DIELECTROPHORESIS OF CELLS

HERBERT A. POHL *and* JOE S. CRANE

From the Department of Physics, Oklahoma State University, Stillwater, Oklahoma 74074. Dr. Pohl's present address is the Cavendish Laboratory, University of Cambridge, Cambridge, England. Dr. Crane's present address is the Department of Physics, Cameron State College, Lawton, Oklahoma 73501.

ABSTRACT Dielectrophoresis, the motion produced by the action of nonuniform electric field upon a neutral object, is shown to be a simple and useful technique for the study of cellular organisms. In the present study of yeast (*Saccharomyces cerevisiae*) using a simple pin-pin electrode system of platinum and high-frequency alternating fields, one observes that the collectability of cells at the electrode tip, i.e. at the region of highest field strength, depends upon physical parameters such as field strength, field uniformity, frequency, cell concentration, suspension conductivity, and time of collection. The yield of cells collected is also observed to depend upon biological factors such as colony age, thermal treatment of the cells, and chemical poisons, but not upon irradiation with ultraviolet light. Several interesting side effect phenomena coincident with nonuniform electric field conditions were observed, including stirring (related to "jet" effects at localized electrode sites), discontinuous repulsions, and cellular rotation which was found to be frequency dependent.

INTRODUCTION

In the study of living organisms, numerous techniques are now available. It is our purpose to add a new and simple physical technique, *dielectrophoresis*, to the array of methods available for the study of small organisms. It is based upon the fact that cells with different electrical characteristics will behave differently in a nonuniform electric field. It is an essential feature of this method that the cell behavior is that of a *neutral* body acted upon by a divergent field.

The effect of a nonuniform electric field on an ideal particle (e.g., a perfect insulator) which is free to move, depends upon the charge and upon the dielectric constant of the particle considered. If the particle possesses a net charge then there is an electrostatic interaction between the charge and the field, resulting in particle motion. This resulting motion is known as *electrophoresis*. If the material contains permanent dipoles, they will tend to become aligned with the field and will then experience a further force in the direction of the strongest field. That material will also contain dipoles induced by the field, and these too will normally be forced in the direction

of increasing field strength. The translational motion of a neutral particle due to the interaction of a nonuniform electric field with all of its dipoles, either permanent or induced, and expressed through the dielectric constant, has been defined as *dielectrophoresis* (Pohl, 1951). The force associated with this motion is termed the dielectrophoretic force. Dielectrophoresis is normally a much smaller effect than electrophoresis.

If the particle is not in a vacuum, but rather in some suspending fluid, then the field also attracts the fluid. The resultant force on the particle in this case is the difference between the forces on each constituent separately, and is proportional to the difference in their dielectric constants.

What has been said so far applies to the case of simple "ideal" materials, i.e., to solids and fluids which are perfectly insulating; but real matter conducts, sometimes weakly, sometimes strongly. This greatly complicates the previous simple picture. If one were to try to predict dielectrophoretic behavior using only the simple "perfect dielectric" model, he must be prepared to often find contradictory behaviors in dealing with actual materials. In actual liquids, for example, the field distribution is often strongly different from that supposed from first considerations, as Felici and coworkers have shown (Felici, 1967; Briere and Gosse, 1968 *a*, *b*). In addition to field alteration effects, there are a number of other phenomema which appear to complicate matters, such as rectification, interfacial polarization or Maxwell-Wagner-Sillars polarization (von Hippel, 1954), and electrical double layers. Progress in the application of dielectrophoresis to actual systems, especially living systems, has had therefore to depend heavily upon the experimental side, with theory often acting in an a posteriori fashion. The present studies on living cells were based upon much prior work with inanimate systems (Pickard, 1961; Pohl, 1951; Pohl, 1958; Pohl and Schwar, 1959; Pohl, 1960 *a*; Hawk, 1967; Pohl and Plymale, 1960; Pohl and Schwar, 1960; Feeley, 1969; Chen, 1969; Pohl, 1960 *b*; Pohl, 1968; Verschure and Ijlst, 1966), and by preliminary studies on yeast (Pohl and Hawk, 1966; Crane and Pohl, 1968) and on thrombocytes (Hawk, 1967).

