Pattern formation by swimming microorganisms

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Abstract

In still water, the motion of many species of bacteria and algae is biased in a particular direction by the action of gravity or the presence of oxygen, light, or nutrients. A sufficiently dense population of such organisms alters the motion of the fluid in which the organisms are suspended which in turn alters their behavior, leading to a fluid instability known as bioconvection. In this paper, I discuss experimental observations of bioconvection and models that have been proposed to describe it. The emergence of the bioconvective instability from the equations of fluid mechanics and those describing organism behavior is demonstrated. Pattern formation and the existence of topological phase transitions are discussed.

1 Introduction

Microorganisms are the backbone of every ecosystem on Earth. Algae are responsible for a large portion the oxygen in the air that we breathe and are the major producers driving the food chain in many aquatic environments, while bacteria found in water and moist soil are necessary for recycling of nutrients from dead plants and animals. Hence, understanding the behavior of these creatures is essential for the sustainable development of humankind. Many microorganisms are motile: the alga *Chlamydomonas nivalis* swims with two flagella which propel it through water at a speed as much 10 body lengths per second, while the common soil bacterium *Bacillus subtilis* has a large number of flagella (Fig. 1) which allow it to move at similar speeds relative to its body length[1]. Evolution has developed a number of mechanisms that compel microorganisms to swim in certain directions depending on the ambient conditions. In some cases, the mechanisms by which one direction is favored over another is well-understood. Gyrotactic organisms, such a *C. nivalis*, on average swim upward in still water due to the fact that their center of mass is located behind their center of buoyancy. On the other hand, the mechanism that biases the motion of oxytactic organisms so that they swim towards favorable oxygen concentration is not known. Like gyrotactic organisms, oxytactic organisms also have a tendency to swim upward, in this case because oxygen diffuses into the water from the surface while they consume the oxygen at lower depths. Despite sharing a tendency swim upward, the details of the specific taxis lead to substantially different behavior as we will see later. A tendency to swim upward is also seen in phototactic organisms as they seek favorable light intensity. Most microorganisms respond to more than one taxis; for example, *C. nivalis*, being photosynthetic, is phototactic as well as gyrotactic.

While many microorganisms swim upward, they are also more dense than water. An instability therefore develops as the organisms collecting at the surface cause the density of the fluid to become larger there than below. This instability is referred to as bioconvection due to its similarity with thermal (Rayleigh-Bénard) convection. Bioconvection has been observed in nature[6] and has been studied in the laboratory for almost a century due to the richness of the phenomenon and its implications for ecology and society. First, the motion may be essential to the function of the organism's ecosystem; for example, the development of bioconvection may allow oxytactic organisms to increase the supply of oxygen in the water[2] , enhancing their ability

