HHS Public Access

Author manuscript

ACS Synth Biol. Author manuscript; available in PMC 2023 May 18.

Published in final edited form as:

ACS Synth Biol. 2022 November 18; 11(11): 3564–3574. doi:10.1021/acssynbio.2c00411.

Agent-based Modeling of Microbial Communities

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Abstract

Microbial communities are complex living systems that populate the planet with diverse functions and are increasingly harnessed for practical human needs. To deepen the fundamental understanding of their organization and functioning as well as to facilitate their engineering for applications, mathematical modelling has played an increasingly important role. Agent-based models represent a class of powerful quantitative frameworks for investigating microbial communities owing to their individuality nature in describing cells, mechanistic characterization of molecular and cellular processes, and the intrinsic ability to produce emergent system properties. This article presents a comprehensive review for recent advances on the agent-based modeling of microbial communities. It surveys the state-of-the-art algorithms employed to simulate intracellular biomolecular events, single-cell behaviors, intercellular interactions, and interactions between cells and their environments which collectively serve as the driving forces of community behaviors. It also highlights three lines of applications of agent-based modeling, including the elucidation of microbial range expansion and colony ecology, design of synthetic gene circuits and microbial populations for desired behaviors, and characterization of biofilm formation and dispersal. The article concludes with a discussion of existing challenges, including the computational cost of the modeling, and potential mitigation strategies.

Keywords

microbial communities; synthetic biology; mathematical models; individual-based modeling; computational simulations; agents

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Author Contributions

All of the authors wrote, edited, and approved the paper.

Conflict of Interest

The authors declare no competing financial interest.

Supporting Information

Additional mathematical details for the architecture and implementation of ABMs.

Microbial communities are ubiquitous in natural and anthropogenic environments. Examples of the former include water, soil and the animal and human bodies.[1,2] The latter category comprises biotechnological applications, such as wastewater treatment and biocatalysis[1], and other industrial processes for the production of chemicals[3] and biofuels[4]. While microbial communities can be harnessed for the benefit of mankind through rational engineering,[5–8] they can also be harmful by causing infections that are difficult to treat with antibiotics,[9] contaminating medical devices and implants[1], and causing food spoilage.[10] A fundamental understanding of how microbial communities colonize and grow in various environments is essential for effectively manipulating them so as to maximize their benefits while mitigating their adverse effects. Mathematical modelling is playing an increasingly important role for this purpose. While modelling has been extensively used to interpret and explain experimental results, it can also be used to optimize experimental design[11] or make predictions about the spatiotemporal dynamics of microbial communities that can be tested experimentally. A wide variety of modelling approaches have been applied to study microbial communities. They include[11] primary, secondary and tertiary models,[12] empirical equations,[13] mechanistic[14] models, flux balance analysis,[15] reactive transport models,[16] Bayesian network models,[17] neural networks,[18] cellular automata[19] and agent-based models (ABMs).[20]

An important distinguishing characteristic of the various model classes is the spatial and temporal scales at which they operate. While metabolic models[14,15] are primarily concerned with the effect of intracellular processes upon cell physiology, population-level models seek to elucidate the effect of variables such as temperature, pH and nutrient concentration upon the growth of entire colonies that typically contain over 10^{10} cells.[21] Moreover, models that include diffusion and reaction of substrates need to resolve timescales as small as 10^{-3} seconds whereas biomass growth and decay typically occurs over several hours or days.[19,22] Recently, there has been much interest in developing hybrid models that integrate several sub-models operating at different length and time scales.[22–24] Agent-based models (ABMs), which are the focus of the present review, constitute one such class of hybrid models.

ABMs represent a microbial colony as a group of discrete agents with predefined attributes and whose interactions are governed by a set of rules. Each agent may represent an individual cell or a group of cells.[11] The objective of an ABM is to explain how macroscopic colony properties emerge as a result of microscopic interactions among agents, whose individual characteristics may exhibit a significant degree of variability. Their abilities to naturally incorporate heterogeneity in microbial colonies make ABMs the natural choice of model whenever such variability is known to play a significant role in the phenomenon of interest. This can happen for several reasons such as chemical concentration gradients, adaptation to local environmental conditions, stochastic gene expression and genotypic variation.[25] The ease of incorporating any number of physical, chemical and biological processes of interest is another tremendous advantage of ABMs that enable such models to be readily adapted to study microbial spatiotemporal dynamics in diverse settings and to provide mechanistic insights into the origins of system properties. Finally, this class of models can be useful for deriving additional insight into dynamics that is unavailable from experimental data. For example, detailed comparison of the results of ABMs in the presence

and absence of hydrodynamic interactions have been instrumental in understanding their effects upon collective oscillations[26] and chemotactic cell migration[27] in dense bacterial suspensions. ABMs also enable one to monitor transient changes of characteristics such as growth rate in different population sub-groups,[28] which may be difficult to achieve experimentally.

This review begins with a survey of the architecture of ABMs and the manner in which they are implemented. This is followed by the applications of ABMs for the disciplines of microbial range expansion and colony ecology, synthetic biology regarding the design and engineering of synthetic populations for spatiotemporal patterns, and biofilm formation and dispersal. Finally, challenges in the application of ABMs and corresponding potential solutions are explored.

ARCHITECTURE AND IMPLEMENTATION OF ABMS

An ABM typically includes several sub-models of processes occurring at multiple dimension and time scales. The components of an ABM can be broadly classified into molecular events, single-cell behaviours, intercellular interactions and interactions between cells and their environment (Figure 1). A wide variety of modelling approaches have been used to incorporate these components into the various ABMs presented in the literature to date. The focus of this section is to evaluate the modelling tools available for each component and discuss their advantages and disadvantages.

Biomolecular events.

ABMs are capable of incorporating several types of intracellular biomolecular events including gene regulation,[23] signal transduction,[29,30] metabolic reactions and evolutionary processes. Gene regulation is the control of gene expression that specifies the production of proteins that serves as functional biomolecules, such as enzymes and transporters, for various intracellular processes. Signal transduction is the propagation of external and internal information through the conformational transition or abundance change of biomolecules for cellular decision making or functional realization, such as chemotaxis of microbial cells in the presence of chemical gradients.[29,30] Metabolic reactions characterize the conversion of materials and the production and consumption of energy inside the cell, and underlie metabolite synthesis and secretion, cellular growth and division, and other phenomena such as bioluminescence.[31] Seemingly diverse, these molecular events can all be described using a system of coupled ordinary differential equations (ODEs) comprising of one equation for each molecular species of interest in each agent.[32] The intracellular dynamics are coupled to the rest of the simulation by making the rates of change of each intracellular variable dependent upon one or more external variables such as the local nutrient, metabolite or toxin concentration or forces exerted by neighbouring cells.[33] An alternative, and possibly more realistic method of simulating intracellular molecular events, is to use delay differential equations (DDEs).[23] While this method accounts for the influence of environmental conditions upon each agent’s physiological state over a period of time, the simulation may produce divergent results due to intracellular chemical concentrations being

reset during cell division.[23] In such cases, the parameter values in the model may need to be carefully tuned to ensure that the results are meaningful.

Compared with gene regulation, signal transduction and metabolic reactions that occur over relatively short time scales typically, the change in the genetic make-up of cells due to natural selection typically occurs much longer.[21] Such mode of molecular events can be modelled by using random variables to represent agent genotypes and allowing for random mutations during the cell division process. The occurrence of mutations may affect one or more cellular functions such as the ability to produce public goods[34] or toxins[35] depending upon the modelling objectives.

Single-cell behaviours.

The most characteristic nature of ABMs is the individual-based treatment of cells. Agents can represent individual cells in some models[22] or a group of cells in others[36]. In addition, agents can be spherical[22,24] or rod-shaped[37,38] in the former case but they are always spherical in the latter. Different ways of modelling can lead to a trade-off between spatial resolutions and computation cost. Namely, the coarse-grained approach of representing a group of cells with an agent has lower computational cost; meanwhile, it also has a lower spatial resolution.