The reaction of biological materials to electric fields was probably first studied by Muth (1927), who subjected fat particle emulsions to high frequencies and noticed pearl-chain formation (the end-to-end attachment of the particles which resulted in a formation similar to that of a chain of pearls). Liebesny (1939) also observed these formations for erythrocytes in high-frequency fields. Heller and coworkers (1960) studied the responses of various organisms to high field strengths in the frequency range of 10^5–10^8 Hz. They observed pearl-chain formation, orientation, preferential movement, rapid rotation, and frequency optima for alignment. Schwan and coworkers (Saito et al., 1966) have presented theoretical treatments for pearl-chain formation and orientation of biological particles. Schwan (1957) gave a review of the electrical properties of tissue and cell suspensions. His reports of extraordinarily high (effective) dielectric constants (10^2–10^4) for cell and tissue sus-

pensions prompted Pohl and Hawk (1966) to apply selective dielectrophoresis to such a suspension. It was found that by the use of a proper combination of frequency and solvent conductivity, the purely physical phenomenon of dielectrophoresis could be used to distinguish between living and dead yeast cells, and even to bring about physical separation. A more quantitative study of the solution resistivity and of the frequency response was reported by Crane and Pohl (1968).

The present study extends the work of Crane and Pohl and investigates in detail the response of yeast cells to a nonuniform electric field as affected by many parameters. In the present paper we shall describe in detail the necessary equipment and procedures necessary to ensure meaningful results, then go on to describe the observed response of yeast to nonuniform electric fields and its dependence upon various parameters at our control.

EXPERIMENTAL

The configuration of the electrodes used is an important parameter. A nonuniform field will be produced by any electrode design other than parallel plates, but certain shapes are to be preferred. For mathematical simplicity of treating the results, cylindrical or spherical geometries are desirable, and easily constructed. A special shape of electrodes capable of giving uniform force, and called the isomotive configuration, is also useful (Pohl, 1968). Configurations which approximate the cylindrical or spherical geometries which can be readily constructed include a wire perpendicular to a flat plate (pin-plate), and two parallel wires side by side (wire-wire). Pohl and Hawk (1966) used a pin-plate configuration. For the present work, a pin-pin design which minimizes Wien effect rectification was chosen. The rounded pin tips act essentially as two separated spheres. Near the tips this field can be shown to be approximately equivalent to that produced by concentric spheres, an approximation to be made later in calculations.

The pin-pin electrode arrangement is shown in Fig. 1. The electrodes were made from 22-gauge, 0.51 mm diameter platinum wire mounted inside a cylindrical well in a Plexiglas plate. The plate dimensions were 36 × 76 × 3.4 mm, for mounting on a microscope stage. The cylindrical well which holds the suspension is 1.7 mm deep and 4.0 mm across. The pin

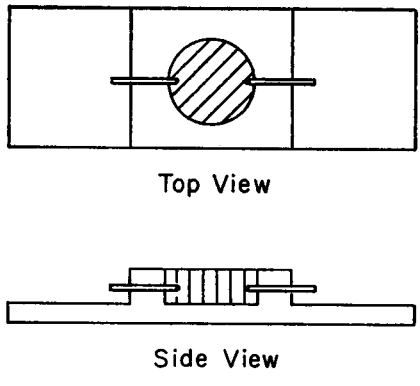

FIGURE 1 Diagram of pin-pin dielectrophoresis cell.

tips were made approximately spherical by abrasion in a lathe, polished with $\frac{1}{4}\mu$ diamond dust, and mounted 2.75 mm tip-to-tip through holes in the side of the well.

Voltage was supplied to the electrodes from varied sources. For the range 5 Hz to 600 kHz, a Hewlett-Packard 200 CD audio oscillator (Hewlett-Packard Co., Palo Alto, Calif.) with a Lafayette KT 615 audio amplifier (Lafayette Instrument, Lafayette, Ind.) was used. At 2.55 MHz, a specially designed oscillator providing up to 210 v was designed. Higher frequencies were supplied by a Heathkit DX-60B transmitter (Heath Company, Benton Harbor, Mich.) using at 7.334 MHz crystal for frequencies of 7.334, 14.7, 22.0, and 29.4 MHz. An Ameco TX-62 transmitter was used for 50.7 and 152 MHz, supplying up to 50 v. Excess power of the transmitter was dissipated by a dummy load (Heathkit Cantenna). A General Radio 1209-C oscillator (General Radio Co., Concord, Mass.) supplied up to 8 v at frequencies from 260 to 910 MHz.

The frequency of the field supplied to the electrodes was monitored by an oscilloscope to assure absence of contamination by harmonics or 60-cycle signal. Voltage was determined using a Hewlett-Packard 410B vacuum tube voltmeter (Hewlett-Packard Co.).