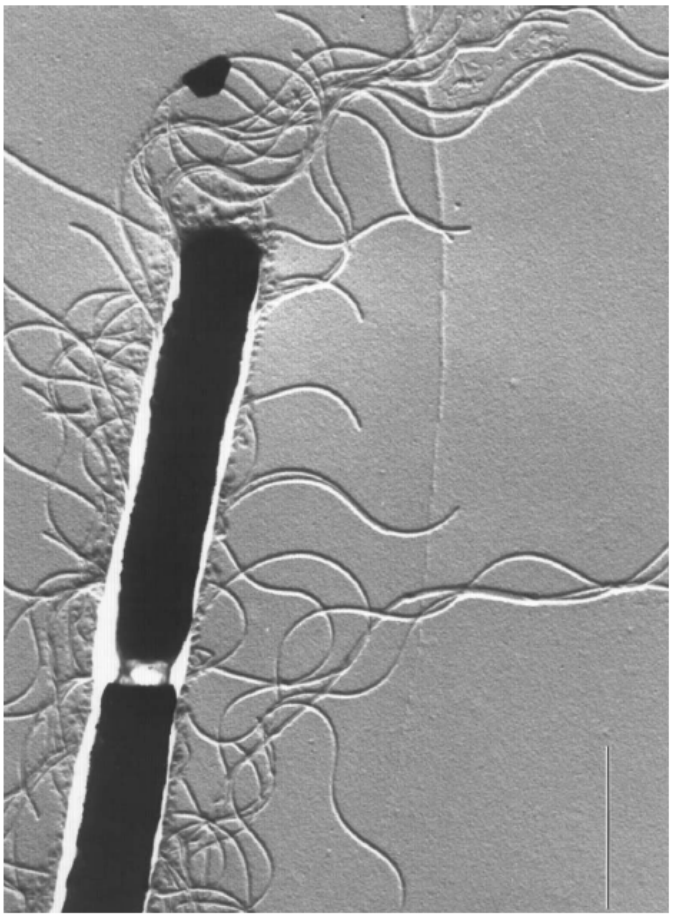

Figure 1: Electron micrograph of oxytactic bacterium *Bacillus subtilis*. Notice the many flagella. Figure from Ref. 1.

and that of other aerobic organisms to thrive in oxygen poor environments. Furthermore, bioconvection patterns concentrate the organisms into dense regions separated by regions with few organisms, facilitating mating or controlling predator-prey relationships[2]. Second, studying bioconvection can grant some insight on the mechanism underlying the particular taxis of some organism. Observations of bioconvection patterns imply that the motion of gyrotactic organisms does not depend on pressure, as was assumed for some such organisms[2]. Third, bioconvection could be exploited for technological purposes. Bioconvection has already been used as a simple method to separate organisms with different taxis or behaviors[2]. Finally, bioconvection has a number of aspects that make it distinct from thermal convection and theoretically interesting. Organisms are not identical particles; hence, any parameters that model their physical properties are strictly distributions. Furthermore, swimming organisms are self-propelled so that their correlated motion leads to unusual flow properties, such as non-Gaussian superdiffusion ($< r^2 > \sim t^\alpha$, $1.5 < \alpha < 2.0$)[3].

2 Experiment

That suspensions of microorganisms are subject spontaneous pattern formation was known as long ago as 1848 and the first quantitative study of the phenomena was published in 1911[2]. Most experiments begin with an initially uniform suspension of the microorganism of interest, which is obtained by stirring. The fluid, which subsequently becomes quiescent, spontaneously develops the bioconvective instability as the microorganisms swim guided by their various taxes. If one wishes to study the behavior due to a particular taxis, precautions to exclude other taxes. For example, in studies of gyrotaxis *C. nivalis* placed in the dark to avoid phototactic effects. In the earliest studies, the experiments focused on the determining the conditions under which the patterns became visible. The patterns are apparent due to the fact that the organism densities are many orders of magnitude larger in the down flowing regions than in the others. It was discovered that for a fixed initial concentration of *Tetrahymena pyriformis* there exists a value of the chamber depth below which no patterns form[2]. This critical chamber depth was also seen to increase with the initial concentration of the organism. Descriptions of the patterns themselves referred only approximate length scales of the patterns or were purely qualitative. However, with the

advent of digital image analysis it became possible to quantitatively extract the Fourier components of the patterns[4]. Images were taken of the patterns from above and two-dimensional discrete Fourier transforms were performed, allowing the prominent wavelengths to be determined and showing their dependence on time and the depth of the microbial layer[4]. Such analyses are necessary to explore the emergent properties of bioconvection, but do not uniquely determine the behavior of the microorganisms that give rise to it.

The motion microorganisms can studied indirectly by tracking the motion of passive particles, which are larger and more easily visible than the organisms themselves. Wu and Libchaber[7] used micrometer-sized polystryrene beads and tracked their motion with CCD camera. The statistics of the beads' motion have important implications for the behavior of the microorganisms. However, as with the work of Kessler et al.[11], there is some suggestion that the presence of the spheres altered the cells' orientation. Hence, results of the indirect studies can be difficult to interpret as the passive tracers may significantly alter the properties of the system to be measured. However, the possibility that the beads affect the organism's orientation could prove important in understanding their behavior and might be elucidated by combining the passive particle methods with direct methods discussed below.

