Applications of individual-based modelling in microbial ecology

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ABSTRACT

Individual-based modelling (IbM) allows description of differences between individuals in a spatially heterogeneous environment - the typical case in microbial ecology. We present the prototype of the first spatially explicit individual-based model of bacterial growth. The simulation reproduced the desired bacterial growth properties correctly. In order to describe the dependence of cell size on growth rate at the single cell level, we implemented a mechanistic version of Donachie's conceptual model of cell division. Surprisingly, it appeared incomplete and had to be replaced by a descriptive version. Random variation of cellular parameters as well as spatial heterogeneity of substrate concentration each led to a complete loss of synchrony of the simulated culture. We propose new measures for growth synchrony and spatial heterogeneity. The model prototype shows the feasibility of this novel approach. It will be extended to become a generic tool for simulating all aspects of microbial growth under real life conditions while our focus will be on social behaviour in biofilms.

Introduction

Individual-based models are population or ecosystem models that do not state or prescribe any properties of the population they model. Rather, they describe all the actions of the organisms and their interactions with the environment and each other. The population structure and dynamics emerges from this. Since the population is built from the individuals, the differences between individuals and the spatial heterogeneity can be readily accounted for.

The aim of our research is to understand the complex adaptive *macroscopic* systems we find in microbial ecology in terms of the simpler *microscopic* constituents. It is hoped that this approach will lead to a unified theory of all such systems, including colonies, biofilms, granules, digester sludge, marine snow, etc. One could even argue that cancer is a special case of a colony of eukaryotic cells growing in a highly structured environment. Within this framework, the role of individual-based modelling (IbM) is the electronic synthesis of the complex systems from the empirical knowledge about the constituents. This reconstruction of a complex system by computer simulation will tell us whether our knowledge about the constituents is good enough for understanding the whole and, more importantly, address the question of how the actual complex system is selected from all the possible ones.

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The Model

BacSim is the first spatially explicit IbM of bacterial growth. It is an extended and modified version of Gecko, an 'ecological flight simulator' which has been used to study ecosystem dynamics such as trophic interactions in food webs [1]. Gecko has been written in the object-oriented language Objective-C using the Swarm toolkit for multi-agent simulations under development at the Santa Fe Institute, New Mexico, USA [4].
BacSim represents each simulated bacterial cell, called agent, "electronically" as an object instance of an object-oriented program. These agents exist in a continuous 2D space and have free range and extent - they occupy and compete for space. Time is discrete and agents repeat their schedule of activities once every time-step (Fig. 1). For substrate diffusion, a lattice with variable grid granularity and overall size is used to simulate diffusion gradients quantitatively. Agents take up substrate from the lattice elements in which they are located. As we want BacSim to be easily applicable to a large range of bacteria, the number of parameters used was kept to a minimum (Table 1).

Results

The simulator output faithfully reproduced all input parameters (Table 1). For growth rate dependent cell size variation, a conceptual model of cell division proposed by Donachie [2]

Table 1. Parameters used as input in model construction and the resulting model output. We have chosen reliable values typical for *E. coli*. If uptake is to be described by the Best equation, three instead of two parameters are needed for uptake. For surface area calculations, an average eccentricity of the cells of 4.4 [2] was used. Units given are as used in the model and are appropriate for the microbial scales of time and space.

Parameter	Units	Input	Model output ± SD
μ_{max}: maximum growth rate	min^{-1}	0.0205	$0.020428 \pm 1.7 \cdot 10^{-8}$
K_s: half saturation constant	fg fl^{-1}	$2.34 \cdot 10^{-3}$	$2.3328 \cdot 10^{-3} \pm 8.2 \cdot 10^{-9}$
Y_{max}: apparent yield at μ_{max}, Corrected for maintenance	$\frac{\text{fg dry mass}}{\text{fg glucose}}$	0.4444	$0.4437 \pm 8.0 \cdot 10^{-5}$
m: apparent maintenance rate at $\mu = 0$	$\frac{\text{fg glucose}}{\text{fg dry mass} \cdot \text{min}}$	$6 \cdot 10^{-4}$	$5.918 \cdot 10^{-4} \pm 2.2 \cdot 10^{-6}$
$\overline{V_u}$: median cell volume at $\mu = 0$	fl	0.4	$0.396 \pm 1.8 \cdot 10^{-4}$
Time for replication + cell division (only mechanistic Donachie models)	min	60	Not applicable
Minimal cell size	fl	0.1	Not applicable
Cell density (dry mass)	fg fl^{-1}	290	Not applicable
D: diffusion coefficient for glucose	μm^2 min^{-1}	40680	Not applicable

