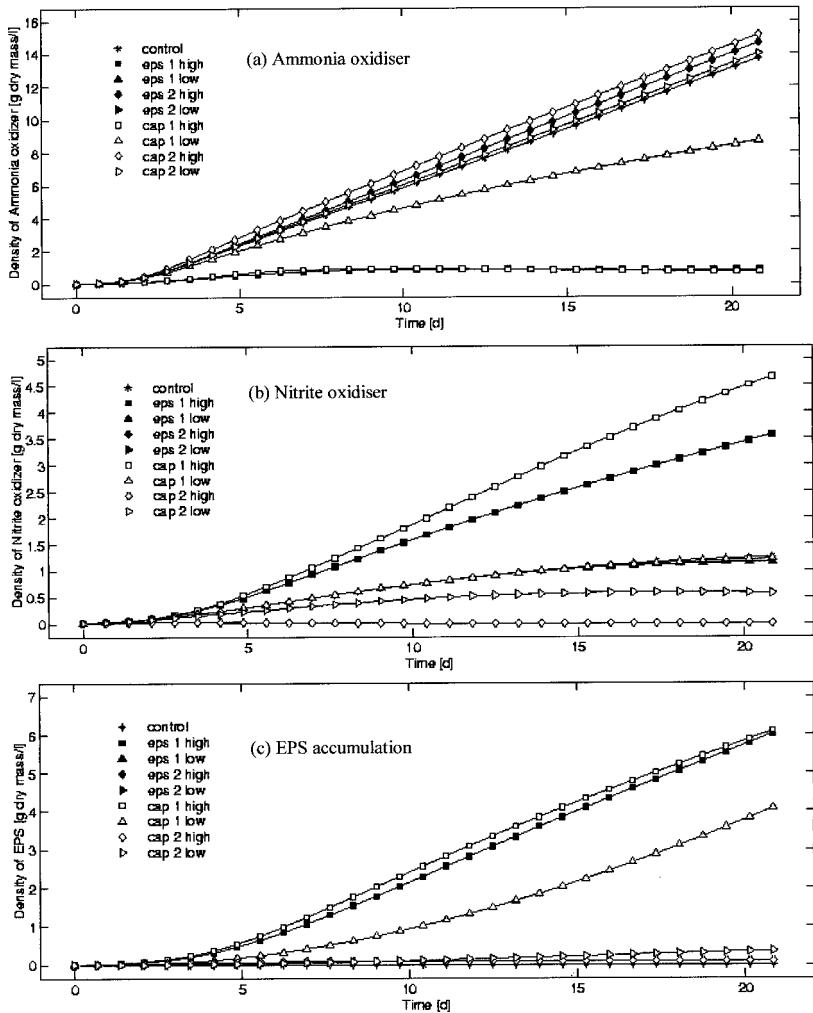
A moving T-test of the relative residuals picks up those stretches of a time series which dif-



**Figure 1** Biofilm structures from different EPS production scenarios after 30,000 min (21 days), including oxygen concentration contours in mg/l. The biofilm grows upwards from the substratum (bar at the bottom, length 200 μm) towards the bulk liquid (highest contour line). The light grey fog is EPS, the lighter cells are the EPS producing ammonia oxidisers and the darker cells are the nitrite oxidisers, which appear somewhat brighter where immersed in EPS

for significantly from the control, but often trends can be parallel or interwoven despite being significantly different. Therefore, we introduce as a further requirement a 5% divergence of trends, before we regard differences as relevant.