Finally, a direct statistical analysis of microorganism trajectories became possible only relatively recently with the use of computer image-recognition software recorded the position of the cells from the output of a CCD camera in real time[1]. This pioneering study was able to validate some theoretical results; however, the small area in which the organisms were tracked did not allow trajectories longer than a few seconds to be recorded and the time resolution was low (about 13 Hz). Laser velocimetry allowed to Vladimirov[8] to gather much more complete data, tracking hundreds of organisms at once. Such direct methods offer an unambiguous way to understand the behavior the organisms. Perhaps by combining direct tracking with the passive particle methods described above it might be possible to connect the microscopic behavior of the system to macroscopic consequences more easily.

2.1 Theory

Bioconvective pattern formation has been studied through continuum models analogous to those of Rayleigh-Bénard convection. Following Hill and Pedley[1], we separate the system into volume elements large enough that they contain many organisms but much smaller than any length scales as-

sociated with the flow. Assuming a dilute suspension of organisms so that interactions between organisms can be ignored and volume that they occupy is negligible compared to the volume of fluid in a cell, the velocity of the flow must satisfy the continuity ($\nabla \cdot \mathbf{u} = 0$) and the Navier-Stokes equations. We make the approximation that the change in the fluid density due to the organisms is only significant in the buoyancy term to obtain

$$\rho \frac{\mathrm{D}\mathbf{u}}{\mathrm{D}t} = -\nabla P_e + n\mathbf{g}(\rho - \rho_{\mathrm{org}}) + \mu \nabla^2 \mathbf{u}.$$

Here $\mathrm{D}\mathbf{u}/\mathrm{D}t$ represents the material derivative $\partial_t \mathbf{u} + (\mathbf{u} \cdot \nabla)\mathbf{u}$, P_e is the excess pressure, $n(\mathbf{r}, t)$ is the number density of organisms, g is the acceleration of gravity, $\rho - \rho_{\mathrm{org}}$ is the difference in density between the water and the organism, and μ is the viscosity of the fluid. Because the time scales of the organisms' life cycles are much longer than the typical period of the bioconvective flows, we assume conservation of organisms. The organism flux is sum that due to random diffusion $-D \cdot \nabla n$, where D is the diffusivity tensor, and the bias due to the various taxes $n\mathbf{V}$, where $\mathbf{V}$, is the average velocity. The equation of organism conservation is thus

$$\frac{\mathrm{D}n}{\mathrm{D}t} = -\nabla \cdot (n\mathbf{V} - D \cdot \nabla n).$$

We then assume that the direction of swimming, $\mathbf{p}$ executes a random walk subject to deterministic restoring force, leading to a Fokker-Planck equation for the probability density function ($f(\mathbf{p})$) of the swimming direction:

$$\frac{\partial f}{\partial t} + \nabla_p \cdot (\dot{\mathbf{p}} f) = D_{\mathrm{rot}} \nabla_p^2 f.$$

We assume that the rotational diffusivity, D_{rot}, is isotropic. Although the simplest models assume a tendency toward upward swimming irrespective of the flow around the organism, the mechanism of gyrotaxis implies that the $\dot{\mathbf{p}}$ evolves due to gravitational and viscous torques:

$$\dot{\mathbf{p}} = \frac{\rho g a}{\mu \gamma} \mathbf{p}_\perp + \frac{1}{2} \omega \times \mathbf{p} + \epsilon \mathbf{p} \cdot E \cdot (I - \mathbf{pp})$$

assuming organisms with a spheroidal shape and where γ is a parameter related to the timescale over which the reorientation occurs, a is the distance between the center of mass and the center of buoyancy, $\mathbf{p}_\perp$ is the projection

of the orientation in the plane normal to gravity, $\omega(\mathbf{r}, t)$ is the vorticity, ϵ is the eccentricity of the spheroid, and E is the rate of strain tensor. On the other hand, for oxytactic organisms it has been assumed that average velocity is proportional to the gradient of the oxygen concentration, $V = \chi \nabla C$, with the oxygen concentraion too obeying a diffusion-advection equation:

$$\frac{\mathrm{D}C}{\mathrm{D}t} = D_{\mathrm{O2}} \nabla^2 C - Kn,$$

where K is the rate at which the organisms consume bacteria. However, Hill and Pedley[1] note no published work has included the action of viscous torques for oxytactic organisms as has been done for gyrotactic oxygen.

