

GYROTACTIC BUOYANT CONVECTION AND SPONTANEOUS PATTERN

FORMATION IN ALGAL CELL CULTURES

John O. Kessler

Department of Physics
University of Arizona
Tucson, Arizona 85721
U.S.A. *

Abstract

Regular convection patters may form spontaneously in isothermal liquids which contain swimming microorganisms. The energy for this dissipative process is supplied by the swimmers. Individual cell trajectories are guided by gravity and vorticity so that cells accumulate toward regions of the liquid where the downstreaming velocity is a maximum. This concentrative mechanism, named "gyrotaxis", has been proven by the demonstration that swimming cells focus at the axis of a downward cylindrical Poiseuille flow of the cell culture. Since the density of the cells exceeds that of the liquid in which they swim, gyrotaxis reinforces vorticity. This convection pattern producing system has been named "Gyrotactic Buoyant Convection (GBC)". At sufficient average cell concentration, GBC can cause localised intermittent concentration pulses.

INTRODUCTION

The direction of locomotion of single algal cells is affected by gravity and the velocity field of the aqueous growth medium in which they swim. This modification of the orientation of individual cell trajectories can result in cell concentrative effects and

* Visiting at the Department of Applied Mathematics and Theoretical Physics, Cambridge University, Silver Street, Cambridge CB3 9EW, England until June 1984.

cooperative interaction which generates fluid convection patterns.

Cultures of *Dunaliella*, a single cell green alga, were generally used in the experiments reported. The cells are approximately ellipsoidal in shape. They contain a relatively dense posterior chloroplast and two thin anterior flagella which are used for forward locomotion. The cells usually vary in diameter from 5 to 15 μm and the flagella are of similar lengths. Swimming speeds as high as 100 $\mu\text{m s}^{-1}$ are observed. The average is somewhat less, perhaps 50 $\mu\text{m s}^{-1}$. Because of uneven flagellar motion, which may be purposeful or accidental, swimming trajectories may be intermittent, curved or irregular, and many cells rotate axially in the course of their forward progress. The culture medium used in experiments quoted here approximates seawater (1.03 gm cm^{-3}), and the average density of the cells is about 10% greater than that of the medium, as determined by observation of the sedimentation of killed cells.

It is observed that *Dunaliella* populations tend to swim upward. This behaviour is generally called "negative geotaxis". For concreteness, one may imagine the cells as rotationally symmetric around the swimming axis, but with a centre of mass located a distance L behind their geometric centre [1]. The cells then have a mass moment $\underline{M} = m\underline{L}$, where \underline{M} points to the rear of the cell, and m is the cell mass. The gravitational torque $\underline{M} \times \underline{g}$ tends to orient the cells toward an upright position. If the cells swim forward with velocity \underline{V}_C , one may write $\underline{M} = -\gamma \underline{V}_C$. Generally γ will be a tensor which depends on various other externally supplied stimuli, as well as the fluctuating internal condition of the cell. Here, for simplicity, γ will be considered a time independent scalar. That assumption implies that the cells will swim strictly upward in a still fluid environment, which is, in fact, the average behaviour under uniform illumination.

GYROTAXIS AND FOCUSING

An object, such as an algal cell, which is placed in a fluid with vorticity will experience a torque $\beta \nabla \times \underline{v}$, where \underline{v} is the fluid velocity. It will be assumed for simplicity that β is a scalar proportional to viscosity μ and the third power of an effective cell dimension which includes the effect of the ellipsoidal or irregular shape and the moving flagella. Combining the gravitational and fluid dynamic torques one obtains the total torque [2]

$$\underline{T} = \beta \nabla \times \underline{v} - \gamma \underline{V}_C \times \underline{g} . \quad (1)$$

Under steady state conditions \underline{T} vanishes, except for cell swimming fluctuation effects. The resultant trajectories produce a cell concentrative phenomenon that will be called "gyrotaxis", because of the crucial role of vorticity [3]. The average velocity of

cells when $T=0$ will be denoted by \underline{v} , without subscript c.