After introducing the definition of agents, it is necessary to identify the properties assigned to each agent. Primary properties included in ABMs are the agents' types, sizes and mechanical properties such as their forces, positions and velocities. It is also needed to specify the rules governing the temporal evolution of these properties. One prominent trait of agents is their movement, which can be broadly classified as passive or active. The former is the result of mechanical interactions with other agents and environmental forces arising from impenetrable walls. As explained in the next section, mechanical interactions may be modelled using rule-based approaches that forbid agent overlap or by solving the equations of motion determined by Newton's Laws. Models employing rod-shaped agents typically use the latter approach due to the need to simulate both rotational and translational motion. On the other hand, active motion, better known as cellular motility, arises from internal propulsive forces. Bacteria such as *E. coli* bear the ability to actively swim in liquid medium, by switching their state between running or tumbling[27] to achieve motion in a random walk pattern. In the running state, the flagella rotate in counter-clockwise to form a bundle, such that a net propelling force is generated. By contrast, in the tumbling state, the flagella rotate in clockwise and the cell changes it orientation randomly. The associated propelling force and corresponding cell velocity and reorientation may be determined using a rule-based approach[39] or a random number generator.[29,30,40] Chemotaxis is the behaviour that a cell actively moves towards a higher concentration of an external attractant such as methyl-aspartate or serine for *E. coli*. In the absence of attractant gradient, the probability of the cell in the running state is constant. However, when the cell senses the gradient, the running probability is increased according to the level of gradient. Such a cellular movement behaviour is governed by an intracellular signalling network, which is typically modelled as a set of ordinary differential equations (ODEs). Notably, while most modern ABMs[22,24] simulate agent motion in continuous space, lattice-based models have also been used.[32]

In addition to movement, most ABMs incorporate agent growth and reproduction[22–24,41] while some also simulate decay and death.[22,24] Agents' growth rate is typically determined by the local concentration of one or more nutrients according to the Monod equation. Each agent has a pre-defined maximal size above which it divides into two agents. The positions and sizes of the two resulting agents are usually determined stochastically. Agent death is simulated by the deletion of agents whose sizes fall below a threshold value.[22,24]

Cell-cell interactions.

Interactions between agents can be either physical or chemical in nature. Physical interactions refer to the excluded-volume interactions between agents as well as between agents and simulation boundaries where there are impenetrable walls. Two distinctive methods have been adopted to model such interactions. The first involves implementing special algorithms that prevent cell overlap.[24,28,36] These algorithms typically involve computing a displacement vector that accounts for the overlaps of a target agent with its neighbours and translating the agent's position by that vector.[24] This process is performed for each agent in a simulation and multiple iterations of the entire cycle may be necessary to completely resolve overlaps between all pairs of agents.[36] In the second approach, one evaluates the total force exerted upon each agent by its neighbours and uses this information to update the cells' velocity and position according to Newton's laws.[22,37,38,42,43] Forces between cells are typically evaluated using the Hertzian model.[22,37,38,42,43] In the case of non-spherical agents, it is also necessary to update their angular velocities and orientations by evaluating torques.[37,38,42,43] While the first method has been widely used for its ease of implementation and lower computational cost,[24,36,44–46] it is not readily generalizable to the case of non-spherical agents.

In addition to physical interactions, agents may also interact among themselves by secreting diffusive public goods, metabolites and toxins into the extracellular space.[47] Such chemical interactions are simulated by solving partial differential equations (PDEs) that describe the variation of chemical concentrations over time and across space. Here, the variation typically arises as a result of chemical diffusion, production and consumption. In cases with fluid flow, the PDE for each chemical would contain additional terms describing transport due to convection. A wide variety of methods are available to solve the PDEs including nonlinear multigrid,[44] Fourier transform,[35] marker-and-cell,[22] and lattice Boltzmann[19] methods. The local concentrations of various chemicals at an agent's location are used to update its properties at each time-step.

In microbial communities, chemical interactions may occur over a wide range of spatial scales. For example, toxins secreted by a cell only affect neighbouring cells in the case of contact-dependent inhibition[48,49] but may also result in the death of distant cells in the case of long-range inhibition.[50,51] Both situations can be modelled in ABMs by setting the relevant chemical diffusion coefficients to an appropriate value. In addition to chemical interactions, conjugation represents another important class of interactions in microbial communities that involves transfer of genetic material between cells.[28] It is typically simulated by identifying potential recipients in the vicinity of each donor and assigning a probability for successful plasmid transfer between agents. Additionally,

chemical interactions are shown highly variable over time,[52] which can be potentially characterized with ABMs.

Microbial communities often develop into biofilms which contain extracellular polymeric substances (EPS). EPS fulfils various functions such as maintaining the cohesiveness of the biofilm and providing nutrients for cells.[47] Special algorithms are used to simulate physical and chemical interactions between agents in biofilms. Models using spherical agents typically simulate EPS production through the accumulation of an outer EPS shell upon each producing agent.[22,24] The thickness of the shell affects the strength of excluded volume interactions between EPS-containing agents. A portion of the EPS belonging to each agent is regularly released to the environment where it exists as an agent of the EPS type.[22,24,28,38,53,54] These EPS particles can undergo excluded volume interactions with all other agents in the simulation. Some studies have also used continuum methods to simulate the modification of physical interactions between agents due to EPS.[55–57] The presence of EPS also modulates chemical interactions by reducing the diffusion coefficients of various chemicals by up to 40%.[25]

Cell-environment interactions.

ABMs of microbial communities typically incorporate several interactions between agents and their environment. The most common process in this category is chemical exchange, which includes uptake of nutrient and antibiotics and the release of metabolic by-products. In cases where the effect of spatiotemporal variation in nutrient concentration is the primary objective of investigation,[54] it is necessary to solve the necessary PDEs as described above. In some studies,[24] the concentration of available nutrient was varied periodically with time to examine the effect of such variation upon the rate of colony growth. However, if one is simulating colony growth in a nutrient rich environment where there are no significant substrate concentration gradients, the simulation protocol can be simplified by omitting these equations and assuming a constant growth rate for each agent.[42,58]

In the context of biofilms, erosion and detachment constitute another important class of agent-environment interactions that usually arises due to the physical effects of fluid flow and is responsible for the removal of biomass. In the case of models that do not explicitly include fluid dynamical effects, the rate of biomass removal is usually determined using an empirical detachment function.[24] Li *et al.*[59] examined the effect of using different detachment functions upon the biofilm morphology. In mechanistic models that incorporate the effect of fluid dynamics,[22,54] biomass detachment arises as a result of the drag force experienced by cells due to local velocity gradients. Fluid dynamical effects can be simulated using computational fluid dynamics (CFD)[22] or lattice Boltzmann[19,36] methods. Additionally, the interactions between cells and the environment may also be altered by the presence of EPS. EPS facilitates the adhesion of cells upon the substratum in the early stages of colonization and acts as a scaffolding that maintains the cohesiveness of biofilms and limits the ease with which environmental effects such as fluid flow can remove biomass from biofilms.[47] These functions of EPS can be incorporated into an ABM by modelling its effect upon physical interactions using the methods described above. Moreover, EPS may improve cells' tolerance to antimicrobial agents by reducing their diffusion rate in biofilms.

The four classes of forementioned processes and interactions occur over a vast range of length and time scales. To address the computational cost, a sequential solution procedure is often employed that uses timesteps of different orders of magnitude when updating the simulation variables associated with the different processes.[19,22] This approach assumes that all faster processes have reached pseudo-equilibria while all slower processes are 'frozen' in time when simulating a sub-model associated with a particular process. Such a multi-timestep algorithm is essential for realizing simulations at experimentally relevant length and time scales, especially when modelling biofilms. Mathematical details regarding the implementation of ABMs can be found in the Supplementary Information.

Emergent properties of microbial communities.

Microbial communities have a remarkable ability to undergo spontaneous self-organization and form distinctive patterns, whose emergence depends upon environmental conditions as well as the social interactions between the species comprising the colony.[60] ABMs can be viewed as *in silico* experiments that shed insight upon the manner in which population level characteristics naturally emerge as a result of microscale interactions within and among agents in a heterogeneous population. For example, relating to the spatial segregation of mutually inhibiting species through toxin production,[37] pattern formation due to engineered gene circuits[61] and genetic sectoring during range expansion[62], ABMs can all be potentially applied. Indeed, they have been successfully used to predict the spatial structure of microbial colonies at long time intervals as well as to investigate the dynamics of the self-organization and pattern formation process[61,63]. It is also possible to carry out quantitative comparisons of the extent of self-organization for different values of model parameters by evaluating quantities such as the radial distribution function and static structure factor.[38]

A wide variety of other population level characteristics can also be examined. Simulations of biofilms are typically concerned with the values of morphological parameters such as the height, roughness and biomass area density[22] as well as the expansion rate in one or more directions.[64] Parameters describing the population composition such as species fractions, diversity and relative fitness of different species are of particular interest in simulations involving multiple species[24,53]. Models that study how selective pressures determine the genotypes of agents in spatially structured environments examine the number of species with various genotypes at different time points.[34,35] In addition to the above variables, examining the fluid velocity and nutrient concentration profiles can provide useful insight into the biophysical mechanisms responsible for heterogeneity within a population.[22]

APPLICATIONS OF ABMS

Microbial range expansion and colony ecology.

Microbial social interactions such as competition and cooperation can play an important role in shaping the emergent properties of microbial ecosystems. Over the last decades, ABMs have been widely used to investigate the emergent ecosystem properties during population growth in space as a result of the user-defined interactions at the micro-scale. A set of software packages have been developed for this purpose including AgentCell,[65] BSim,[23,66] BNSim,[67] and gro.[68]

Blanchard *et al.*[37] employed an ABM to systematically characterize the six fundamental types of social interactions for a simple two-species ecosystem, including neutralism, commensalism, amensalism, competition, mutualism, and parasitism.[69] Their model incorporated various processes occurring at different length and time scales including intracellular biomolecular events, cell elongation and division, cell movement and rotation due to physical contacts between cells, chemical exchange between cells and environment, and diffusion of extracellular molecules. Each cell was modelled as a rigid rod surrounded by an elastic cylinder shell with two half-spherical caps. Collision-induced forces between cells were simulated using the Hertzian model.