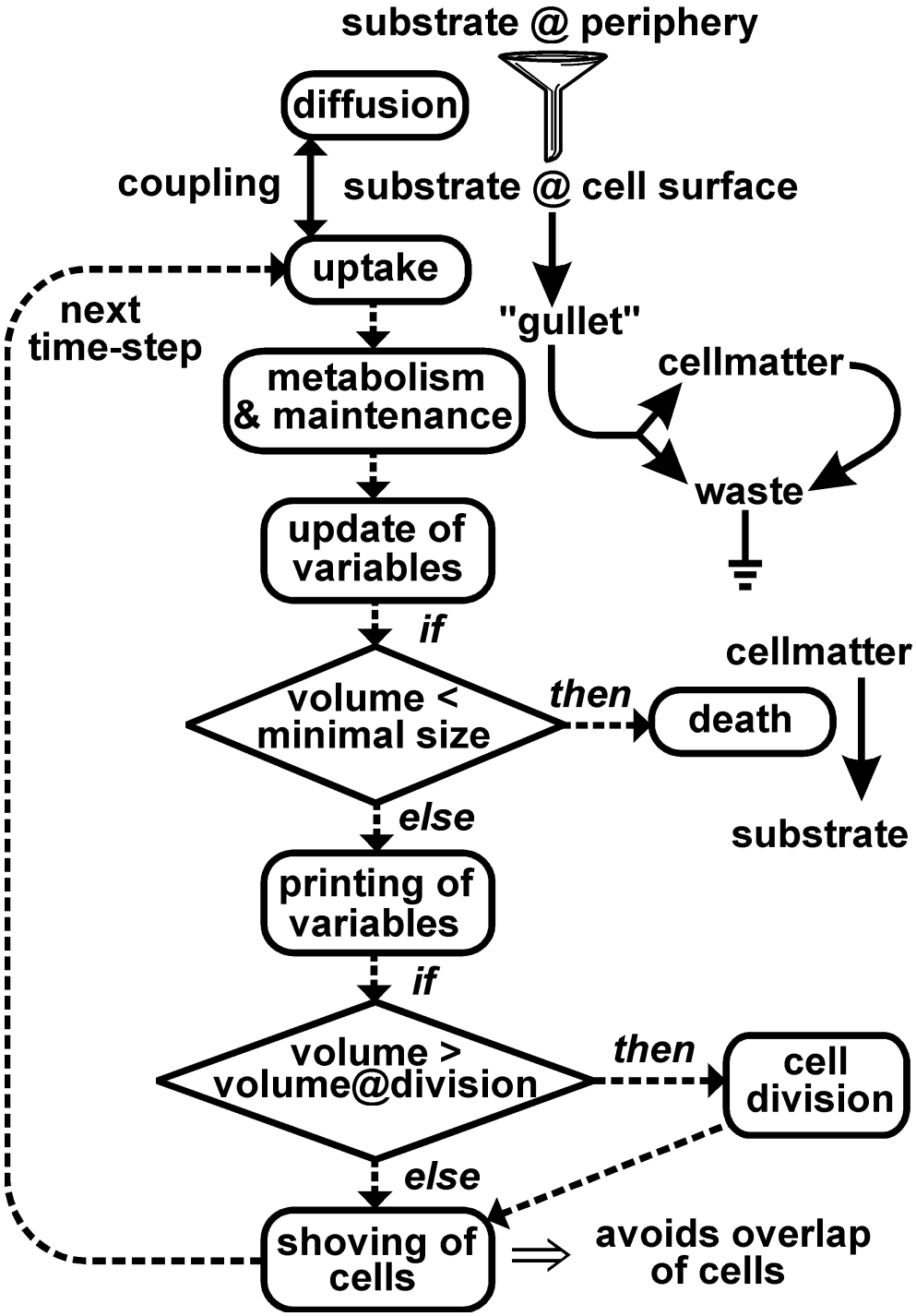