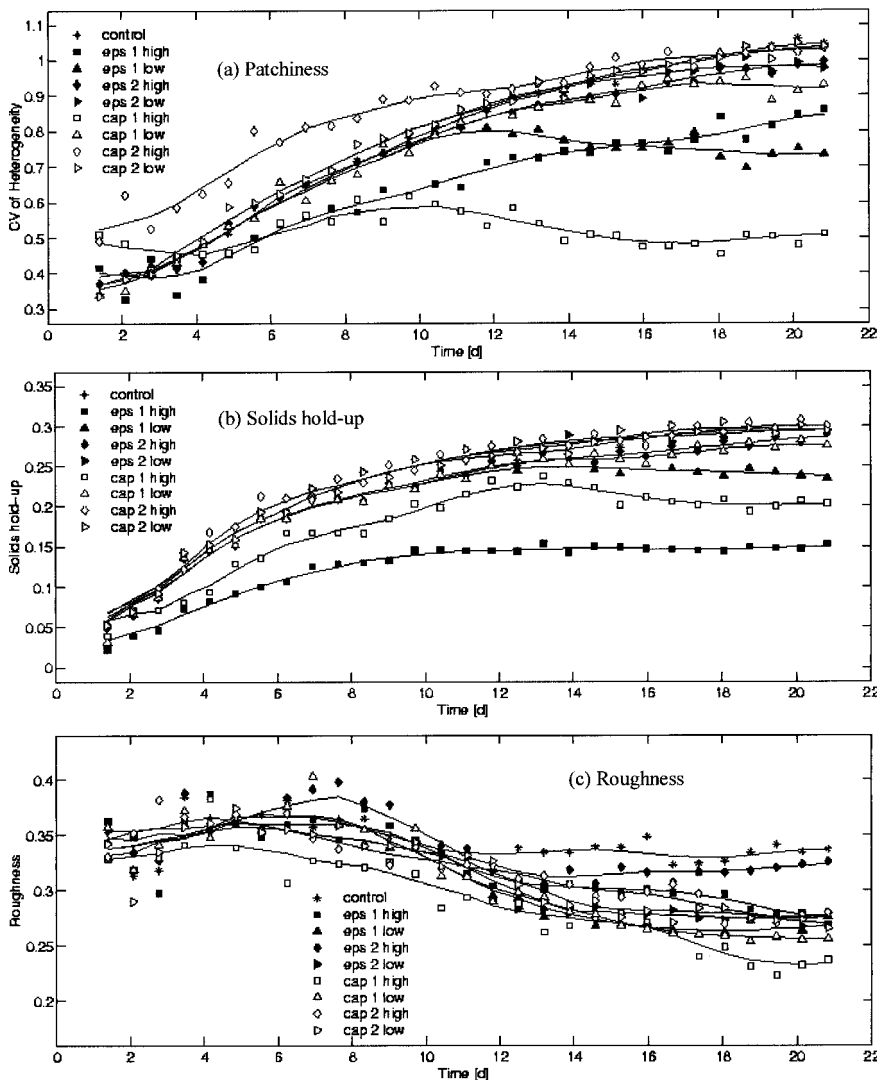
The structure of the biofilm was dramatically influenced by EPS production or capsule formation (Figure 1). The ammonia oxidising species, which is first in the food chain, dominated the biofilm if no EPS was produced and was thus the majority species. In all cases, oxygen was the limiting substrate for both species, while oxygen penetration was much deeper where considerable amounts of EPS had accumulated. The mixing of the two species was rather limited. Capsule formation led to more uniformly spaced cells compared with EPS production. In the case of high EPS production rates, cell density was very low at the biofilm bottom. This is due to the death of those cells that have spent more energy on



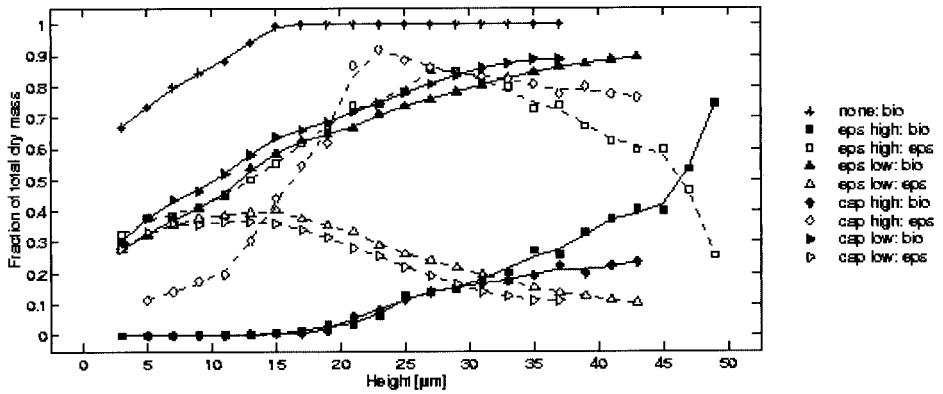
**Figure 2** Growth curves for (a) ammonia and (b) nitrite oxidisers as well as (c) EPS accumulation for the different EPS production scenarios. Explanation of the legends: "eps 1 high" means EPS production by species 1 in the food chain (ammonia oxidiser) at a high rate; "cap 2 low" means capsule formation by species 2 in the food chain (nitrite oxidiser) at a low rate. Solid symbols for EPS production and open symbols for capsule formation. Quadrangles for high production rates and triangles for low production rate. Symbols for species 2 are rotated symbols for species 1. Note that often the traces for EPS production are covered by the traces for capsule formation

EPS synthesis than they can gain at the low oxygen tensions in the depth of the biofilm. The cells of the non-producing species are dragged along with the flow of EPS production in the expanding biofilm. EPS material enters the periphery of clusters of non-producing cells. If cells are sticky, clustering is more pronounced as cells do not tend to be swept away from each other in areas of EPS production.