By simulating colony expansion on a 2D plain from a well-mixed, equal relative abundant initial colony, the spatial patterns of a series of microbial colonies were analysed (Figure 2A). Depending on the type of interactions, colonies showed distinct spatial characteristics at growing fronts. Specifically, communities with asymmetrical social interactions (commensalism, amensalism and predation) yielded unbalanced structures with the species benefiting relatively from interactions dominants the colonies; by contrast, communities with symmetrical interactions (neutralism, cooperation and competition) gave rise to balanced spatial structures. The authors further analysed the patterns of microbial colonies by comparing the surviving lineage and sector number as measurable indicators of cellular social interactions. Notably, among the three symmetrical cases, mutualism resulted in the most sectors, neutralism had the second most, and competition yielded the least.[37]

In addition to social interaction between microbial species, motility and chemotaxis play critical roles in microbial spatial structuring. To quantitatively understand and characterize bacterial motility and chemotaxis, ABMs have been applied for *in-silico* explorations. Kalinin *et al.*[29] and Jiang *et al.*[30] constructed an ODE-based mathematical description of bacterial chemotactic sensing in controlled chemical concentration gradients, and showed with simulations that the drift velocity–the magnitude of velocity vector along the gradient–remains constant in fixed exponential gradients but decreases in linear gradients. Based on the findings, an ABM was recently developed to enable an individual-based, population-level modelling of chemotactic microbial populations in space (Figure 2B).[70] In another effort, Micali *et al.*[40] used a modified version of the ABM, Rapidcell,[40] along with experiment to characterize the drift velocity in exponential gradient of *E. coli*, through which they uncovered that the drift velocity changes as a function of local chemical concentration. With these explorations, the contributions of bacterial motility and chemotaxis on colony expansion were subsequently revealed by Cremer *et al.*[71] who employed ABM as a validation tool in conjugation with PDE-based modelling and quantitative experiments.

Meanwhile, recent studies have applied ABMs to simulate microbial communities for understanding the relationship between cell morphology and spatial colony pattern,[72] thermodynamics and microbial growth[73], and the cell density-dependent toxin production for spatial expansion.[74] These models have the intrinsic ability to incorporate the phenotypic heterogeneity of growing microbial populations.[75] Moreover, a recent ABM software ARCADE was developed to present the emergence of bacteria population in dynamic microenvironments, which provides a rule-based, on-lattice framework that involves bacteria, immune cells and tissue.[32]

Patterns and dynamics of engineered populations.

Another line of ABM applications lies in the design of engineered organisms for synthetic biology purposes.[76,77] Compared with other tools, ABMs confer a detailed, mechanistic description of the molecular and cellular processes from gene expression to chemical and mechanical interactions, cell motility and chemotaxis, and cell-environment coupling, thereby enabling the generation of emerging spatiotemporal community properties from microscopic interactions. Thus, in theory, ABMs allow researchers to quantitatively describe gene circuits, their coupling to hosts and environments[78] for quantitative and predictive analysis of gene circuits.

One great example illustrating such applications is BSim,[66] a flexible computational tool for agent-based, multiscale simulations of microbial populations. Treating microbes as autonomous agents, BSim describes realistic intracellular molecular processes such as gene regulation with ODEs and captures dynamic, heterogenous and three-dimensional environments with user-defined meshes. It allows to explore the relationship between microscopic driving forces of agents and macroscopic collective behaviors of an entire population. The tool was used to simulate four spatially structured populations, that were engineered to fulfil the EQUAL Boolean function, and a growing engineered population that carries a synthetic three-gene circuit with an oscillatory protein production. For the both cases, Bsim yielded steady-state outputs that are consistent with experimental results, which illustrated the power of the tool in quantitatively modeling engineered strains and enhancing system robustness through the integration of circuit dynamics, population statistics and spatial organization.

To expand its application regime, BSim was recently upgraded to yield a new version, BSim 2.0,[23] by allowing the definition of the realistic, three-dimensional morphology of individual cells and the specification of physical parameters of realistic environments during experimental setups such as microfluidic devices. Compared to other software packages, the updated tool addressed the lack of fundamental features that are crucial to realistic description of physical interactions among cells and between the cells and their environments. To illustrate its utility, the tool was applied it to simulate an engineered bacterial consortium which involves coupled positive and negative feedback loops and produces oscillatory population dynamics in a microfluidic chip (Figure 3A). The simulations successfully reproduced the population-level oscillation of the engineered consortium. Additionally, the simulations revealed a high-level sensitivity of system dynamics to the parameters associated with signaling chemicals and boundary conditions of the environment, and further uncovered the impacts of spatial segregation of the populations on the oscillatory dynamics. Together, the consistency of the simulations with the corresponding experiments demonstrated BSim 2.0 as a useful tool for the engineering of gene regulatory circuits in multi-strain consortia.

To facilitate the advance of synthetic biology, researchers have also explored the potential of ABMs for guiding rational gene circuit design. In a recent study,[79] Leaman *et al.* experimentally characterized the spatiotemporal onset dynamics and emergent properties of an engineered bacterial population that contains quorum-sensing regulated gene expression. In parallel, they built an agent-based model that describes quorum sensing and biophysical

interactions among cells and between cells and environments (Figure 3B). Impressively, the model successfully predicted the activation time of quorum sensing with a remarkable degree of accuracy (<7%) for various cell densities and uncovered a power law pattern of gene activation time with respect to the initial cell density. In addition, the model was successfully utilized to guide the design of ribosome binding site, the nucleotide sequence located at the upstream of an mRNA molecule and critical for the initiation of translation, which resulted in predictable, controllable tuning of the expression activation time over a 30-fold range. The study served as a compelling example of ABMs which quantitatively recapitulates not only the intracellular temporal modulation of gene expression but also the spatiotemporal dynamics of microbial populations. It also illustrated the promise of model-guided, predictive design of synthetic circuits, a milestone for the design, construction, and optimization of synthetic microbial systems.

Biofilm formation and dispersal.

The realistic modelling of biofilms represents one of the most exciting and important applications of ABMs (Figure 4). The iDynoMiCS[24] software package is a comprehensive tool available for biofilm simulation. It was developed based on several previous work over the preceding decade.[44–46,55,56,80–82] Meanwhile, it contains several novel features such as the incorporation of a pressure field to describe the shrinkage of mature biofilms, continuous release of EPS into the extracellular domain by individual agents, and metabolic switching in response to changes in environmental conditions.

The utility of this tool was illustrated using two case studies, the first of which investigated the relative abundance of three different species that took varying amounts of time to switch from aerobic to anerobic metabolism or vice versa in response to periodic variations of oxygen concentration in the environment.[24] It was found that the fastest responding species always dominates the biofilm in the absence of a metabolic cost for switching pathways, particularly when the frequency of oxygen pulse was high. However, when the cost associated with switching was greater for species with a shorter response time, the optimal switching strategy was found to depend upon the pulse frequency. While the fastest responding species was still favored in cases where the oxygen concentration changed very rapidly, the species with an intermediate value of response time had the highest relative abundance at low pulse frequencies.

Leveraging iDynoMiCS, Merkey *et al.* further studied plasmid invasion of biofilms[28]. By performing a sensitivity analysis of model parameters, they found the time delay between the points when a cell first receives a plasmid and begins to participate in horizontal gene transfer (HGT) of that plasmid to other cells to be the most important parameter that dictates the speed of plasmid invasion. Other parameters that significantly affected the invasion speed include the probability of successful transfer, scan speed, EPS yield and pilus reach. They also found that plasmid invasion is easier for a young biofilm that is growing rapidly as compared to a mature biofilm due to the higher rate of cell division in the former case. Moreover, a stronger dependence of HGT upon local growth rate reduced the depth of penetration of the plasmid into the biofilm. Finally, the propagation of the plasmid

throughout the population was favored by the initial placement of the single donor cell in the interior of the biofilm where it is surrounded by a greater number of potential recipients.

The first model capable of simulating all scales of processes occurring in a biofilm with a purely mechanistic approach was proposed by Li *et al.*[22] Known as NUFEB, this package is built upon the large-scale atomic/molecular massively parallel simulator (LAMMPS),[83] an open-source molecular dynamics software. One of its key advantages over other tools is that biofilm detachment arises naturally as a result of the drag forces experienced by cells near the biofilm surface, which avoids the need of empirical functions for modeling the phenomenon. In addition, the tool incorporates realistic fluid dynamics through dynamic coupling of the LAMMPS simulation with SediFOAM,[84] a computational fluid dynamics software. Moreover, the framework has been shown to exhibit a good parallel performance and allow to be scaled up for the simulation of large systems containing up to 10^7 cells.