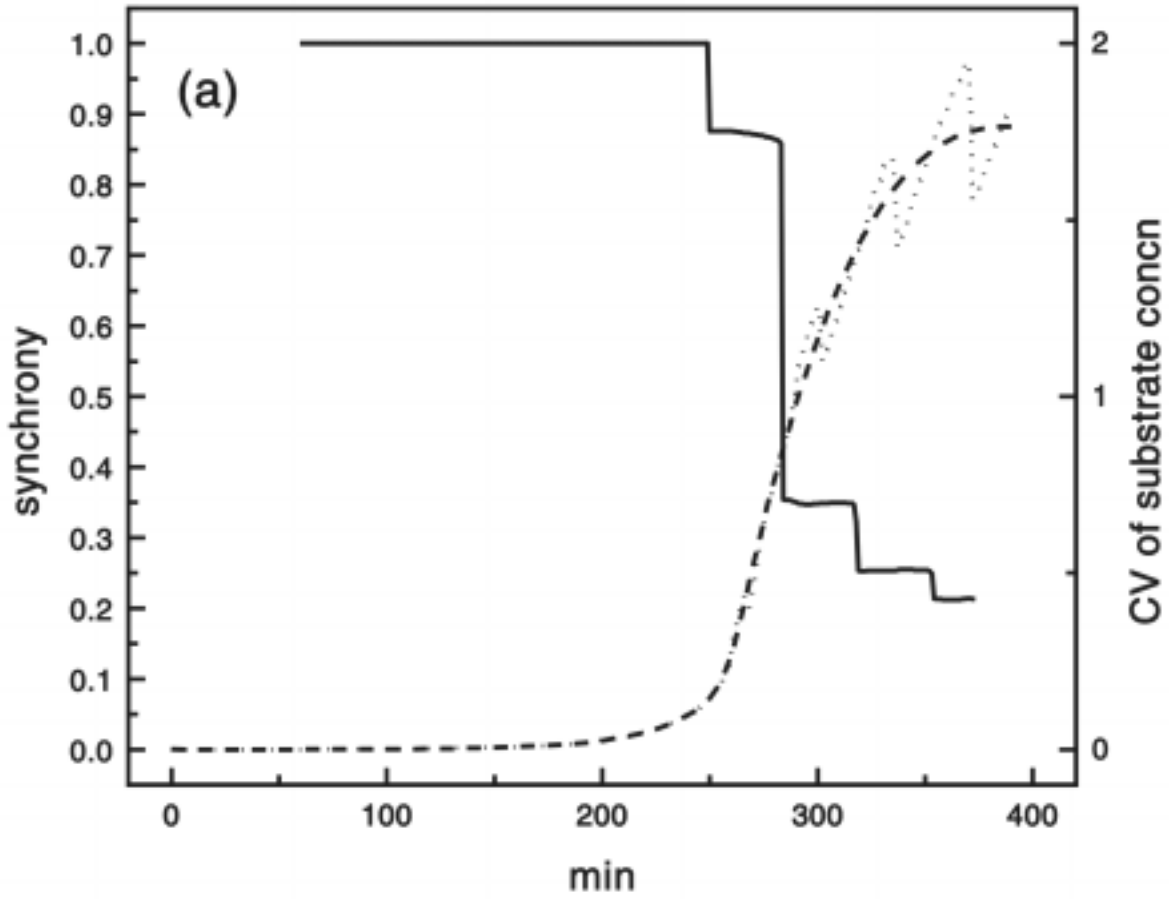
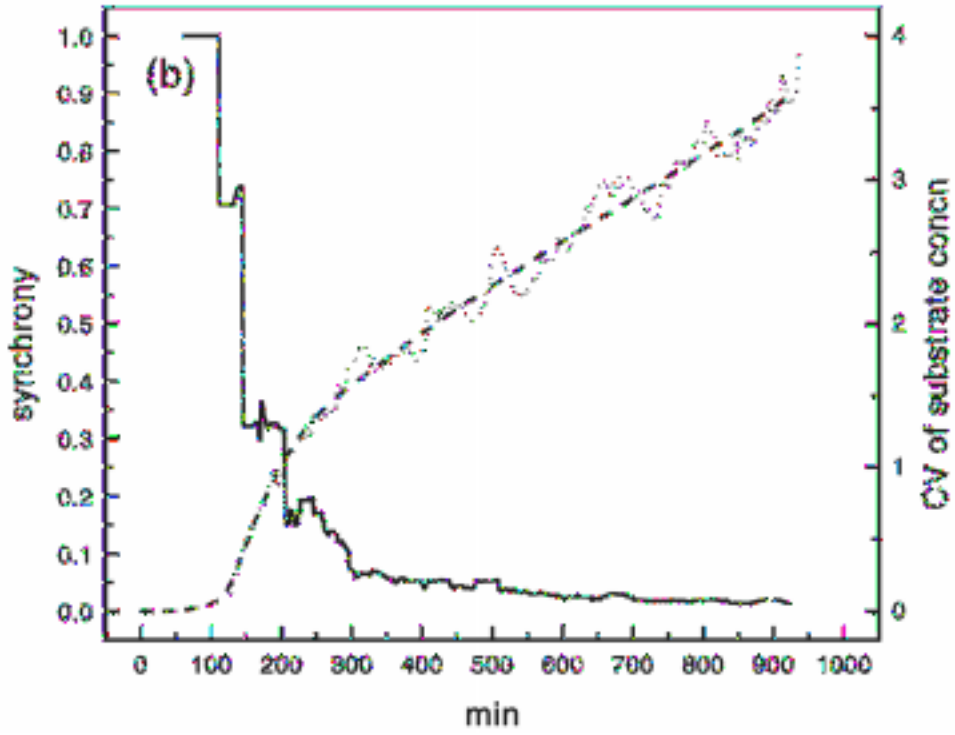
