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THE ROTATING SPECTROMETER: NEW BIOTECHNOLOGY FOR CELL SEPARATIONS

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16. Abstract

An instrument for biochemical studies, called the rotating spectrometer, separates previously inseparable cell cultures. The rotating spectrometer is intended for use in pharmacological studies which require fractional splitting of heterogeneous cell cultures based on cell morphology and swimming behavior. As a method to separate and concentrate cells in free solution, the rotating method requires active organism participation and can effectively split the large class of organisms known to form spontaneous patterns. Examples include the biochemical "star," an organism called *Tetrahymena pyriformis*. Following focusing in a rotated frame, the separation is accomplished using different radial dependencies of concentrated algal and protozoan species. The focusing itself appears as concentric rings and arises from the coupling between swimming direction and Coriolis forces. A dense cut is taken at varying radii and extraction is replenished at an inlet. Unlike standard separation and concentrating techniques such as filtration or centrifugation, the instrument is able to separate motile from immotile fractions. For a single pass, typical split efficiencies can reach 200-300% compared to the inlet concentration.

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I. INTRODUCTION

Most algal and protozoan concentration techniques treat the organisms passively, relying on their density for centrifugation or size for filtration. The recent advent of a gyrotactic spectrometer has introduced new ways to separate organisms based on more subtle specifications, such as swimming speed or morphology [1]. However, even this technique is known to focus gyrotactic organisms only--namely organisms with their bouyant center displaced from their mass center or equivalently, bottom heavy species. This new class of separation has not proven successful for separating many organisms of biochemical significance [2], most notably a "biochemical star" protozoa called *Tetrahymena*. This organism, in particular, has numerous applications in drug testing and vitamin assays [3]; its biochemistry corresponds remarkably to that found in humans and rats.

The rotating spectrometer described herein (Fig. 1) accomplishes separation/ concentration using radial dependencies of different microorganisms (including *Tetrahymena)*. This focusing into concentric rings (Fig. 2) arises from the coupling between swim directions and the Coriolis force in a rotating frame. A dense cut of a particular organism is taken at various radii and this extraction is replenished at an inlet. The apparatus has been demonstrated to concentrate the following test organisms: algae (*Gonium pectorale, Euglena gracilis, Euglena gracilis* var. *bacillarus, Polytomella parva*) and protozoans (*Tetrahymena pyriformis*). This separation is accomplished independent of whether the organisms are gyrotactic.

Unit operation requires filling of a rotating chamber (Fig. 1) with organisms and solution to be separated and concentrated (at >2 x 10^5 organisms/ml). This initial concentration can be accomplished by standard means of either filtration or centrifugation; both have been employed successfully as detailed in section III data collection. After the turntable begins rotation, dense organism rings form spontaneously in 5-30 seconds. A dense cut is taken using a point scoop at the desired radii. The apparatus can be stopped for refilling or more solution can be added continuously at an inlet. This process can be repeated in conjunction with other separation techniques.

Experiments described elsewhere [4] were the first to find this new rotating bioconvective structure, namely an ordered set of concentric rings. This memorandum describes the design and performance of a rotating spectrometer which uses this ring structure to split microorganism cultures. The spectrometer design incorporates the

following features: (1) it can be used with any class of organisms known to bioconvect (and not merely gyrotactic swimmers); (2) samples are easily removed and replenished owing to the device's top-loading option; (3) the selectivity of the dense cut or split can be tuned with respect to rotation speed and fluid height to take optimal test samples; (4) because all organisms are known not focus to a centerline (as in previous gyrotactic spectrometers), several cuts can be taken at different radii that show different organism properties; and (5) small (< 4 cm^3) and large (> 100 cm^3) samples can be separated or concentrated. Small sample requirements can prove advantageous, particularly if measurements incorporate several process steps which one wishes to monitor, but not subject the entire sample to an intrusive concentrative scheme. It is worth noting that in the limit of dead cells, cultures do not separate or concentrate in a rotating frame.

Tuning of a separation can be accomplished in many ways. When coupled to the rotating turntable, a voltage reducer varies angular speed continuously from rest to maximum (in the present case, 4.7 rad s^{-1}). Fluid depth can be controlled by either top-loading or inlet flow regulation. Finally, the time of sample taking can be varied, such that the concentrated stream is more or less diffuse.