NUFEB has also been utilized to simulate biofilm growth under varied nutrient conditions[54]. Specifically, the study revealed that the biofilm surface is smooth and compact in the rich case but rough and wavy in the poor case, in agreement with previously reported results[19]. It also showed that smooth biofilms were obtained if the nutrient source was located at the substratum instead of above the top surface even if the nutrient concentration was relatively low. This was due to the fact that the nutrient is more evenly distributed throughout the biofilm at the substratum. Additionally, the introduction of shear flow was shown to significantly reduce a biofilm's surface roughness and porosity, which is because increasing shear rate reduced the average volume of detached biomass segments although it also resulted in a greater frequency of detachment events.

More recently, the utility of NUFEB was demonstrated for the simulation of complex biofilms containing nitrifiers, heterotrophs and EPS.[22] The study showed that, although heterotrophs grew faster than nitrifiers in the early stages of biofilm growth, they were eventually outcompeted by nitrifiers due to changes in the chemical environment within the reactor as reported by previous experimental efforts.[85] In addition, the study revealed that biofilms can develop a wavy surface profile due to the non-uniform initial distribution of microbes upon the substratum. Moreover, this case study demonstrated the feasibility of NUFEB for simulating large biofilm systems containing up to 2.3×10^7 cells with efficient, highly parallelized algorithms without the need for coarse graining.

CONCLUSION

In this article, we reviewed ABMs of microbial communities by outlining the fundamental algorithms underlying the individual-based description of diverse molecular and cellular processes of microbial populations and highlighting the applications of ABMs for microbial range expansion and spatial ecology, patterns and dynamics of engineered populations, and biofilm formation and dispersal. Despite tremendous and versatile utilities of ABMs, there are several outstanding challenges in the field. One main issue is that the computational cost of ABMs is high compared to models that directly study population level characteristics[21]. This is particularly true for simulations that consider three-dimensional space or incorporate fluid dynamics. Although state-of-the-art computational algorithms enable the simulation

of systems containing up to 10^7 cells, the simulated population size is still several order of magnitude smaller than many biological systems such as the human microbiome or wastewater treatment facilities.[86] To address the challenge, alternative techniques are being actively pursued with the goal to generate comparable results as ABMs but at a significantly lower computational cost. One such approach makes use of statistical emulators that reformulate the ABM into a more computationally tractable model, which allows to accurately predict the values of metrics such as floc size, biofilm roughness and diversity that are essential for the design and operation of bioreactors for wastewater treatment.[86] If the goal is to investigate the spatiotemporal dynamics of microbial range expansion, one can also use PDE-based models derived from continuum mechanics principles to determine the colony expansion rate.[61] This approach was demonstrated to produce results equivalent to those obtained from an ABM and to accurately simulate pattern formation in a microbial colony containing an engineered gene circuit.[61] Notably, in addition to reducing computational cost, the relatively simple structures of these models may facilitate the development of biophysical insights into system dynamics.

Other challenges of ABMs include the need for prior knowledge of systems being modelled, the difficulty of obtaining biophysical insights, and the need to consider the effect of agent shape. Before constructing an ABM, it is necessary to have a good understanding of the experimental system being modelled to ensure that all relevant processes are included in the model[21]. It is also helpful to identify the qualitative effects of model parameters upon the variables of interest.[21] However, this may not always be possible. Often, several rounds of model redesign and cross-validation with experiment are necessary before satisfactory results are obtained. The complexity and high level of microscopic details involved in the development of ABMs is another potential challenge. Often, the effects of model input upon the output are not clear, which can hinder the extraction of biophysical insights from the modelling process.[20] Finally, most ABMs have used use spherical or cylindrical agents to represent individual cells for computational convenience.[22,24,28,53,54] However, microbes in nature have diverse shapes and asymmetrical divisions. In addition, it has been shown that cells' shape can affect the organization of microbial communities, such as the structure of a growing biofilm in the presence of nutrient concentration gradients.[72] Further research is thus required to expand the capacity of ABMs for different cellular shapes.

While ABMs have been developed and used in studying microorganisms for over two decades, their full potential is only just beginning to be realized. As ABMs become increasingly realistic and scalable, their usage is expected to become increasingly popular, not only for the purpose of explaining experimental results but also for guiding and optimizing experimental design. It is also expected to facilitate the engineering of complex ecosystems such as industrial bioreactors and the human microbiome toward diverse applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgement

This work was supported by the National Institutes of Health (GM133579), National Science Foundation (1553649) and Department of Energy (DE-SC0019185).

References

(1). Flemming HC; Wingender J; Szewzyk U; Steinberg P; Rice SA; Kjelleberg S Biofilms: An Emergent Form of Bacterial Life. Nat. Rev. Microbiol 2016, 14 (9), 563–575. 10.1038/nrmicro.2016.94. [PubMed: 27510863]

(2). Hall-Stoodley L; Costerton JW; Stoodley P Bacterial Biofilms: From the Natural Environment to Infectious Diseases. Nat. Rev. Microbiol 2004, 2 (2), 95–108. 10.1038/nrmicro821. [PubMed: 15040259]

(3). Shahab RL; Brethauer S; Davey MP; Smith AG; Vignolini S; Luterbacher JS; Studer MH A Heterogeneous Microbial Consortium Producing Short-Chain Fatty Acids from Lignocellulose. Science 2020, 369, 1073. 10.1126/science.abb1214.

(4). Bao T; Feng J; Jiang W; Fu H; Wang J; Yang ST Recent Advances in N-Butanol and Butyrate Production Using Engineered Clostridium Tyrobutyricum. World J. Microbiol. Biotechnol 2020, 36 (9), 1–14. 10.1007/s11274-020-02914-2.

(5). Brenner K; You L; Arnold FH Engineering Microbial Consortia: A New Frontier in Synthetic Biology. Trends Biotechnol. 2008, 26 (9), 483–489. 10.1016/j.tibtech.2008.05.004. [PubMed: 18675483]

(6). Grosskopf T; Soyer OS Synthetic Microbial Communities. Curr. Opin. Microbiol 2014, 18 (100), 72–77. 10.1016/j.mib.2014.02.002. [PubMed: 24632350]

(7). Deter HS; Lu T Engineering Microbial Consortia with Rationally Designed Cellular Interactions. Curr. Opin. Biotechnol 2022, 76, 102730. 10.1016/j.copbio.2022.102730.

(8). Johns NI; Blazejewski T; Gomes ALC; Wang HH Principles for Designing Synthetic Microbial Communities. Curr. Opin. Microbiol 2016, 31, 146–153. https://doi.org/10.1016/j.mib.2016.03.010. [PubMed: 27084981]

(9). Andersson DI Persistence of Antibiotic Resistant Bacteria. Curr. Opin. Microbiol 2003, 6 (5), 452–456. https://doi.org/10.1016/j.mib.2003.09.001. [PubMed: 14572536]

(10). Dzianach PA; Dykes GA; Strachan NJCC; Forbes KJ; Perez-Reche FJ; Pérez-Reche FJ Challenges of Biofilm Control and Utilization: Lessons from Mathematical Modelling. J. R. Soc. Interface 2019, 16 (155), 20190042. 10.1098/rsif.2019.0042.

(11). Esser DS; Leveau JHJ; Meyer KM Modeling Microbial Growth and Dynamics. Appl. Microbiol. Biotechnol 2015, 99 (21), 8831–8846. 10.1007/s00253-015-6877-6. [PubMed: 26298697]

(12). Oscar TP Development and Validation of Primary, Secondary, and Tertiary Models for Growth of Salmonella Typhimurium on Sterile Chicken. J. Food Prot 2005, 68 (12), 2606–2613. 10.4315/0362-028X-68.12.2606. [PubMed: 16355832]

(13). Du J; McGraw A; Hestekin JA Modeling of Clostridium Tyrobutyricum for Butyric Acid Selectivity in Continuous Fermentation. Energies 2014, 7 (4), 2421–2435. 10.3390/en7042421.

(14). Dillard LR; Payne DD; Papin JA Mechanistic Models of Microbial Community Metabolism. 2021. 10.1039/d0mo00154f.

(15). Bhowmik P; Rajagopal S; Hmar RV; Singh P; Saxena P; Amar P; Thomas T; Ravishankar R; Nagaraj S; Katagihallimath N; et al. Validated In Silico Model for Biofilm Formation in Escherichia Coli. ACS Synth. Biol 2022, 11 (2), 713–731. 10.1021/acssynbio.1c00445. [PubMed: 35025506]

(16). Chandrasekar A; Binder M; Liedl R; U Berendonk T Reactive-Transport Modelling of Enterococcus Faecalis JH2-2 Passage through Water Saturated Sediment Columns. J. Hazard. Mater 2021, 413, 125292. 10.1016/J.JHAZMAT.2021.125292.