It is observed that the conductivity of the cellular suspensions has considerable effect upon the response to a nonuniform electric field. Precise measurement of it is important, but not easy in biological suspensions. The bridge used here was a General Radio 1650B impedance bridge (General Radio Co.) with a resistance range of 10^{-2}–10^{7} ohms and an accuracy of 1% between frequencies of 20 Hz and 20 kHz. Measurements were usually made at 1000 Hz, although other frequencies were used to check the electrode behavior. An external capacitor was connected across the variable resistance arm of the bridge to aid in nulling. Dipping-type conductivity electrodes were used. Electrodes of stainless steel, carbon, shiny platinum, and platinized (black) platinum were compared. Stainless steel and carbon electrodes gave very poor results, with errors ranging up to 200-fold as judged by comparison of the cell constants at varied frequencies and determined using various concentrations of standard KCl solutions. At very low conductivities, the shiny platinum electrode gave best results; at medium and high conductivities, the platinized platinum electrodes were superior. The latter were selected as the best compromise for the conductivity range of interest here (Robinson and Stokes, 1959).

It should be acknowledged that electrode polarization could affect our voltage determinations, especially at low frequencies, inasmuch as the test cell is provided with smooth platinum electrodes.[1]

Cell suspension concentrations were determined using an optical density apparatus requiring 1 ml of liquid, and calibrated independently by counting procedures. Cell concentrations of 2×10^{6} cells/ml were normally used, with the concentration determined to within $\pm 5\%$.

The direct observation of the dielectrophoresis was made with a microscope having a calibrated reticule. The average length of the chains of cells collected was determined after a fixed time interval, and is reported as the "yield" or "dielectrophoretic collection rate" (DCR).

In the limit of very low concentrations, the "yield in unit time" can be expressed more simply as the number of cells which gather in unit time and per unit length of the electrode,

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[1] After the completion of this study, one reviewer helpfully pointed out that a measurement of the actual voltage drop across the cell under constant AC current conditions measured over a wide range of frequencies will aid in determining the contribution of electrode polarization to the voltage drop in the cell. The authors agree that this is an excellent suggestion but feel that the observed constancy of the measured conductance with frequency using the smooth Pt electrodes in the conductance cells indicates that such error is slight here even in the dielectrophoresis test cell.

at the region of maximum field intensity. This has an advantage of minimizing the effects of pearl-chain formation on the observed dielectrophoretic collection rate. It opens the problem of statistical variations in the counting and related errors, however. The dilute system counting procedure may be especially useful where but small numbers of cells are available (say, less than about 1000–10,000 per ml).

Of the various experimental errors, the largest single source is that of measuring the yield at a given time. We estimate that a scatter of some 15–20% in the reproducibility of the yield exists. The frequencies are known to the nearest ±2%, the voltages to some ±3–5%.

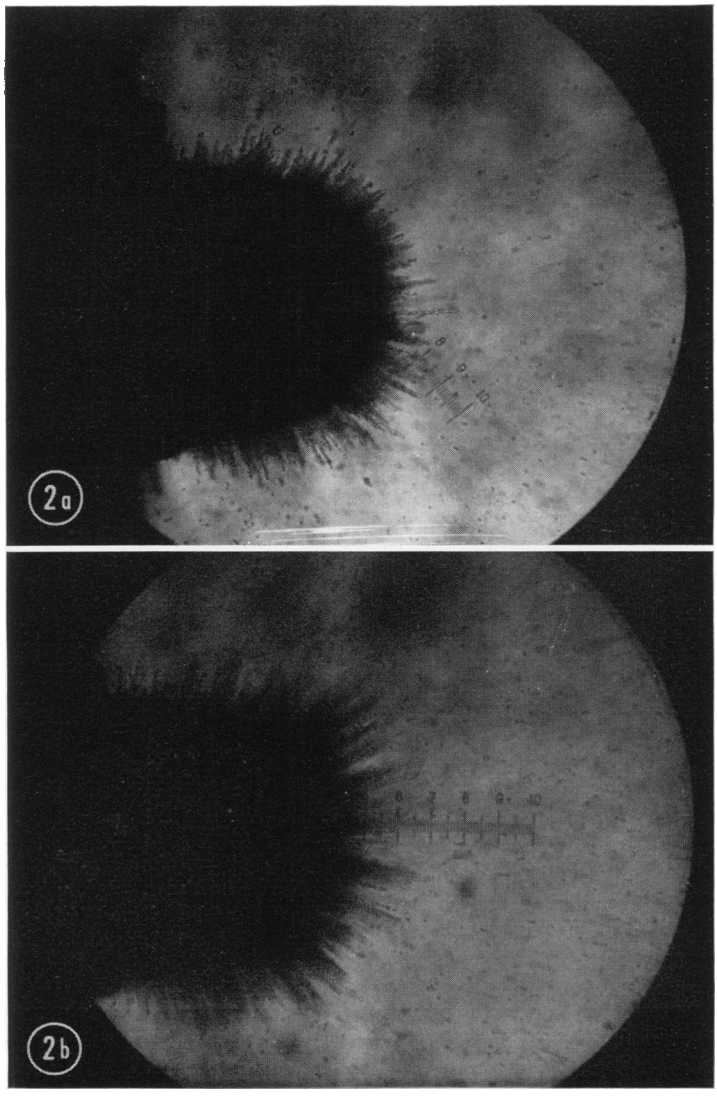

FIGURE 2 Photographs of yeast collecting.