One may consider the simple special case of a cell culture slowly flowing through a cylindrical tube. In conventional coordinates (r, ϕ, z) ,

$$\underline{v} = -v_o (1 - \frac{r^2}{R^2}) \hat{z}, \quad (2)$$

where v_o is the maximum velocity in this downward Poiseuille flow, R is the tube diameter, r the local radius, and \hat{z} the upward unit vector. Since $\underline{g} = -g\hat{z}$ and $\nabla \times \underline{v} = -(2rv_o/R^2)\hat{\phi}$, Eq. 1 with $T=0$ specifies the radial component of \underline{v} , $v_r = -(2rv_o\beta)/(\gamma g R^2)$. The orientation angle θ of the cell trajectory, relative to \hat{z} , is given by $\sin\theta = (2rv_o\beta)/(mgLR^2)$, using $\gamma = mL/V$. It can be shown that the azimuthal component of \underline{v} vanishes.

This derivation shows that a cell culture in a downward cylindrical Poiseuille flow will focus at the centre of the tube. The only assumptions needed are the simple fluid dynamical and gravitational torques and an absence of strong free will on the cells' part. The effect has been observed, Fig. 1. For the experimental conditions $v_o \approx 10^{-1}$ cm s⁻¹, $V \approx 10^{-2}$ cm s⁻¹, and travel distance 3 mm, a focus time of 100 s is predicted, and that is in the observed range. The parameter β was estimated by $3\pi\mu a^3/2$, where $\mu = 10^{-2}$ gm cm⁻¹ s⁻¹, $a = 5 \times 10^{-4}$ cm. The cell density is ≈ 1 gm cm⁻³ and L was estimated at 0.01a, as a result of observing the spontaneous rotation of killed cells.

It might be thought that the observed cell focusing phenomenon is due to some particle-flow-in-tube effect, i.e. the theory given is sufficient to yield focusing, but is it necessary? a simple vertical tall glass U-tube in which a quantity of algal culture is displaced to one leg and allowed to flow slowly into the other (by an air valve connected to the end of one leg of the apparatus) provides a simple proof of the necessity of the theory. Eq. (2) holds on the downflow side; on the upflow side the sign is changed, v_z is positive. Then the sign of v_r changes, which implies radially outward movement of the cells. This effect is indeed observed: initially there is visible focusing only on the downflow side. Eventually cells are seen to accumulate around the periphery of the ascending liquid column. The direction of \underline{g} relative to the flow direction is crucial, as is the active swimming of the cells. Dead cells do not focus.

CONVECTION PATTERN FORMATION

Regions of the fluid where cell concentration differs from the average sink or rise, since concentration is directly proportional

to density. This buoyant convection is reinforced by gyrotaxis: cells accumulate toward sinking fluid, thereby increasing vorticity. The process will be named Gyrotactic Buoyant Convection (GBC). The concentration variations which occur in GBC are reduced by random cell swimming and by cell-cell collisions. GBC generates convection/concentration patterns (Figs. 2 and 3) and streamers (Fig. 4) near the bottom of a cell culture. "Bioconvection" due to upswimming of a cell population, but neglecting gyrotaxis, has been described previously [4]; it cannot account for the type of streamers observed or for focusing.

To describe GBC from a continuum viewpoint, and in the Boussinesq approximation, the forcing term to be added to the Navier-Stokes equation is $gnU\Delta\rho$, where n is the cell concentration, U the cell volume, and $\Delta\rho$ the difference between cell and aqueous medium densities. The volume fraction nU ranged from 10^{-5} to 10^{-3} in experiments where pattern formation was observed.

The cell flux j includes the gyrotactic term $n\underline{v}$ and a diffusive term to account for random behaviour and collisions then

$$\frac{Dn}{Dt} = -\nabla \cdot j = -\nabla \cdot (n\underline{v} - D\nabla n) , \quad (3)$$

where \underline{v} is obtained from Eq. 1 with $T=0$. The Navier-Stokes equation is

$$\frac{D\underline{v}}{Dt} = -\nabla p + g(\rho_o + nU\Delta\rho) , \quad (4)$$

where ρ_o is the growth medium density and p is pressure. An approximate solution can be obtained for steady state in a slot, at a horizontal plane of symmetry, with the assumptions $v_z = v_z(x)$, $n = n(x)$ and $j_x = 0$. Then

$$n = n_o \exp \left(-\frac{\beta}{\gamma g D} v_z \right) . \quad (5)$$

Putting this result into Eq. (4), with $D/Dt = 0$, and using appropriate boundary and fluid conservation conditions, one obtains (numerically) a standing wave pattern with v_z nearly sinusoidal. The concentration n is therefore periodic and shows exponential maxima which account for the sharp appearance of the convection patterns (Fig. 2) which are visualized by the cell concentration. The length and velocity scales derived from these equations agree with experiment, for reasonable assumptions of the parameters. Further discussion is too lengthy for inclusion here.