(17). Sazal M; Mathee K; Ruiz-Perez D; Cickovski T; Narasimhan G Inferring Directional Relationships in Microbial Communities Using Signed Bayesian Networks. BMC Genomics 2020, 21 (6), 663. 10.1186/s12864-020-07065-0. [PubMed: 33349235]

(18). Li X; Rizik L; Kravchik V; Khoury M; Korin N; Daniel R Synthetic Neural-like Computing in Microbial Consortia for Pattern Recognition. Nat. Commun 2021, 12 (1), 3139. 10.1038/s41467-021-23336-0. [PubMed: 34035266]

(19). Picioreanu C; Van Loosdrecht MCM; Heijnen JJ Effect of Diffusive and Convective Substrate Transport on Biofilm Structure Formation: A Two-Dimensional Modeling Study. Biotechnol. Bioeng 2000, 69 (5), 504–515. 10.1002/1097-0290(20000905)69:5<504::AID-BIT5>3.0.CO;2-S. [PubMed: 10898860]

(20). Hellweger FL; Clegg RJ; Clark JR; Plugge CM; Kreft J-UU Advancing Microbial Sciences by Individual-Based Modelling. Nat. Rev. Microbiol 2016, 14 (7), 461–471. 10.1038/nrmicro.2016.62. [PubMed: 27265769]

(21). Ferrer J; Prats C; López D Individual-Based Modelling: An Essential Tool for Microbiology. J. Biol. Phys 2008, 34 (1–2 SPEC. ISS.), 19–37. 10.1007/s10867-008-9082-3. [PubMed: 19669490]

(22). Li B; Taniguchi D; Gedara JP; Gogulancea V; Gonzalez-Cabaleiro R; Chen J; McGough AS; Ofiteru ID; Curtis TP; Zuliani P NUFEB: A Massively Parallel Simulator for Individual-Based Modelling of Microbial Communities. PLOS Comput. Biol 2019, 15 (12), e1007125. 10.1371/journal.pcbi.1007125.

(23). Matyjaszkiewicz A; Fiore G; Annunziata F; Grierson CS; Savery NJ; Marucci L; di Bernardo M BSim 2.0: An Advanced Agent-Based Cell Simulator. ACS Synth. Biol 2017, 6 (10), 1969–1972. 10.1021/acssynbio.7b00121. [PubMed: 28585809]

(24). Lardon LA; Merkey BV; Martins S; Dötsch A; Picioreanu C; Kreft J; Smets BF IDynoMiCS : Next-Generation Individual-Based Modelling of Biofilms. Environ. Microbiol 2011, 13 (9), 2416–2434. 10.1111/j.1462-2920.2011.02414.x. [PubMed: 21410622]

(25). Stewart PS; Franklin MJ Physiological Heterogeneity in Biofilms. Nat. Rev. Microbiol 2008, 6 (3), 199–210. 10.1038/nrmicro1838. [PubMed: 18264116]

(26). Chen C; Liu S; Shi XQ; Chaté H; Wu Y Weak Synchronization and Large-Scale Collective Oscillation in Dense Bacterial Suspensions. Nature 2017, 542 (7640), 210–214. 10.1038/nature20817. [PubMed: 28114301]

(27). Colin R; Drescher K; Sourjik V Chemotactic Behaviour of Escherichia Coli at High Cell Density. Nat. Commun 2019, 10, 5329. 10.1038/s41467-019-13179-1. [PubMed: 31767843]

(28). Merkey BV; Lardon LA; Seoane JM; Kreft JU; Smets BF Growth Dependence of Conjugation Explains Limited Plasmid Invasion in Biofilms: An Individual-Based Modelling Study. Environ. Microbiol 2011, 13 (9), 2435–2452. 10.1111/j.1462-2920.2011.02535.x. [PubMed: 21906217]

(29). Kalinin YV; Jiang L; Tu Y; Wu M Logarithmic Sensing in Escherichia Coli Bacterial Chemotaxis. Biophys. J 2009, 96 (6), 2439–2448. 10.1016/j.bpj.2008.10.027. [PubMed: 19289068]

(30). Jiang L; Ouyang Q; Tu Y Quantitative Modeling of Escherichia Coli Chemotactic Motion in Environments Varying in Space and Time. PLoS Comput. Biol 2010, 6 (4), e1000735. 10.1371/journal.pcbi.1000735.

(31). Djezzar N; Inaki FP; Djedi N; Duthen Y A Computational Multiagent Model of Bioluminescent Bacteria for the Emergence of Self- Sustainable and Self-Maintaining Artificial Wireless Networks. Informatica 2019, 43 (3), 395–408.

(32). Yu JS; Bagheri N Agent-Based Models Predict Emergent Behavior of Heterogeneous Cell Populations in Dynamic Microenvironments. Front. Bioeng. Biotechnol 2020, 8, 249. 10.3389/fbioe.2020.00249. [PubMed: 32596213]

(33). Zhao J; Cao Y; DiPietro LA; Liang J Dynamic Cellular Finite-Element Method for Modelling Large-Scale Cell Migration and Proliferation under the Control of Mechanical and Biochemical Cues: A Study of Re-Epithelialization. J. R. Soc. Interface 2017, 14 (129), 20160959. 10.1098/rsif.2016.0959.

(34). Wang M; Liu X; Nie Y; Wu XL Selfishness Driving Reductive Evolution Shapes Interdependent Patterns in Spatially Structured Microbial Communities. ISME J. 2021, 15 (5), 1387–1401. 10.1038/s41396-020-00858-x. [PubMed: 33343001]

(35). Doekes HM; de Boer RJ; Hermsen R Toxin Production Spontaneously Becomes Regulated by Local Cell Density in Evolving Bacterial Populations. 2019, 15 (8). 10.1371/journal.pcbi.1007333.

(36). Graf von der Schulenburg D; Pintelon TRR; Picioreanu C; van Loosdrecht MCM; Johns ML Three-Dimensional Simulations of Biofilm Growth in Porous Media. AIChE J. 2009, 55 (2), 494–504. 10.1002/aic.

(37). Blanchard AE; Lu T Bacterial Social Interactions Drive the Emergence of Differential Spatial Colony Structures. BMC Syst. Biol 2015, 9 (1), 59. 10.1186/s12918-015-0188-5. [PubMed: 26377684]

(38). Ghosh P; Mondal J; Ben-Jacob E; Levine H Mechanically-Driven Phase Separation in a Growing Bacterial Colony. Proc. Natl. Acad. Sci. U. S. A 2015, 112 (17), E2166–E2173. 10.1073/pnas.1504948112. [PubMed: 25870260]

(39). Charteris N; Khain E Modeling Chemotaxis of Adhesive Cells: Stochastic Lattice Approach and Continuum Description. New J. Phys 2014, 16 (2), 025002. 10.1088/1367-2630/16/2/025002.

(40). Micali G; Colin R; Sourjik V; Endres RG Drift and Behavior of E. Coli Cells. Biophys. J 2017, 113 (11), 2321–2325. 10.1016/j.bpj.2017.09.031. [PubMed: 29111155]

(41). Rudge TJ; Steiner PJ; Phillips A; Haseloff J Computational Modeling of Synthetic Microbial Biofilms. ACS Synth. Biol 2012, 1 (8), 345–352. 10.1021/sb30003. [PubMed: 23651288]

(42). Volfson D; Cookson S; Hasty J; Tsimring LS Biomechanical Ordering of Dense Cell Populations. Proc. Natl. Acad. Sci. U. S. A 2008, 105 (40), 15346–15351. 10.1073/pnas.0706805105. [PubMed: 18832176]

(43). Farrell FDC; Hallatschek O; Marenduzzo D; Waclaw B Mechanically Driven Growth of Quasi-Two-Dimensional Microbial Colonies. Phys. Rev. Lett 2013, 111 (16), 1–5. 10.1103/PhysRevLett.111.168101.

(44). Picioreanu C; Kreft J-U; van Loosdrecht MCM Particle-Based Multidimensional Multispecies Biofilm Model. Appl. Environ. Microbiol 2004, 70 (5), 3024–3040. 10.1128/AEM.70.5.3024-3040.2004. [PubMed: 15128564]

(45). Xavier JB; Picioreanu C; van Loosdrecht MCM A Framework for Multidimensional Modelling of Activity and Structure of Multispecies Biofilms. Environ. Microbiol 2005, 7 (8), 1085–1103. 10.1111/j.1462-2920.2005.00787.x. [PubMed: 16011747]

(46). Xavier JB; Picioreanu C; Abdul Rani S; van Loosdrecht MCM; Stewart PS Biofilm-Control Strategies Based on Enzymic Disruption of the Extracellular Polymeric Substance Matrix - A Modelling Study. Microbiology 2005, 151 (12), 3817–3832. 10.1099/mic.0.28165-0. [PubMed: 16339929]

(47). Flemming HC; Wingender J The Biofilm Matrix. Nat. Rev. Microbiol 2010, 8 (9), 623–633. 10.1038/nrmicro2415. [PubMed: 20676145]

(48). Blanchard AE; Celik V; Lu T Extinction, Coexistence, and Localized Patterns of a Bacterial Population with Contact-Dependent Inhibition. BMC Syst. Biol 2014, 8, 23. 10.1186/1752-0509-8-23. [PubMed: 24576330]

(49). Aoki SK; Pamma R; Hernday AD; Bickham JE; Braaten BA; Low DA Contact-Dependent Inhibition of Growth in Escherichia Coli. Science 2005, 309 (5738), 1245–1248. 10.1126/science.1115109. [PubMed: 16109881]

(50). Celik Ozgen V; Kong W; Blanchard AE; Liu F; Lu T Spatial Interference Scale as a Determinant of Microbial Range Expansion. Sci. Adv 2018, 4 (11), 1–10. 10.1126/sciadv.aau0695.