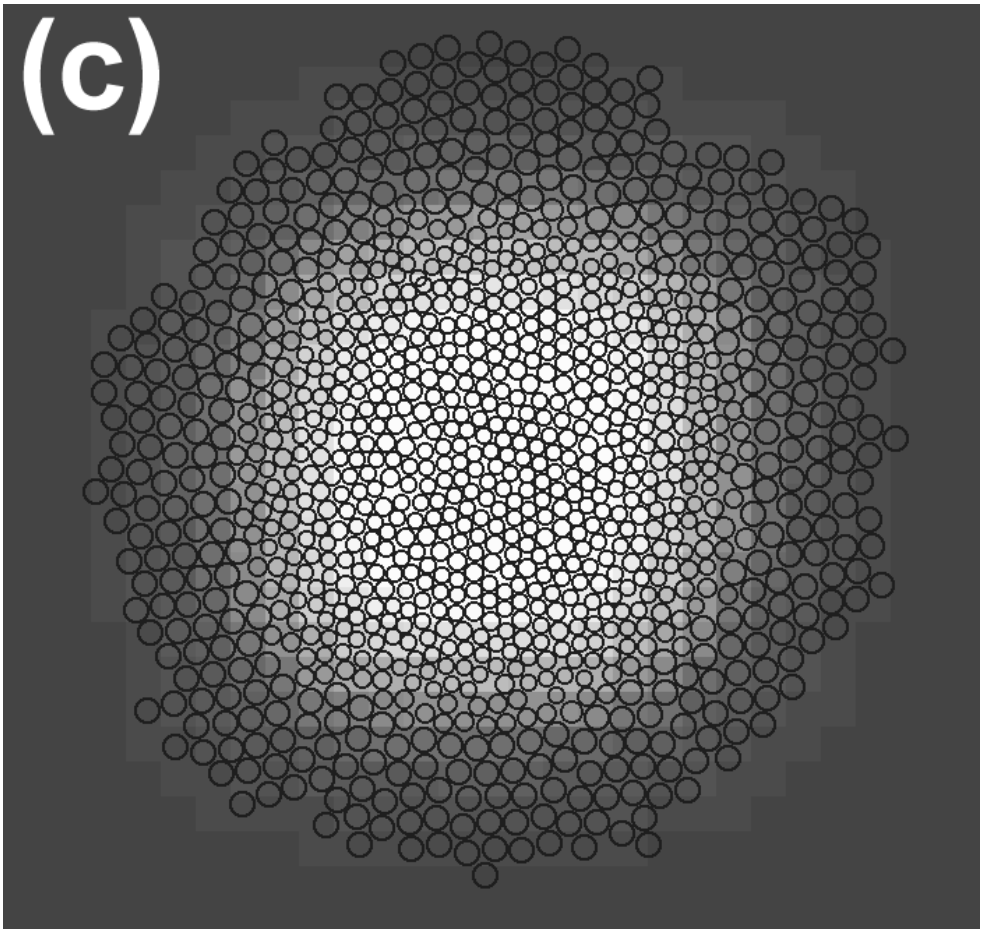