COOPERATIVE EFFECTS IN THE TIME DOMAIN

If $|\underline{M} \times \underline{g}| > > \beta |\nabla \times \underline{v}|$, the cells simply swim upward. If the inequality is reversed, steady gyrotaxis ceases. The cells rotate with an unsteady angular velocity. Steady gyrotaxis requires $\sin\theta \leq 1$.

In the downward Poiseuille flow previously considered, if shear is increased focusing becomes ever less efficient as $\sin\theta \leq 1$ applies to fewer and fewer members of the cell population. For the case of efficient focusing, with $\sin\theta < 1$ initially, as cells stream to the tube axis, the cell concentration n , and hence the fluid mass density, increases there, while decreases occur toward the periphery. Thus the velocity profile changes from parabolic to centrally peaked. This effect increases $\nabla \times \underline{v}$ locally and can lead to $\sin\theta > 1$. The result is the appearance of cell concentration pulses, centred on the flow axis of symmetry, see Fig. 5. Pulse initiation is probably due to oscillations of cell concentration near the axis, as $\sin\theta$ oscillates around unity. A secondary effect arises from differences in sinking velocity of cell concentration pulses of different sizes. Large pulses catch up with smaller ones and swallow them. The cell pulses themselves have a vortex structure which appears similar to that of thermals. If conditions are well regulated and steady, cell concentration pulses occur at approximately regular intervals. Tall cell cultures containing spontaneously generated convection patterns include GBC streamers which contain cell concentration pulses very similar to those observed in the tubular flow.

OTHER ORGANISMS AND CONDITIONS

There are many macroscopic and microscopic fluid dynamical aspects of gyrotaxis and GBC which need extensive investigation. The types of patterns which can be obtained may be altered with weak directional illumination; they also vary from one organism to another. Eventually, it will be possible to infer microscopic information concerning swimming behaviour or cell geometry from observation of the macroscopic patterns. Experiments in the present series have also demonstrated that not only *Dunaliella* but *Euglena*, *Chlamydomonas*, and *Carteria* all exhibit GBC. This statement is made on the basis that cultures of each of these organisms exhibit spontaneous streamer generation near the bottom of the culture-containing vessel.

DISCUSSION

The spontaneous formation of geometric patterns by micro-organism containing fluids has features of both equilibrium and

nonequilibrium phase transitions. The phenomenon may certainly be called "synergetic" in the sense that millions of independent cells work together coherently [5] coupled through the properties of the fluid and gravity.

Superficially, GBC seems analogous to Rayleigh-Bénard convection. Actually there is a big difference. In GBC, nothing is transported through the fluid layer, and the energy supplied to operate the dissipative structure is stored and transduced within entities that take part in the internal dynamics. This situation is typical for biological systems. It can definitely not be claimed that dissipative structure is associated with improving any macroscopic throughput.

A somewhat closer physical correspondence is found in ferromagnetism. One might consider vorticity as analogous to a mean magnetic field which exerts a torque on subentities which, acting cooperatively, produce more vorticity. The gravity field, in this conception, merely provides a directional framework for the action, much like the crystal lattice does in ferromagnetism. Certainly, the ferromagnetism analogy is "good" in the sense that there is no throughput, that there is a spontaneous appearance of domains in the absence of an external field (\underline{H} or $\nabla \times \underline{v}$), and that an external field (e.g., a supplied Poiseuille flow-associated $\nabla \times \underline{v}$) can produce a single domain. The laser is another metaphor. The pumping is internal, \underline{g} corresponds to cavity quality and vorticity is analogous to the radiation field.

APPLICATIONS

The work with *Dunaliella* was begun because this organism has remarkable environmental adaptability and potential commercial utility [6,7,8]. GBC allows cells to traverse vertical distances more quickly than they can swim individually. This effect is one aspect of cell harvesting and may also be of importance in algal ecology. Gyrotaxis could account for some of the plankton-concentrative effects in wind-driven Langmuir convection and will prove useful in cell separations based on motility and dynamic properties.