(51). Bassler BL; Losick R Bacterially Speaking. Cell 2006, 125 (2), 237–246. 10.1016/j.cell.2006.04.001. [PubMed: 16630813]

(52). Liu F; Mao J; Kong W; Hua Q; Feng Y; Bashir R; Lu T Interaction Variability Shapes Succession of Synthetic Microbial Ecosystems. Nat. Commun 2020, 11 (1), 1–13. 10.1038/s41467-019-13986-6. [PubMed: 31911652]

(53). Jayathilake PG; Jana S; Rushton S; Swailes D; Bridgens B; Curtis T; Chen J Extracellular Polymeric Substance Production and Aggregated Bacteria Colonization Influence the Competition of Microbes in Biofilms. Front. Microbiol 2017, 8 (SEP). 10.3389/fmicb.2017.01865.

(54). Jayathilake PG; Gupta P; Li B; Madsen C; Oyebamiji O; González-Cabaleiro R; Rushton S; Bridgens B; Swailes D; Allen B; et al. A Mechanistic Individual-Based Model of Microbial Communities. PLoS One 2017, 12 (8), 1–26. 10.1371/journal.pone.0181965.

(55). Alpkvist E; Picioreanu C; van Loosdrecht MCM; Heyden A Three-Dimensional Biofilm Model With Individual Cells and Continuum EPS Matrix. Biotechnol. Bioeng 2006, 94 (5), 961–979. [PubMed: 16615160]

(56). Alpkvist E; Klapper I Description of Mechanical Response Including Detachment Using a Novel Particle Model of Biofilm/Flow Interaction. Water Sci. Technol 2007, 55 (8–9), 265–273. 10.2166/wst.2007.267.

(57). Jin X; Marshall JS; Wargo MJ Hybrid Model of Bacterial Biofilm Growth; Springer US, 2020; Vol. 82. 10.1007/s11538-020-00701-6.

(58). Scott SR; Din MO; Bittihn P; Xiong L; Tsimring LS; Hasty J A Stabilized Microbial Ecosystem of Self-Limiting Bacteria Using Synthetic Quorum-Regulated Lysis. Nat. Microbiol 2017, 2 (June), 1–9. 10.1038/nmicrobiol.2017.83.

(59). Li C; Zhang Y; Yehuda C Individual Based Modeling of Pseudomonas Aeruginosa Biofilm with Three Detachment Mechanisms. RSC Adv. 2015, 5 (96), 79001–79010. 10.1039/c5ra11041f.

(60). Ben-Jacob E; Cohen I; Levine H Cooperative Self-Organization of Microorganisms. Adv. Phys 2000, 49 (4), 395–554. 10.1080/000187300405228.

(61). Cao Y; Neu J; Blanchard AE; Lu T; You L Repulsive Expansion Dynamics in Colony Growth and Gene Expression. PLoS Comput. Biol 2021, 17 (3), 1–12. 10.1371/JOURNAL.PCBI.1008168.

(62). Hallatschek O; Hersen P; Ramanathan S; Nelson DR Genetic Drift at Expanding Frontiers Promotes Gene Segregation. Proc. Natl. Acad. Sci. U. S. A 2007, 104 (50), 19926–19930. 10.1073/pnas.0710150104. [PubMed: 18056799]

(63). Rudge TJ; Federici F; Steiner PJ; Kan A; Haseloff J Cell Polarity-Driven Instability Generates Self-Organized, Fractal Patterning of Cell Layers. ACS Synth. Biol 2013, 2 (12), 705–714. 10.1021/sb400030p. [PubMed: 23688051]

(64). Warren MR; Sun H; Yan Y; Cremer J; Li B; Hwa T Spatiotemporal Establishment of Dense Bacterial Colonies Growing on Hard Agar. Elife 2019, 8, 1–47. 10.7554/eLife.41093.

(65). Emonet T; Macal CM; North MJ; Wickersham CE; Cluzel P AgentCell: A Digital Single-Cell Assay for Bacterial Chemotaxis. Bioinformatics 2005, 21 (11), 2714–2721. 10.1093/bioinformatics/bti391. [PubMed: 15774553]

(66). Gorochowski TE; Matyjaszkiewicz A; Todd T; Oak N; Kowalska K; Reid S; Tsaneva-Atanasova KT; Savery NJ; Grierson CS; di Bernardo M BSim: An Agent-Based Tool for Modeling Bacterial Populations in Systems and Synthetic Biology. PLoS One 2012, 7 (8), e42790. 10.1371/journal.pone.0042790. [PubMed: 22936991]

(67). Wei G; Bogdan P; Marculescu R Efficient Modeling and Simulation of Bacteria-Based Nanonetworks with BNSim. IEEE J. Sel. Areas Commun 2013, 31 (12), 868–878. 10.1109/JSAC.2013.SUP2.12130019.

(68). Gutiérrez M; Gregorio-Godoy P; Pérez Del Pulgar G; Munoz LE; Sáez S; Rodríguez-Patón A A New Improved and Extended Version of the Multicell Bacterial Simulator Gro. ACS Synth. Biol 2017, 6 (8), 1496–1508. 10.1021/acssynbio.7b00003. [PubMed: 28438021]

(69). Faust K; Raes J Microbial Interactions: From Networks to Models. Nat. Rev. Microbiol 2012 108 2012, 10 (8), 538–550. 10.1038/nrmicro2832. [PubMed: 22796884]

(70). Ni C; Lu T Individual-Based Biophysical Modelling of Spatial Dynamics of Chemotactic Microbial Populations. ACS Synth. Biol 2022.

(71). Cremer J; Honda T; Tang Y; Wong-Ng J; Vergassola M; Hwa T Chemotaxis as a Navigation Strategy to Boost Range Expansion. Nature 2019, 575 (7784), 658–663. 10.1038/s41586-019-1733-y. [PubMed: 31695195]

(72). Smith WPJ; Davit Y; Osborne JM; Kim WD; Foster KR; Pitt-Francis JM Cell Morphology Drives Spatial Patterning in Microbial Communities. Proc. Natl. Acad. Sci. U. S. A 2017, 114 (3), E280–E286. 10.1073/pnas.1613007114. [PubMed: 28039436]

(73). Gogulancea V; González-Cabaleiro R; Li B; Taniguchi D; Jayathilake PG; Chen J; Wilkinson D; Swailes D; McGough AS; Zuliani P; et al. Individual Based Model Links Thermodynamics,

Chemical Speciation and Environmental Conditions to Microbial Growth. Front. Microbiol 2019, 10 (AUG), 1871. 10.3389/FMICB.2019.01871/BIBTEX. [PubMed: 31456784]

(74). Blanchard AE; Liao C; Lu T An Ecological Understanding of Quorum Sensing-Controlled Bacteriocin Synthesis. Cell. Mol. Bioeng 2016, 9 (3), 443–454. 10.1007/s12195-016-0447-6.

(75). Lu T; Shen T; Bennett MR; Wolynes PG; Hasty J Phenotypic Variability of Growing Cellular Populations. Proc. Natl. Acad. Sci 2007, 104 (48), 18982–18987. 10.1073/pnas.0706115104. [PubMed: 18025471]

(76). Gorochowski TE Agent-Based Modelling in Synthetic Biology. Essays Biochem. 2016, 60 (4), 325–336. 10.1042/EBC20160037. [PubMed: 27903820]

(77). Krishnamurthy EV Agent-Based Models in Synthetic Biology: Tools for Simulation and Prospects. Int. J. Intell. Syst. Appl 2012, 4 (2), 58–65. 10.5815/ijisa.2012.02.07.