Growth of the EPS producing or capsule forming species was reduced, while that of the other species was stimulated (Figure 2). This is due to energy expenditure for EPS synthesis. The extent of EPS accumulation was higher at lower rates of EPS production when the minority species was producer. This was due to lower growth rates, also caused by the energy cost of EPS synthesis, at higher EPS production rates. The patchiness of the biofilm, as measured by the variability (CV) of the spatial heterogeneity within the biofilm, was decreased if considerable amounts of EPS were produced (Figure 3). The cell density of the biofilm (solids hold-up) and the roughness of the biofilm surface were lowered by EPS



**Figure 3** Biofilm shape parameters from different EPS production scenarios. (a) Patchiness (coefficient of variation of spatial heterogeneity), (b) solids-hold up (1-porosity), and (c) surface roughness. Legends as in Figure 2

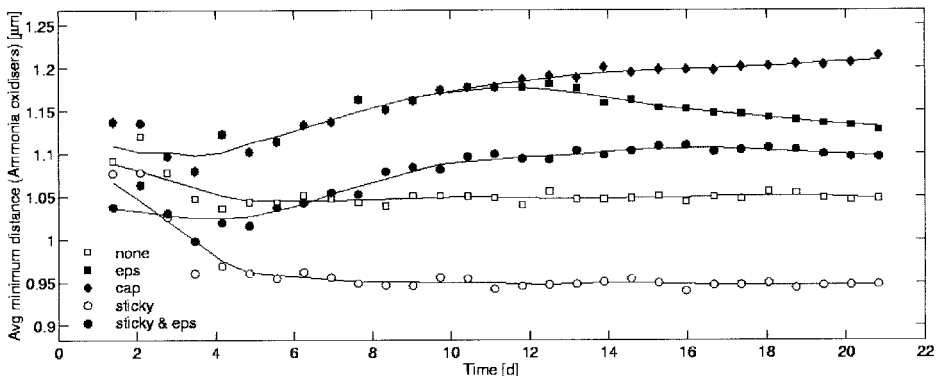


**Figure 4** Vertical gradients of biomass and EPS from different EPS production scenarios after 21 days ("eps high:" means EPS production by ammonia oxidiser at a high rate and "cap low:" means capsule formation by ammonia oxidiser at a low rate). Explanation of symbols: biomass traces (bio) are solid with full symbols; EPS traces (eps) are dashed with open symbols

production and capsule formation (see Picioreanu *et al.* (1998b) for definitions of these parameters). The vertical profiles of EPS density (Figure 4) showed a peak in the middle of the biofilm, whereas the biomass density of the EPS producing majority species was highest at the biofilm-liquid interface. The non-producing species became overgrown by the majority species and was mainly starving at the bottom of the biofilm. Production of EPS, and even more so the formation of capsules, decreased clustering (increased the average minimum distance between cells, Figures 1 and 5). Introduction of binding forces between like cells increased clustering.

### Conclusions

Because EPS production costs energy, too high a production rate leads to a decrease in the growth rates of the producers. EPS accumulation can likewise be reduced by too high production rates since less energy is available to make more cells. Therefore, reports of the *extent* of EPS produced are specific for the growth conditions used in contrast to *rates* and *yields*, which are more general and should be the parameters of choice in studies of EPS



**Figure 5** Clustering of cells as dependent on EPS production (eps) or on capsule formation (cap) as well as on the presence of forces binding the producing cells to each other (sticky). Explanation of the legend: solid symbols for EPS production or capsule formation; angular symbols for non-sticky cells and round symbols for sticky cells

production. Due to competition, EPS production by one species stimulates growth of the other. A maximum EPS density at the top of the biofilm, as reported by Zhang *et al.* (1998), was not observed. This may be due to decay of EPS in lower layers, as future simulations will investigate. The patchiness and roughness of the biofilm decreased and the porosity of the biofilm increased due to EPS formation. Introduction of binding forces between like cells increased clustering.

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