ACKNOWLEDGEMENTS

I should like to acknowledge the support of the University of Arizona Physics Department, the Office of Arid Lands, and the University of Arizona Foundation. I have greatly appreciated the help and advice of S.H. Davis, R.W. Hoshaw, N.R. Mauney, J.W. O'Leary and especially of Dick Kassander, without whom the work would not have happened.

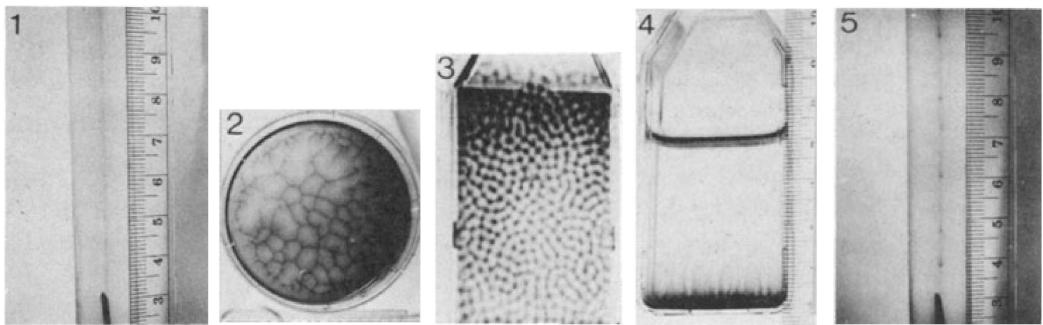


Figure 1. A culture of *Dunaliella tertiolecta* in artificial seawater is flowing through the vertical tube with an average flow rate of approximately 0.5 cm s^{-1} . The darker region on the axis is the column of focused cells.

Figure 2. Convection pattern in a 1 cm layer of a *Dunaliella* culture with cell concentration approximately $10^7 \text{ cells cm}^{-3}$. Cells accumulate in the dark regions which are downwelling. The cell concentration provides the flow visualization. Slowly varying pattern form.

Figure 3. As in Fig. 2, but near steady state (from a TV screen).

Figure 4. GBC streamers form near the bottom of the culture flask. *Carteria sp.*, approximately $10^6 \text{ cells cm}^{-3}$.

Figure 5. As in Fig. 1, at a flow rate of 0.1 cm s^{-1} . Concentration pulses which sink at approximately 0.3 cm s^{-1} .

REFERENCES

1. A.M. Roberts, Geotaxis in Motile Organisms, *J. Exp. Biol.* 53, 687 (1970). Also A.M. Roberts, Hydrodynamics of Protozoan Swimming, in Biochemistry and Physiology of Protozoa, Vol. 4, 2nd Ed., M. Levandowsky and S. Hutner, eds., Academic Press, New York, 1981, pp. 5-66.
2. A similar development is given in A.M. Roberts, the Biassed Random Walk and the Analysis of Microorganism Movement, in Swimming and Flying in Nature, Wu, Brokaw and Brennen, eds., Plenum, New York, 1975, pp. 377-393. A rudimentary version of the theory is given by Lord Rothschild, in Spermatozoan Motility, D.W. Bishop, ed., Am. Assoc. Adv. Sci., Washington, D.C., 1962, pp. 13-29.
3. "Rheotaxis", described by Roberts [3], is one component of gyrotaxis.
4. S. Childress, Mechanics of Swimming and Flying, Cambridge University Press, Cambridge, 1981, provides a list of references.
5. H. Haken, Introductory Remarks, in Evolution of Order and Chaos, H. Haken, ed., Springer Verlag, Berlin, 1982, pp. 1-4.
6. A. Ben-Amotz and M. Avron, Glycerol, β -Carotene and Dry Algal Meal Production by Commercial Cultivation of *Dunaliella*, in Algae Biomass, G. Shelef and C.J. Soeder, eds., Elsevier/North Holland, Amsterdam, 1980.
7. J.O. Kessler, M.D. Hurley, and B. Kingsolver, A Novel Harvest Technology for *Dunaliella* Phycoculture, in The Future of Small Energy Resources, R.F. Meyer and J.C. Olson, eds., UNITAR, McGraw Hill, New York, 1983, pp. 513-516.
8. J.O. Kessler, Algal Cell Harvesting, U.S. Patent 4,324,067, 1982.