II. SAMPLE PREPARATION AND SPECTROMETER CONSTRUCTION

A. Theory of Bioconvective Rings

Bioconvection is a fluid instability akin in some respects to thermal Benard cells in appearance, but driven by microorganisms' metabolic power, not heat. Bioconvective patterns appear in part from the density inversion (heavy over light fluids) which arises naturally from heavy organisms (density, ~1.05 gm cm^{-3}) which swim upward against gravity (negatively geotaxic). An additional feature of some algal patterns is a coupled locomotion called gyrotaxis, or the tendency of bottom heavy organisms to focus spontaneously into falling streams. This effect, although not universal to all bioconvecting organisms, is known to govern some algal patterns via gravity torques exercised in a velocity gradient. A feature not apparent from Fig. 2 is the dynamic nature of a bioconvective instability. Unlike other forms of biological aggregation, the characteristic shape of biconvective patterns--alternating high and low density waves--does not depend on organism cohesion; rather patterns evolve dynamically from competing gravity, diffusion, and upward swimming. Several publications [1-2] have addressed these mechanisms and only the unique features of rotating bioconvection will be dealt with here.

Jeffreys [5] first noted the stabilizing influence of rotation on thermal convection, a result explainable in subsequent theoretical work (see, e.g., Chandresakhar [6]) using the Proudman-Taylor theorem. For sufficiently fast rotation, this theorem constrains an

inviscid fluid to move primarily in the plane perpendicular to the rotation axis. In classical thermal convection, this constraining plane is horizontal (gravity directed normally to unstable fluid layers), such that rotation restricts vertical energy exchange. To counteract this limited energy exchange, however, narrow viscous channels evolve as exceptions to the Proudman-Taylor constraint against vertical transport. In unstable solutions of microorganisms, these viscous channels appear as the patterns shown in Fig. 2.

The experimental parameters of interest can be classified using a bioconvective Rayleigh number

$$Ra = g(\Delta\rho/\rho)h^2/ U\nu, \quad (1)$$

which is the dimensionless buoyancy of organisms heavier than their suspending solution, the Taylor number,

$$Ta = 4\Omega^2 h^4/\nu^2, \quad (2)$$

which is the dimensionless angular speed of rotation, where g is the gravitational acceleration, $(\Delta\rho/\rho)$ the surface density magnification (~0.10), h the fluid depth, U the swimming velocity (~0.1 cm s^{-1}), ν the kinematic viscosity (~0.01 cm^2 s^{-1}), and Ω the angular velocity of the rotating layer. One further scaling relation can be borrowed from work on thermal convection, namely the Ekman length,

$$\lambda = (\nu/\Omega)^{1/2}, \quad (3)$$

which gives a measure of the characteristic distance between rings.

B. Design Hardware

As illustrated in Fig. 1 schematic, the spectrometer consists of a low-aspect ratio cylindrical chamber or vessel with associated electronics and scoop for rotation and separation. The total weight of the prototype is under 10 kg with dimensions of 30 x 30 x 35 cm^3.

As suspended in their growth media, the concentrated organisms are poured into the mounted vessel. A rigid top for each vessel was removable to allow easy exchange of samples and depth variation. Excepting several qualitative observations, all data were collected using an air free-surface. For very shallow samples at high rotation, the free-surface slope was noticeable, although at these depths no bioconvective patterns formed and hence perturbations became of no consequence to actual observations. In all cases, the vessel bottom was flat and centered on a turntable using a high precision (within 5 x 10^{-4} cm) machinist's deflection instrument. The actual sample rested on a larger transparent platform or stage which allowed transmission of backlighting (from two 45^o angled collimated beams). Backlit samples were photographed against a black backdrop using two remotely cooled, incandescent beams. If observations of *Euglena, Gonium,* or other strongly photosensitive species were planned, samples were photographed

against a white background under normal room illumination (approx. 7 lux).

The turntable itself was belt-driven, the angular velocity of which could be varied continuously from 0.5 to 4.71 s^{-1} using a voltage reducer. Between these velocities, the Ekman length was always at least one order of magnitude smaller than the smallest vessel. The other parameter of interest, fluid depth, was varied between 0.6 and 1.9 cm for *Tetrahymena* and 0.5 and 0.8 cm for *Polytomella*. To some degree a limited height range reflects the narrowness in which strong patterns form actively, although the present work did not attempt to span entirely the critical parameters. Even so, for *Tetrahymena pyriformis* (*Polytomella parva*, respectively), experimental conditions yielded bioconvective Rayleigh numbers between Ra = 2.21 x 10^5 and 3.54 x 10^5 (Ra = 1.22 x 10^4 and 3.14 x 10^5) and Taylor numbers between Ta = 2.47 x 10^6 and 11.56 x 10^6 (Ta = 1 x 10^5 and 4 x 10^5).

