CHEMOBIOCONVECTION IN A SUSPENSION OF LUMINESCENT BACTERIA

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Summary We present observations of the formation of plumes in a suspension of *Photobacterium phosphoreum*. This bacterium metabolizes oxygen to produce light, which allows collective motions to be observed. The instability leading to plume formation was at first thought to be attributed to the well-understood phenomenon of chemotactical bioconvection; however under our experimental conditions this bacterium is not motile. We propose that the chemical reaction by which light is produced changes the composition of the fluid in the same way that has been observed in chemoconvection. We seek to explain this phenomenon by performing a variety of experiments together with a theoretical approach, complemented by numerical simulations.

INTRODUCTION

The phenomenon of *bioluminescence* corresponds to the emission of light from living organisms due to chemical reactions. For example, it is well known that species living in dark conditions such as fish and squid can emit light via a symbiotic relationship with the bacterium *Photobacterium phosphoreum* living in their organs. In these bacteria, the production of light is controlled by *quorum sensing*, so that light production only occurs at sufficiently high bacterial concentrations [1].

In *P. phosphoreum*, the reaction by which light is produced is catalysed by the bacterial enzyme *luciferase*, so that reduced flavin mononucleotide ($FMNH_2$) and a long-chain aldehyde (RCHO) are oxidized by molecular oxygen [1]. We have recently observed a novel phenomenon of plume formation in the bioluminescence when a suspension of this bacterium is allowed to uptake oxygen. These plumes are characterised by regions of high bacterial density that appear to drive convective flows.

Bioconvection is the name given to collective motion of microorganism suspensions driven by nonuniformities in the concentration of cells, which are normally denser than the suspension fluid. Bioconvection was first identified in a suspension of upswimming algae, which accumulated near the surface of the chamber, leading to an unstable density stratification [2]. The upswimming can be in response to chemical gradients, light or gravity. It was thought that the plume formation in *P. phosphoreum* might be another case of bioconvection, but this bacterium is non-motile under our experimental conditions.

On the other hand, buoyancy-driven patterns have been observed in abiotic systems when chemical reactions modify the fluid composition. A well studied example is the methylene-blue-glucose system, where oxygen diffuses into the fluid turning a clear component blue, and this interacts with glucose producing a denser component. The resulting pattern has been called *chemoconvection* [3].

Our hypothesis is that the plume formation in *P. phosphoreum* is due to the chemical reaction with oxygen. This is not the only example of *chemobioconvection*: in 2008 Benoit *et al.* reported plume formation in a suspension of non-motile *E. coli* [4]. The aim of our project is to understand how the oxygen consumption is related to the plume formation, designing for that different experiments, a theoretical framework and numerical simulations.

EXPERIMENTAL RESULTS

The experimental setup consisted of a vertical chamber built with glass coverslips. The separation between the two parallel faces is 0.6 cm and is obtained by using two glass spacer tubes. The lateral separation between the two tubes defines the width of the cell, which can be varied in order to observe the dependence of the patterns on the geometry of the chamber, as is known to occur with Rayleigh-Bénard convection. Bacteria are grown in broth to mid-exponential phase and then introduced into the chamber, and observed in total darkness using a Nikon D300s digital camera equipped with a 60 mm f/2.8 macro lens.

Figure 1 shows the temporal evolution of patterns in a chamber 6 cm wide and 3 cm high. Images were taken every 15 seconds with a 10 second exposure time. The system is initially homogeneous since the oxygen is uniformly distributed in the chamber, and thus the entire fluid appears bioluminescent. After a few seconds, dark regions can be observed as a result of the lack of oxygen, while the upper surface is always bright. Surprisingly, bright well-defined plumes can be seen to descend from the top, entraining surrounding fluid into large-scale vortices, and remaining stable for the complete duration of the experiment (hours). Systematic study with open- and closed-top containers strongly indicates that this plume formation is not driven by evaporation or Marangoni stresses.

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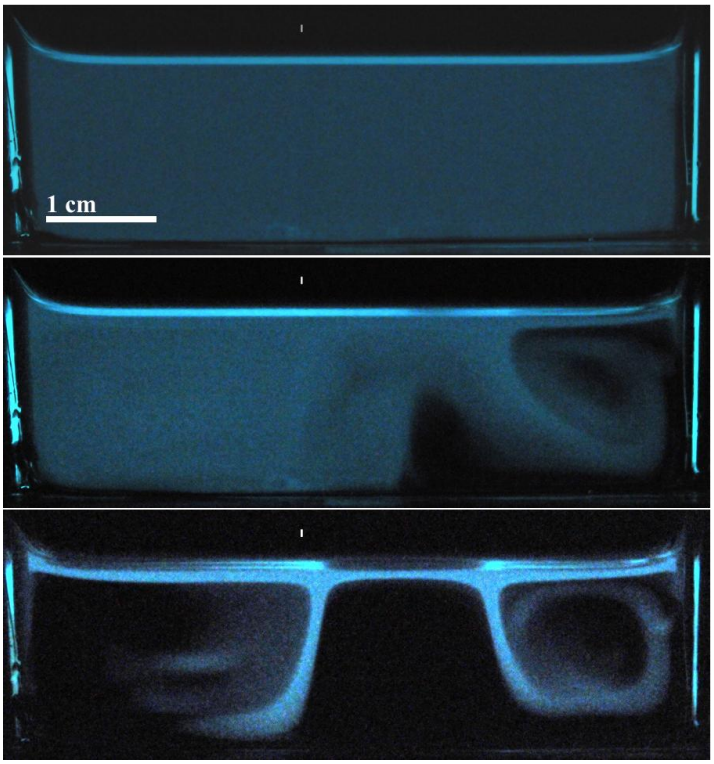

Figure 1. Time sequence of plume formation in a vertical chamber containing *Photobacterium phosphoreum*. The images were taken every 15 sec with an exposure time of 10 sec.

MATHEMATICAL MODEL

The principal feature of the model is to consider the reaction by which the light is produced as the source of buoyancy in the fluid. We hypothesise that, as a product of the reaction with oxygen, a component lighter than the fluid is produced, which acts as a driving force in the Navier-Stokes equation. On the other hand, the oxygen, bacteria and products of the reaction are subjected to diffusion and convection within the fluid. Therefore the equation of motion of the fluid must be coupled with the convection-diffusion equation associated with the chemical species and bacteria.

An important difference between this model and the one proposed by Benoit *et al.* [4] is that in our case the reactions will not be necessarily occurring in the surface. In fact, we expect that the experiments will provide information such as the relation between the amount of oxygen that it is being incorporated in the fluid and the timescale of the emission of light by the bacteria. In addition, we plan to use an oxygen-sensitive dye to clarify this question as well as *in situ* measurements of the consumption rate.

In certain limiting situations analytical progress can be made with the model. More generally the coupled system of equations is studied using a commerical finite-element code (Comsol Multiphysics®), and the results compared with the observations.

References

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