Fig. 1. Graphical pseudo code of bacterial agent activities. Dashed arrows denote the sequence of methods (methods in boxes) carried out each time-step by each agent. The rhomboid if-boxes denote decision methods. The funnel symbolises diffusional substrate flux from source (the reservoir bordering on the diffusion lattice) to sink (the cells in the colony). Simple arrows indicate metabolic conversions. The waste produced from substrate (due to metabolism) and from cell matter (due to maintenance) breakdown is set to zero after conversions (ground symbol). The double-arrow denotes the coupling of diffusion and uptake methods in the program. The shoving of cells avoids the temporary overlap of cells that would otherwise ensue.

was examined. A mechanistic version of the Donachie model led to unbalanced growth at higher growth rates, while including a minimum period between subsequent replication initiations ensured balanced growth only if this period was unphysiologically long. Only a descriptive version of the Donachie model predicted cell sizes correctly. We propose a newgeneric measure of growth synchrony to quantify the loss of synchrony due to random variation of cell parameters or spatial heterogeneity [3]. This degree of synchrony is entirely derived from the growth curve, using the moving standard deviation of the first derivative of the $\log_2$ of the cell number. Variation of the maximal uptake rate completely desynchronises the simulated culture but variation of the volume-at-division does not. We also propose as a new and simple measure for spatial heterogeneity, the standard deviation of substrate concentrations as experienced by the cells [3]. Spatial heterogeneity (Fig. 2) desynchronises population growth by subdividing the population into parts synchronously growing at different rates. At a high enough spatial heterogeneity, the population appears to grow completely asynchronously. Qualitatively, the simulated colony structure at low glucose concentration (0.1 g/l) resembles the fractal colony patterns observed experimentally and with diffusion-limited aggregation models [7], though the mechanisms implemented in BacSim are different.