(78). Sickle JJ; Ni C; Shen D; Wang Z; Jin M; Lu T Integrative Circuit-Host Modeling of a Genetic Switch in Varying Environments. Sci. Rep 2020, 10 (1), 8383. 10.1038/s41598-020-64921-5. [PubMed: 32433471]

(79). Leaman EJ; Geuther BQ; Behkam B Quantitative Investigation of the Role of Intra-/Intercellular Dynamics in Bacterial Quorum Sensing. ACS Synth. Biol 2018, 7 (4), 1030–1042. 10.1021/ACSSYNBIO.7B00406/ASSET/IMAGES/MEDIUM/SB-2017-00406G_M013.GIF. [PubMed: 29579377]

(80). Kreft J-U; Booth G; Wimpenny JWT BacSim, a Simulator for Individual-Based Modelling of Bacterial Colony Growth. Microbiology 1998, 144 (12), 3275–3287. 10.1099/00221287-144-12-3275. [PubMed: 9884219]

(81). Kreft J-U; Picioreanu C; van Loosdrecht MCM; Wimpenny JWT Individual-Based Modelling of Biofilms. Microbiology 2001, 147 (11), 2897–2912. 10.1099/00221287-147-11-2897. [PubMed: 11700341]

(82). Kreft JU; Wimpenny JWT Effect of EPS on Biofilm Structure and Function as Revealed by an Individual-Based Model of Biofilm Growth. Water Sci. Technol 2001, 43 (6), 135–141. 10.2166/wst.2001.0358.

(83). Plimpton S Fast Parallel Algorithms for Short-Range Molecular Dynamics. J Comp Phys 1995, 117, 1–19.

(84). Sun R; Xiao H SediFoam: A General-Purpose, Open-Source CFD–DEM Solver for Particle-Laden Flow with Emphasis on Sediment Transport. Comput. Geosci 2016, 89, 207–219. https://doi.org/10.1016/j.cageo.2016.01.011.

(85). Nogueira R; Elenter D; Brito A; Melo LF; Wagner M; Morgenroth E Evaluating Heterotrophic Growth in a Nitrifying Biofilm Reactor Using Fluorescence in Situ Hybridization and Mathematical Modeling. Water Sci. Technol 2005, 52 (7), 135–141. 10.2166/wst.2005.0192. [PubMed: 16248189]

(86). Oyebamiji OK; Wilkinson DJ; Jayathilake PG; Curtis TP; Rushton SP; Li B; Gupta P Gaussian Process Emulation of an Individual-Based Model Simulation of Microbial Communities. J. Comput. Sci 2017, 22, 69–84. 10.1016/J.JOCS.2017.08.006.

(87). Chen Y; Kim JK; Hirning AJ; Josić K; Bennett MR Emergent Genetic Oscillations in a Synthetic Microbial Consortium. Science 2015, 349 (6251), 986–989. 10.1126/science.aaa3794. [PubMed: 26315440]

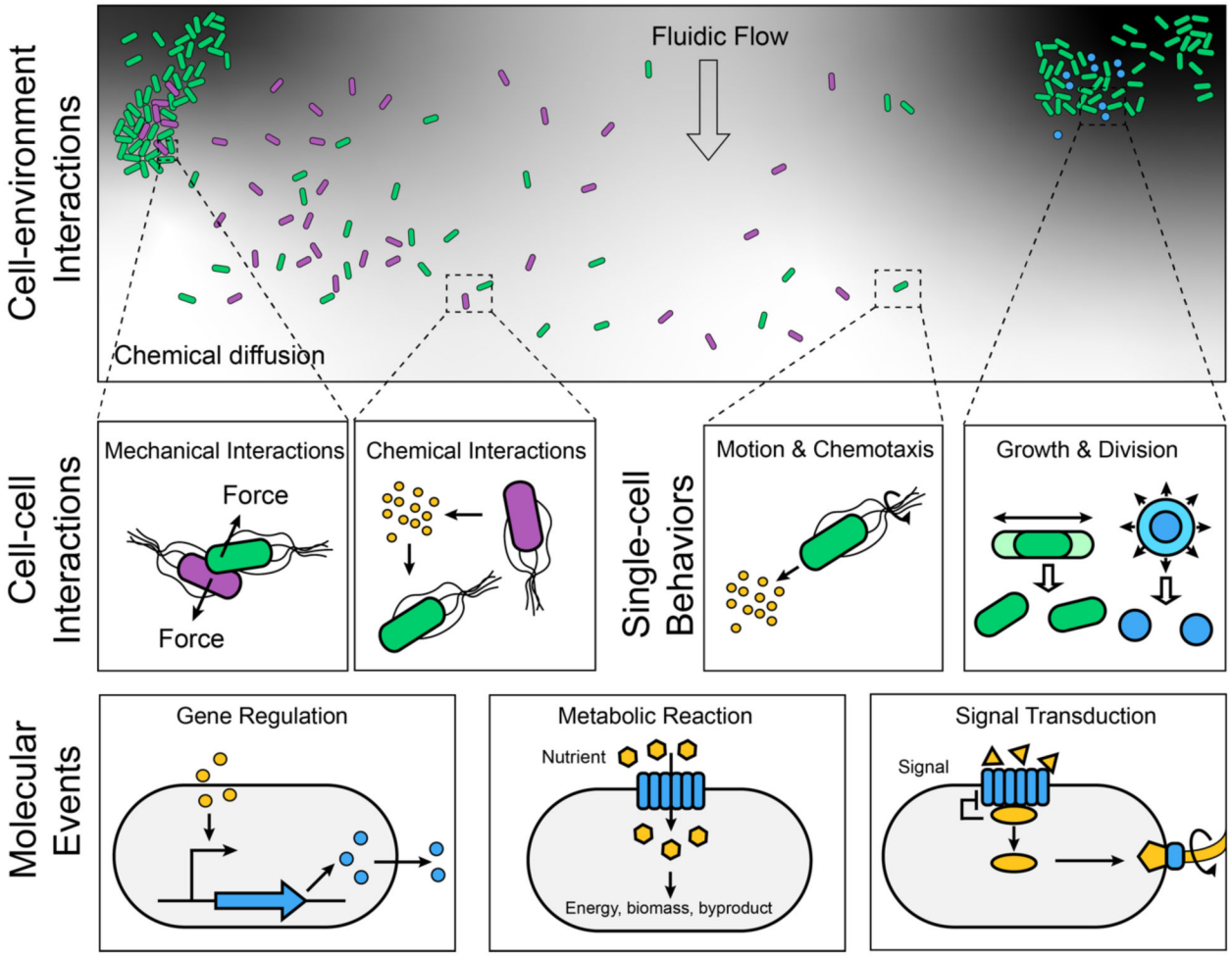

Figure 1: A schematic illustration of the multiscale processes involved in ABMs.

ABMs often contain molecular events, single-cell behaviors, cellular interactions and cell-environment coupling. Molecular events include gene regulation, metabolic reactions, and signal transduction. Single-cell processes include cellular growth, cellular division, and chemotactic migration in response to attractant gradients. Cell-cell interactions include mechanical interactions from mechanical forces exerted by neighboring cells or chemical interactions due to the secretion of toxins or public goods. Cell-environment coupling involve nutrient uptake from the surroundings, biomass dispersal due to fluid flow and others.