Sample splits were taken using visual alignment of pre-evacuated glass pipettes at varying radii. When lowered along a bioconvective ring, the pipette pressure was released manually and a sample was taken. Sample volume could be varied between 0.1 ml to several ml in the smallest rotated chamber (4.8 cm diameter, 1 cm height). No changes in material or chamber dimension were required for separating different species.

The pre-loaded (and unconcentrated) microorganisms were cultured in their growth media [7] in a class 30K clean room held at a constant 20° C and exposed to (7 lux intensity) white fluorescents. A given harvest was filtered using a 0.22 micron mesh to between 1.5 x 10^5 and 2 x 10^6 cm^{-3}, depending on the species and growth time. Hence patterns in these chambers arose as the organized behavior of approximately 10^7 randomly swimming organisms!

III. PERFORMANCE

A. Separation Efficiency

The design of the rotating spectrometer proved capable of concentrating all bioconvecting species attempted, whether gyrotactic or not. Spin-up of the cultures was accomplished in a few seconds and within a minute, a two- to three-fold density magnification was obtained. The voltage reducer allowed optimization of angular speed, such that a given culture could be repeatedly and maximally split.

Spectrometer efficiency was evaluated by comparing motile organism concentrations between an adjacent dense and sparse cut. The concentrative efficiency could be measured using a hemacytometer after a given sample was fixed isotonically with a 5% glutaraldehyde-PBS solution and organisms were counted.

Concentrative factors are shown in Fig. 3 for the non-gyrotactic protozoa,

Tetrahymena pyriformis. Samples were taken in the smallest chamber (4.8 cm diameter) at 0.8 cm fluid depth. For comparison, Fig. 4 concentrative factors were obtained using an identical procedure, except at 0.6 cm fluid depth. The given cut was taken repeatedly from the the third concentric ring in each case at angular speeds varying between 3.5 and 4.7 rad s^{-1}.

Examination of Figs. 3 and 4 indicate that: (1) a maximum in separation efficiency occurs at an intermediate rotation; (2) rotation enhances separation potential compared to the stationary extrapolation to zero rotation; and (3) fluid depth and angular speed are primary design variables to optimize a split. The concentrative factor varies between a two to three times density split. Future publication will address the problem of morphology and swimming behavior within a given set of concentric rings.

The separation time of the spectrometer was also compared for different species [4]. In general, rings of the ciliated algae, *Polytomella parva,* formed more rapidly with decreasing height (to the -4.33 power) and decreasing rotation speed (to the -3.33 power). In general, *Tetrahymena* patterns formed more rapidly with decreasing height (to the -5/3 power) and decreasing rotation speed (to the -4/3 power). In the absence of a more extensive theoretical model, little else can be said about the origin of these statistically well-determined, inter-species relations except to note that faster rotation has less effects on the stability of bioconvective patterns in *Tetrahymena.* This may result from the ability of *Polytomella* to initiate viscous fingers throughout a culture's depth [6] and not just at the surface (as with *Tetrahymena),* such that horizontal rotation constrains their stronger vertical movement to a more limited energy exchange. It is worth noting that as is the case for thermal convection, rotation in general tends to stabilize bioconvection and hence delay the onset of pattern formation.

B. Design Variations

Several variations on the standard rotating spectrometer were examined. The vessel shape and material were varied between a square or rectangular polystyrene dish (3 x 3 cm^2 and 3 x 5 cm^2, respectively) and a glass or polystyrene circular dish with diameter between 3.5 and 8 cm. Non-circular vessels do not affect the separation, except that the maximum ring diameter no longer corresponds with the vessel diameter, but with the largest inscribed circle. No separation differences were noted between glass and polystyrene vessels.

In contrast to results for thermal convection, bioconvective rings patterns were induced even in closed containers without a free surface. Preliminary observations using the green swimming algal species, *Euglena gracilis* var. *bacillaris,* revealed ring patterns in sealed circular dishes (4.7 cm diameter). This allows sterile separations if required to isolate samples from ambient contamination.

IV. DISCUSSION

For all bioconvecting algal and protozoan species tested, rotating separations and density enhnacemnts have been demonstrated. Performance evaluation has centered on concentrating capacity as a function of rotational speed and fluid depth in one of the protozoan known not to focus gyrotactically, *Tetrahymena.* Similar programs are planned for testing different concentrations, mixtures of species, chamber dimensions, etc.

Improvements in hardware design can be realized by coupling the separation with optical density measurements to find local concentrations *in vivo.* This concept would combine automated density technologies, such as electronic Coulter cell counters, with the rotating spectrometer for optimal control.