To perform linear stability analysis, we must calculate steady-state organism concentration $n_0(\mathbf{r})$ about which to perturb. The form of $n_0(\mathbf{r})$ depends on the boundary conditions; it is uniform for an infinitely deep chamber and exponential function of z for a shallow chamber. One can then develop the linearized differential equations for a small disturbance of this state and determine under what conditions linear instabilities occur. The use of linear stability analysis is accompanied by its usual caveats in that little information can be determined about the fate of an unstable system and stability cannot be determined if the critical wave number is zero. Bees and Hill[4] implemented the preceding procedure and, by appropriately adjusting the parameters γ and D, obtained good agreement for the most prominent wavelengths at the onset of bioconvection for *C. nivalis*. However, the values of γ and D need to be determined through direct organism tracking experiments to validate the results.

Linear stability analysis was also performed for the oxytactic system described above[10], revealing the existence of a bioconvective instability. Here the form of the steady state is more complicated: $n_0(z) \sim \sec^2(1/2A(1 - z))$[9]. To predict the forms of the patterns formed by such organisms, a weakly nonlinear theory has been developed[9], predicting stable hexagonal patterns for $D_{O2}/D < 3$ and a nonzero critical wavenumber, which appears to be the experimentally relevant regime. The model also predicts that the first bifurcation will be from the steady state to down hexagons in qualitative, but not quantitative agreement with experiments (Fig. 2) for estimated parameter values[9].

Noever et al. show the existence of a topological phase transition from initially randomly distributed localized regions of down flow into a polygonal net with down flow at the polygon edges in suspensions of the alga

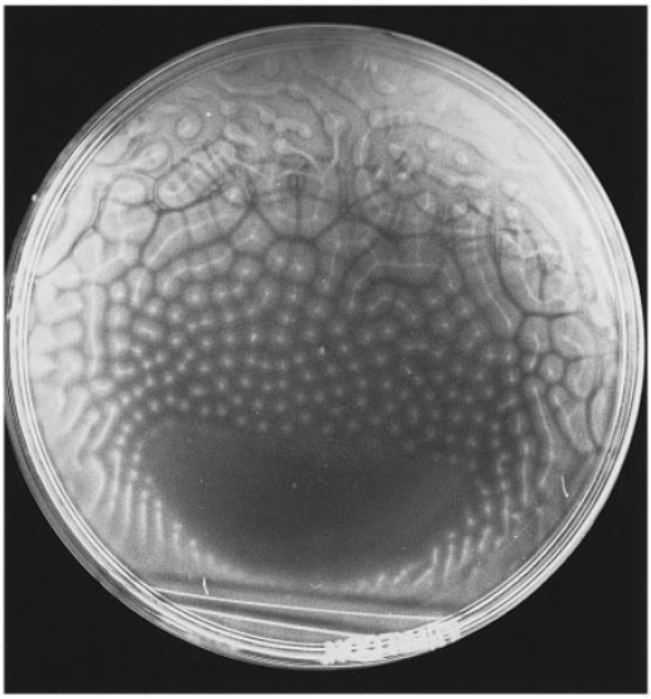

Figure 2: Tilted chamber showing the dependence of the bioconvection pattern of the oxytactic bacterium *Bacillus subtilis*. Figure from Ref. 9.