(b)
synchrony
CV of substrate concn
min
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
4
3
2
1
0
0
100
200
300
400
500
600
700
800
900
1000


(c)

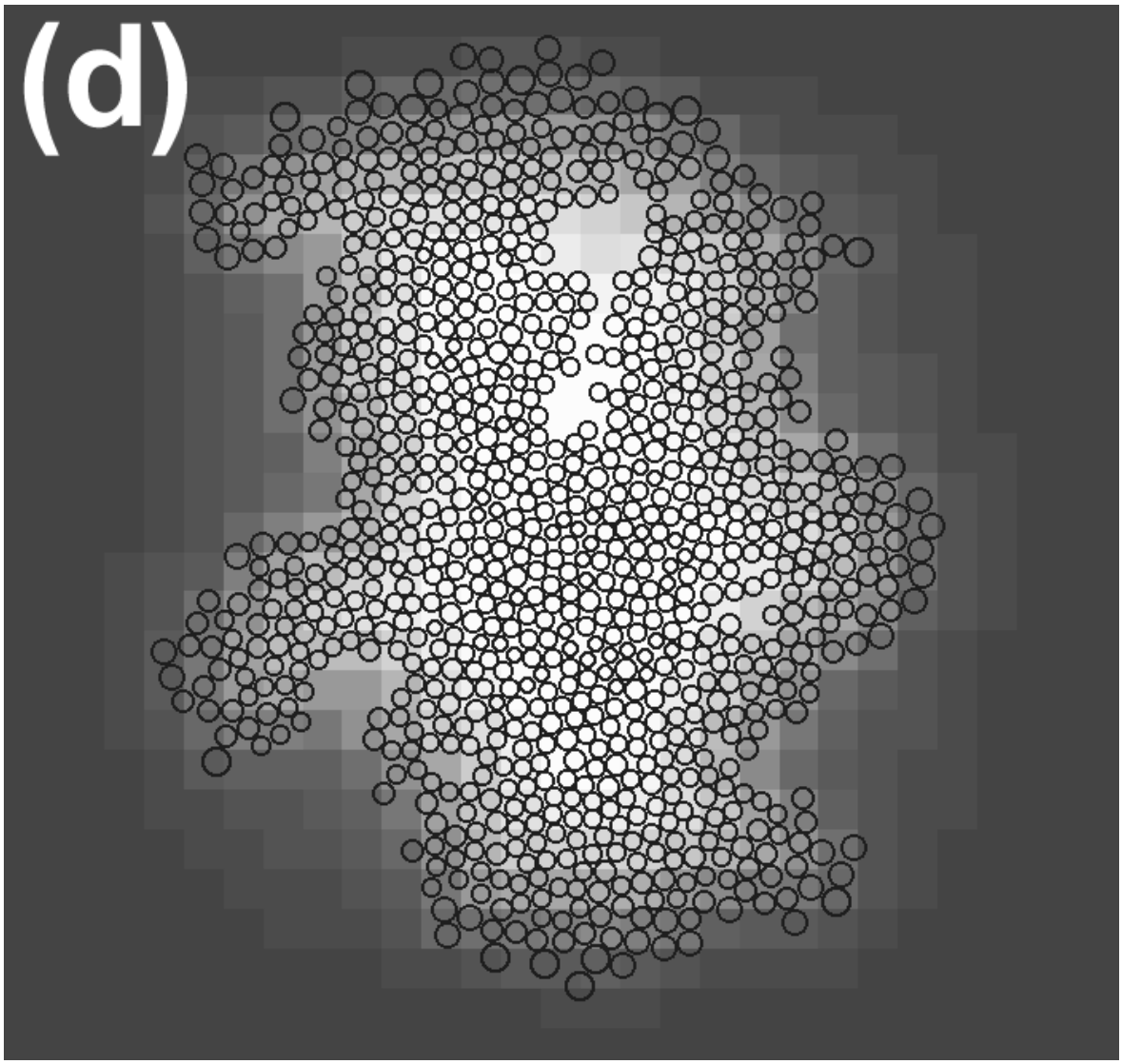


Fig. 2. Simulated colonies growing at various substrate concentrations develop spatial heterogeneity (no random variation of cell properties). **Graphs.** Loss of growth synchrony due to increasing spatial heterogeneity. Glucose concentration was 1 fg/fl (a) and 0.1 fg/fl (b). Degree of synchrony (*mSD(dl)*, ——); spatial heterogeneity (CV (coefficient of variation) of substrate concentration · · ·, spline fit - -). Note the fluctuations due to synchronous growth; bursts of divisions leading to rearrangements of cellular positions cause temporary fluctuations of the CV as a measure of spatial heterogeneity (if calculated by looping through all cells' exterior substrate concentration). Loss of synchrony typically does not follow a simple exponential decay function. **Screen shots.** Spatial heterogeneity at the end of runs (about 1000 cells) at reservoir substrate concentrations of 1 fg/fl (c), 391 min, and 0.1 fg/fl (d), 939 min. At 10 fg/fl, the spatial heterogeneity stays very low (CV <0.1) and is not visible on screen shots, also growth synchrony stays perfect (data not shown). The substrate concentration gradient is visualised as follows: the darker the square, the higher the concentration (logarithmic scale). The substrate concentration in the middle of figures (c) and (d) is too low to support significant uptake rates. Cells in the middle shrink at a maximal rate which is equal to the maintenance rate. The grid size of 2×2 μm used was sufficiently small. Simulations including random variation of cell parameters look very similar, emphasising the importance of spatial heterogeneity in colony development (data not shown).

Conclusions and Outlook

BacSim shows that quantitative IbM of bacteria is feasible and promising [3]. Microscopic simplicity - a few parameters for a very simple black box description of bacterial cells - can be mapped to macroscopic complexity. Growth data can be easily fed into the model and the model output is quantitatively correct. In two cases it was necessary to formulate new measures to describe simulation results (growth synchrony, spatial heterogeneity).

During this study the lack of information on the individual heterogeneity of growth parameters became increasingly apparent to us. Another area where information required for modelling is insufficient, is the volume fraction occupied by cells in colonies and biofilms. Experiments are under way to address this lack of information.

The model will be extended to allow simulation of growth at a larger spatial scale, as well as cell differentiation and pheromone signalling, aiming at modelling multi-species biofilms. This will be achieved partly by merging BacSim with the biofilm model developed in Delft [5, 6] (see also the paper of Picioreanu et al. in these Proceedings).

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