A Typical Dielectrophoresis Experiment with Cells

Let us consider as an example, the determination of the dielectrophoretic response of yeast as a function of frequency, with other variables held constant. Typical values would be a voltage of 20 volts, rms, a suspension conductivity of 10^{-3} mho/m, a concentration of 2.10^6 cells/ml, and a collection time of 2 min.

Yeast cells, *S. cerevisiae*, from a pure colony grown in 4% dextrose–1% peptone solution, are harvested from a growing tube via a sterile bulb pipette and mixed with deionized water. The suspension is centrifuged and the supernatant liquid decanted. The cells are resuspended in fresh deionized water, and again centrifuged. The process is repeated until the conductivity of the suspension, as measured with the black platinum probe and conductivity bridge, is at or below the desired value. Between measurements the probe is kept in a test tube of deionized water to minimize contamination. If the conductivity of the suspension is too low, it can be increased by the addition of dilute KCl solution. If the cells are to be studied in a solution of specified molarity, they would be centrifuged once more and the special solution would be added.

The cell concentration is adjusted by dilution, using the optical density as a guide, and the conductivity rechecked.

The clean and dry electrode chamber is then filled with the suspension. Cleaning is done with rinses of deionized water from a squeeze bottle, followed by drying with an air jet from another squeeze bottle. This drying is necessary to prevent dilution of the suspension when it is placed in the chamber (0.21 ml). The chamber is filled using a syringe graduated in hundredths. After the chamber is filled it is mounted on the microscope stage and the electrical leads connected.

When the selected frequency and voltage of the applied signal is connected in, it is observed that the cells generally migrate to the pin electrode. They attach themselves there in chainlike formations, commonly called pearl-chains (Muth, 1927; Liebesny, 1939; Heller, 1960; Saito and Schwan, 1960), parallel to the field lines. Photographs of typical collection are shown in Fig. 2. The average length of these chains after a given time is designated as the yield, and is measured using the reticule in the microscope. For this particular run, the lengths would be measured at the end of 2 min and the field would be shut off.

Once the measurement is complete, the chamber is removed from the stage, rinsed with a deionized water jet, and dried with air jets. Occasionally not all the cells are removed from the electrode by this process. In this case a gentle brushing with a soft substance such as cotton, lens paper, or a pipe cleaner is necessary, after which the chamber is rinsed and dried, and ready for the succeeding suspension to be examined.

At the end of the run, and if necessary at selected times throughout the experiment, the conductivity and concentration of the suspension are rechecked. The concentration is not likely to change appreciably, but the conductivity may change by 50% over a period of several hours. This is especially true if the conductivity is quite low, say 10^{-4} mho/m. This change is due largely to CO_2 from the air, and to ion leakage from the cells themselves

RESULTS

The dielectrophoretic collection of yeast cells has been studied with reference to two categories of variables, physical and biological. The former includes field strength, frequency, particle concentration, conductivity of the suspension, and elapsed time. The latter includes colony age, chemical treatment, and exposure to heat and to ultraviolet light. The list is not exhaustive, but includes some of the more important

variables, and serves to illustrate the general applicability of dielectrophoresis to cellular suspensions.

In each of the data sets to be presented, the yield is shown as a function of the selected parameter. The yield is the average length of the longest chains attached to the electrodes after a particular time elapsed, and is measured using the reticule with the microscope at 100 power. One division corresponds to an object length of 10.3 μ (about 1.5 yeast cell diameters). The DCR is defined as the yield after a unit elapsed time.

Heating Effects Associated with Aqueous Dielectrophoresis

One source of interference with dielectrophoretic operations is the Joule heating produced by the flow of current (AC or DC) in the region of the electrode tips. If this produces temperature rises of more than a few degrees, thermal convection can become severe enough to disturb the cell collection. The following calculation is given to emphasize the salient parameters involved.