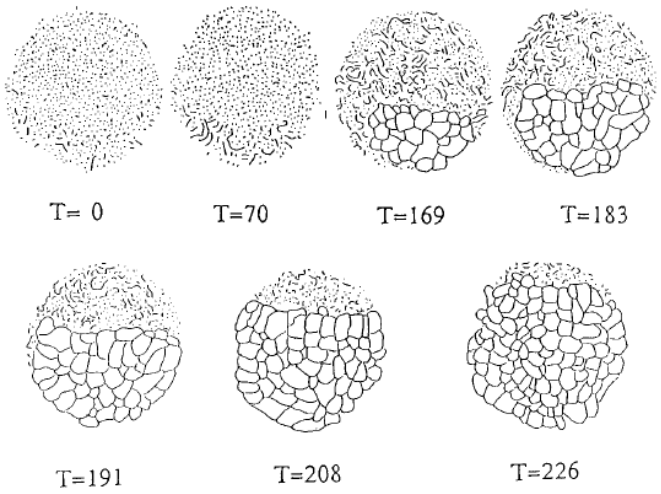

Figure 3: Digitally processed photographs of bioconvection patterns. Dense downward flowing regions are dark, rarefied upward flowing regions are white. A transition from isolated regions of downward flow to a connected polygonal net is seen to occur spontaneously with time. Figure from Ref. 5.

Polytomella parva[5]. Digitally processed photographs of the phenomena are shown in Fig. 3. The bioconvection cluster size is also shown to follow a power-law distribution (Fig. 4, implying that the polygonal is formed by a self-organized mechanism[5].

Surprisingly few, numerical studies of bioconvection have been performed[1], though rich behavior has been seen. Simulations of the continuum equations in 2D suggest that the plumes observed in bioconvection are always transient, providing a reason why attempts at analytic steady-state solutions have failed[1]. It does not appear that any such simulations have been attempted in 3D. Recent numerical schemes in which the positions of individual organisms are explicitly evolved[12] hold much promise in breaching the gap between the microscopic and macroscopic descriptions of bioconvection.

3 Conclusion

Models of bioconvection have been successful in describing many aspects of the phenomenon. However, all of the theoretical models in the literature suffer from one important deficiency: they are valid only for dilute suspensions

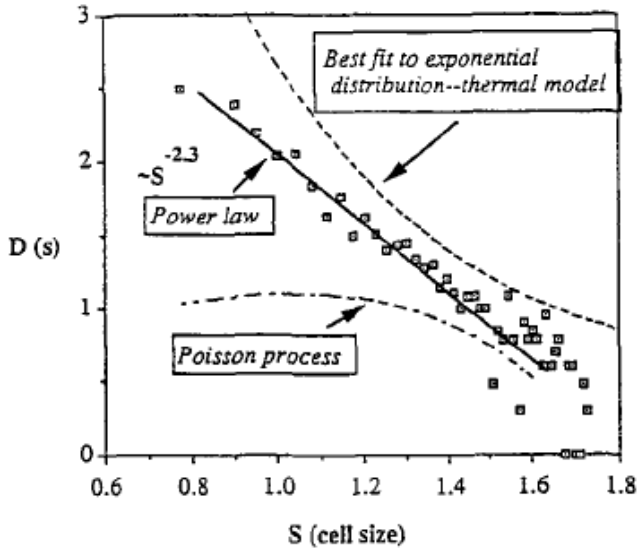

Figure 4: Power-law scaling of bioconvection pattern size. Figure from Ref. 5.

of microorganisms[1]. However, bioconvection patterns by their nature have a large deviations in the density and the theories are likely to fail in the dense down flowing regions of the patterns. Future work should seek to describe bioconvection at organism densities that cannot be considered dilute. Furthermore, the effect of shear flow on oxytactic organisms likely needs to be addressed, as it plays an important role in the onset of bioconvection and the patterns that subsequently form. I would also like to see larger amount of supercomputer time be applied to this problem. It seems that numerical simulation reveal much interesting phenomenon in the regimes where the analytic approximations are no longer valid.

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