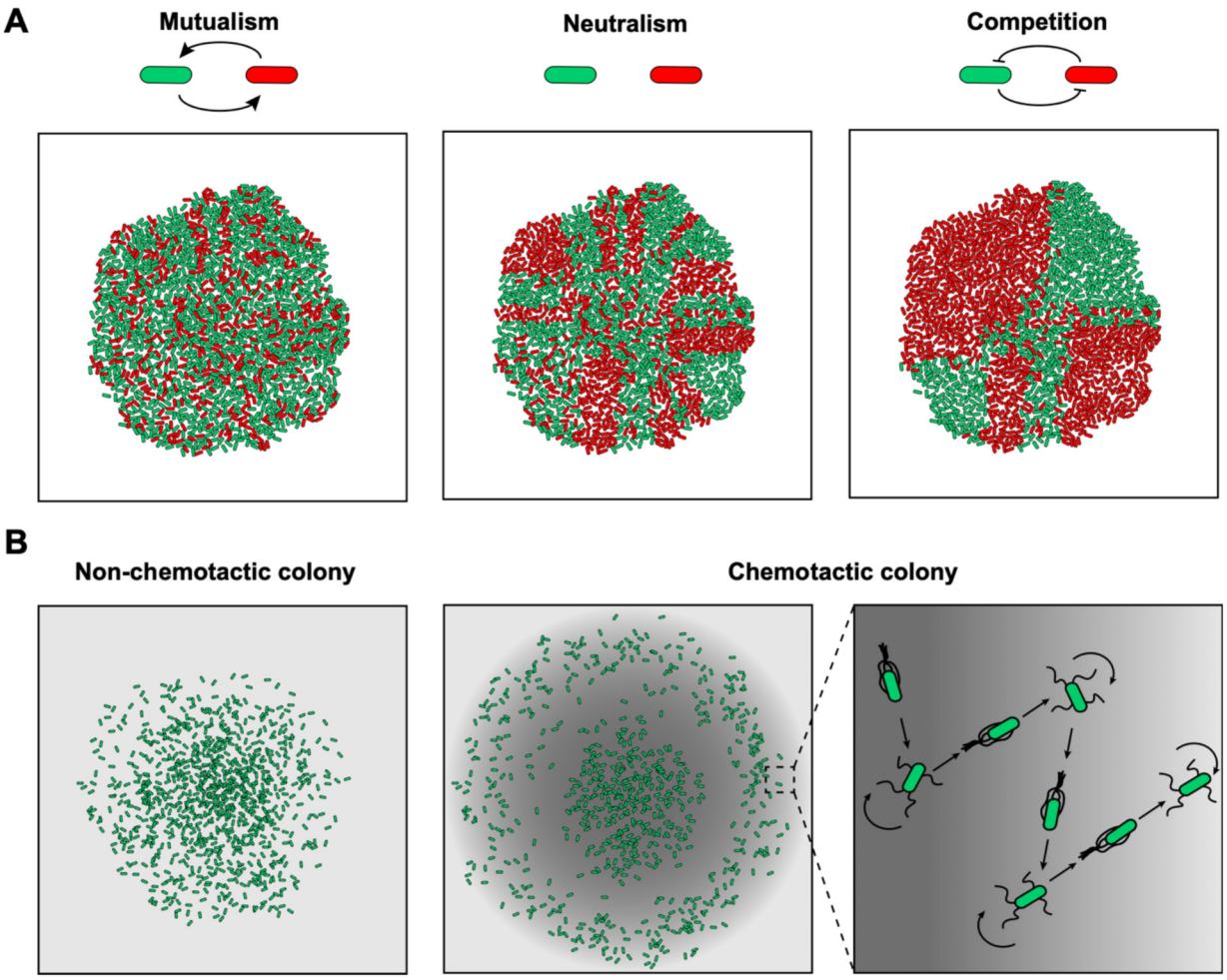

Figure 2: ABMs for studying microbial range expansion and spatial colony ecology.

(**A**) Using an ABM, the functional roles of social interactions in driving microbial range expansion were uncovered.[37] Notably, compared to neutralism, mutualism was shown to promote spatial mixing while competition led to spatial segregation. (**B**) With an ABM, the impacts of chemotaxis on spatial colony structures was illustrated.[70] Compared to the non-chemotactic case, chemotactic populations generated a nutrient gradient that facilitated cellular running, instead of tumbling, and hence promoted spatial expansion.

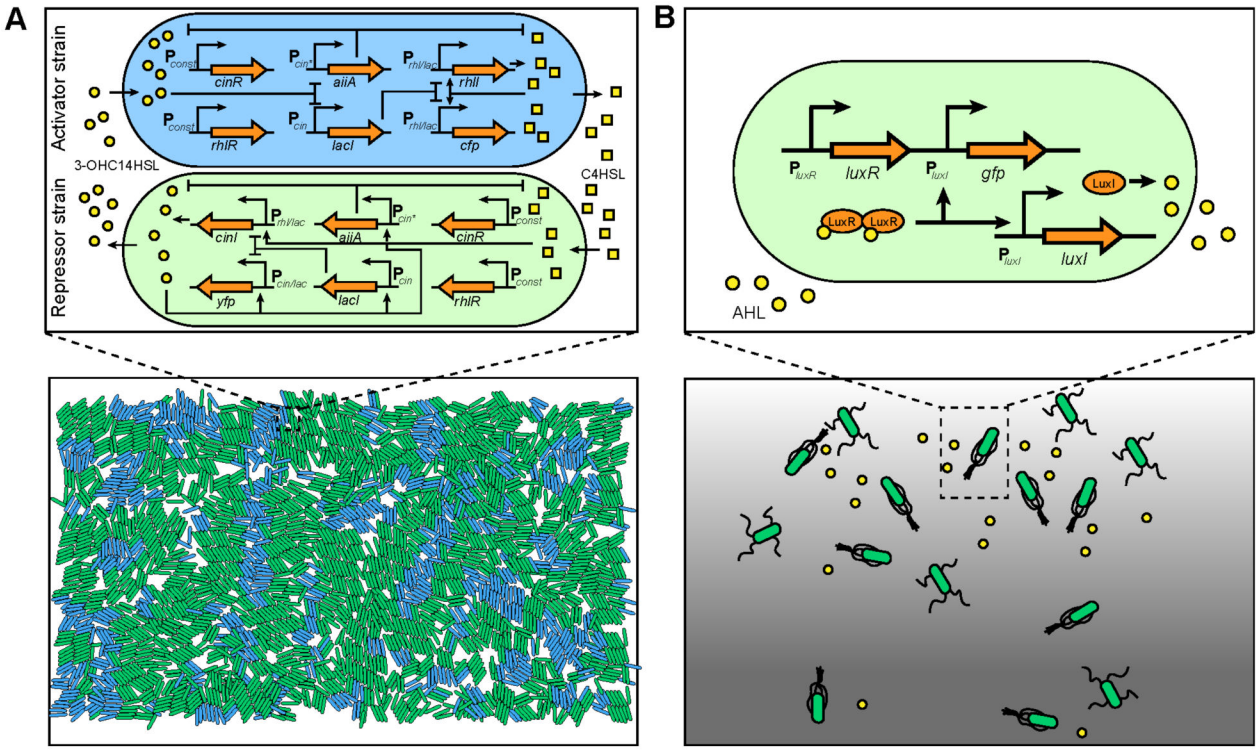

Figure 3: ABMs for capturing and predicting engineered microbial populations carrying synthetic gene circuits.

(**A**) ABM simulations of a synthetic two-species community that involves an activator and a repressor strain[87] (top panel) successfully reproduced an experimentally observed oscillatory population dynamics in a microfluidic device (bottom panel). (**B**) ABM simulations of an engineered bacterial population that contains quorum-sensing regulated gene expression[79] quantitatively captured the time and pattern of the gene activation and further guided the design of ribosome binding site for predictable behavioral modulation.

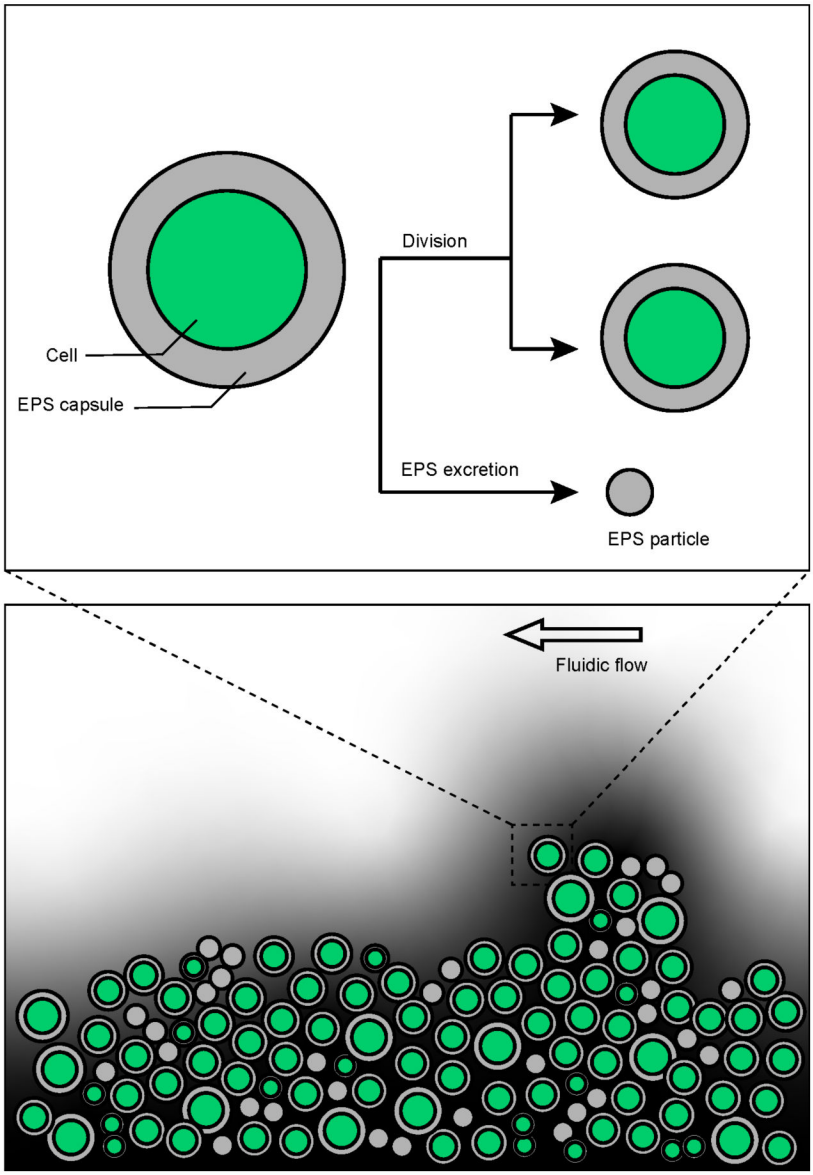

Figure 4: ABMs for biofilm formation and dispersal.

The top panel illustrates the layered structure of agents used in ABM-based biofilm models. Here, each agent (i.e., cell) excretes EPS, which results in the formation of the associated EPS shell as well as individual EPS agents. Each agent can also divide, representing cell division. The bottom panel indicates the effect of fluid flow in shaping the biofilm morphology.