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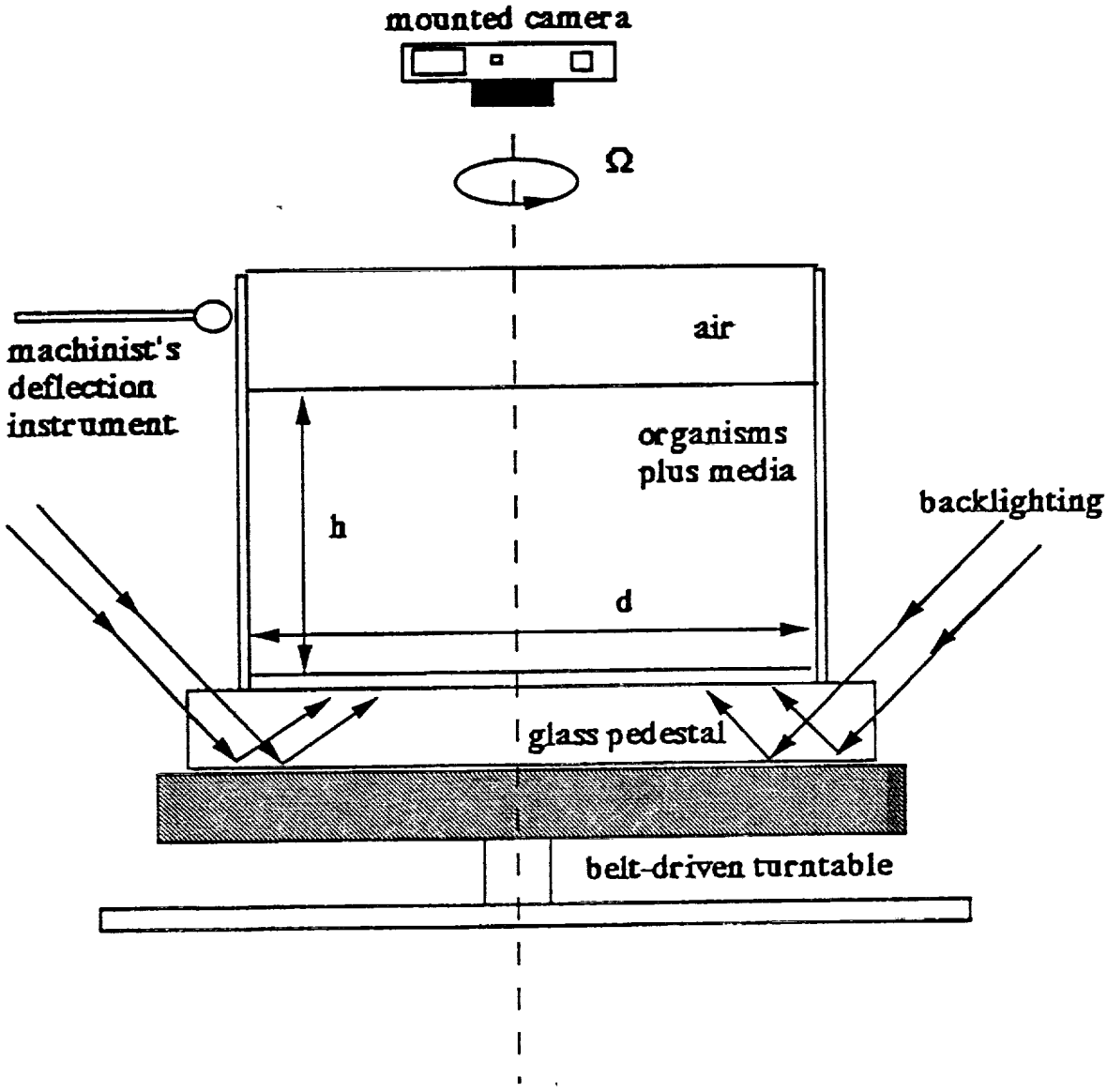

Figure 1. Top and side view of rotating spectrometer showing organism trajectories and turn-table assembly used for microorganism separations and concentrations. h is the fluid depth and d is the chamber diameter.

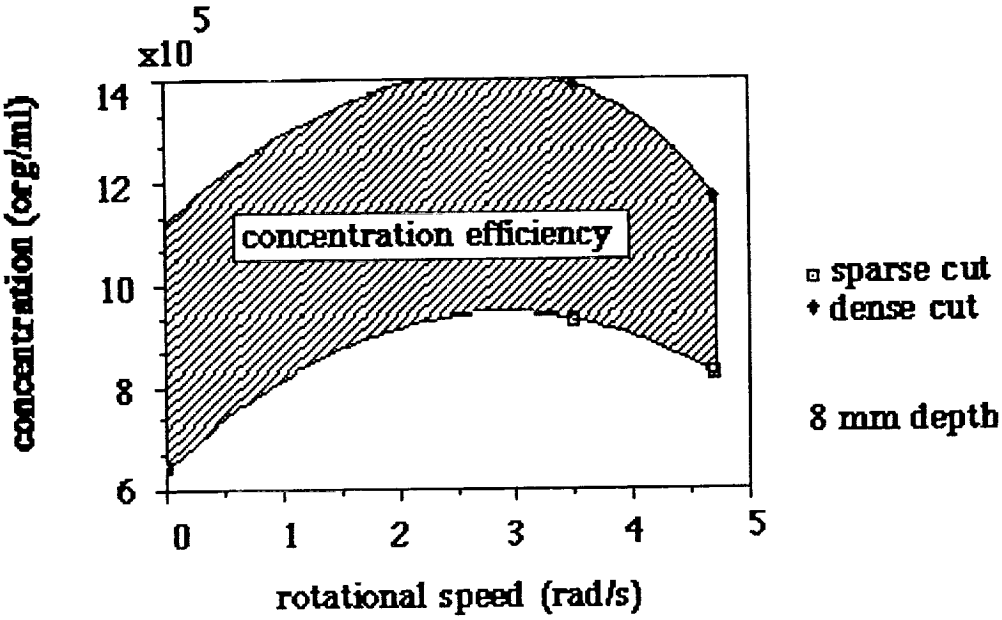

Figure 2. Concentration efficiency as a function of rotational speed at 0.8 cm fluid depth. The top line represents the dense organism cut; the bottom line shows the sparse organism cut. Concentrations are of the order of 1 million organisms per ml.

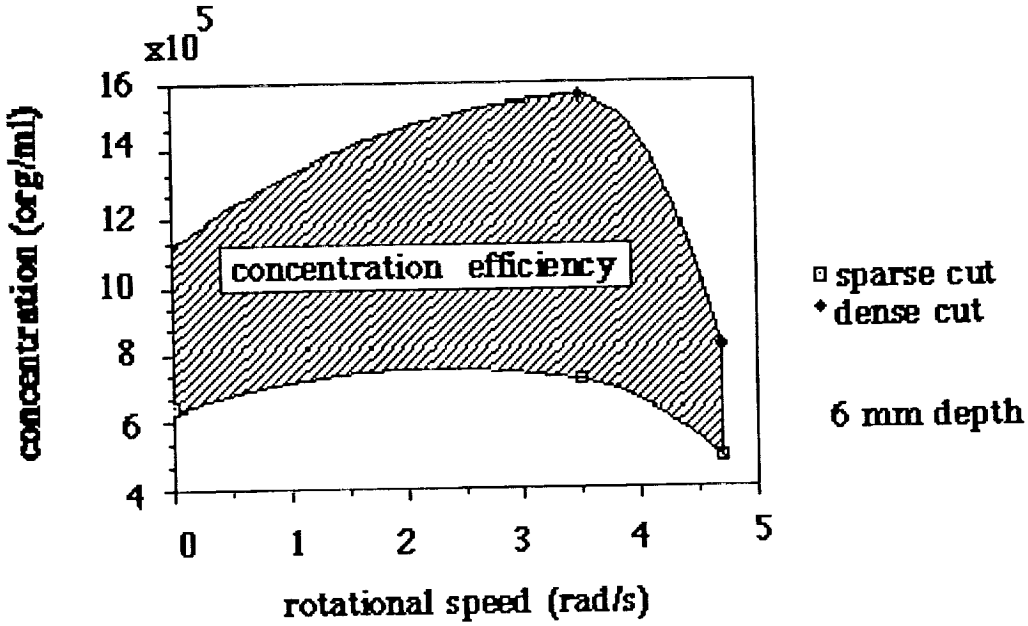

Figure 3. Concentration efficiency as a function of rotational speed at 0.6 cm fluid depth. All other variables besides depth remain constant as shown in Figure 3. Notice the greater concentration factor for shallower dishes.

APPROVAL

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The information in this report has been reviewed for technical content. Review of any information concerning Department of Defense or nuclear energy activities or programs has been made by the MSFC Security Classification Officer. This report, in its entirety, has been determined to be unclassified.

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