The power dw generated in a volume $d\tau$ can be expressed as

$$dw(r) = J(r)^2\rho \cdot d\tau = \sigma[\mathcal{E}(r)]^2 \cdot d\tau,$$

where $J(r)$ is the local current density at r, $\mathcal{E}(r)$ is the local field strength at r, and $\sigma = 1/\rho$ is the specific conductivity. The total power produced, W, in a given volume is

$$W = \int_{\text{volume}} w(r)\, d\tau = \int_{\text{volume}} \sigma \mathcal{E}(r)^2\, d\tau.$$

In steady-state conditions, this heat is conducted outward through a boundary at a distance r from the source centre as

$$W = \lambda \left(\frac{dT}{dr}\right) \times (\text{area at } r).$$

For the case of a spherical electrode of radius r_1 at a potential V_1, surrounded by a grounded ($V_2 = 0$) electrode of radius r_2, the field $\mathcal{E}(r)$ at r is given by

$$\mathcal{E}(r) = r_0 \frac{V_1 r_1 r_2}{r^2(r_2 - r_1)}.$$

The volume element $d\tau = 4\pi r^2\, dr$. Assuming $\sigma(r) = \sigma$, we have

$$W_{\text{gen}} = \sigma \left(\frac{V_1 r_1 r_2}{r_2 - r_1}\right)^2 \int_{r_1}^{r} \frac{4\pi r^2}{r^4}\, dr = 4\pi\sigma \left(\frac{V_1 r_1 r_2}{r_2 - r_1}\right)^2 \left(\frac{1}{r_1} - \frac{1}{r}\right);$$

$$W_{\text{out}} = \lambda \frac{dT}{dr} \cdot 4\pi r^2 = W_{\text{gen}}.$$

Hence

$$\Delta T = \int_{T_1}^{T} dT = T - T_1 = \frac{\sigma}{\lambda}\left(\frac{V_1 r_1 r_2}{r_2 - r_1}\right)^2 \int_{r_1}^{r}\left(\frac{1}{r_1} - \frac{1}{r}\right)\frac{dr}{r^2};$$

$$\Delta T = \frac{\sigma}{\lambda}\left(\frac{V_1 \cdot r_2}{r_2 - r_1}\right)^2\left[1 + \frac{r_1}{r} - \frac{2r_1^2}{r^2}\right] \cong \sigma V_1^2/\lambda.$$

For usual experimental conditions, $r_2 \gg r \gg r_1$, and $\Delta T \cong \sigma V_1^2/\lambda$. For the case of a cylindrical electrode of radius r_1 at a potential V_1 and concentric with a grounded cylinder of radius r_2, the field at a radial distance r is given by

$$\mathcal{E}(r) = \frac{r_0 V_1}{r \ln (r_1/r_2)},$$

and the volume element per unit length is $d\tau = 2\pi r\, dr \times$ (unit length).

A calculation similar to that for the spherical electrode case gives

$$\Delta T = \frac{\sigma}{2\lambda}\left(\frac{V_1}{\ln (r_2/r_1)}\right)^2 (\ln (r/r_1))^2.$$

For the situation $r = r_2$, the maximum temperature rise, ΔT, is

$$\Delta T = \frac{\sigma V_1^2}{2\lambda}.$$

This calculation, evaluated for several cases described in this paper, is instructive. From the results in Fig. 3 we can note that difficulties in the dielectrophoretic collec-

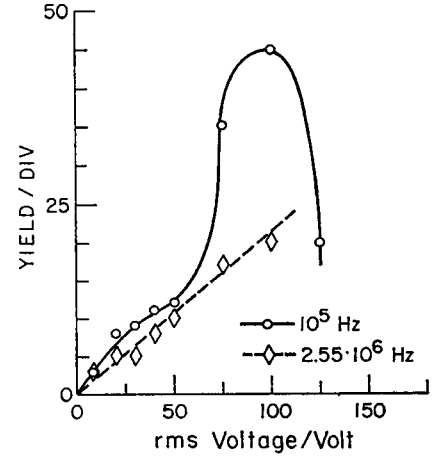

FIGURE 3 Voltage dependence of yield at frequencies of 10^5 Hz and 2.55×10^6 Hz, $\sigma = 6.10^{-4}$ mho/m.

tion occur at about 70–100 v. Using $\lambda H_2O = 5.7 \times 10^{-3}$ w/°C per cm, and $V_1 = 100$ v, $\sigma = 6 \times 10^{-6}$ ohms^{-1} cm^{-1}, we calculate $\Delta T = 10.5$°C. For the relatively serene situation of Fig. 8, say, $V_1 = 20$ v, $\sigma = 8 \times 10^{-6}$ ohms^{-1} cm^{-1}, we calculate $\Delta T = 0.56$°C. We conclude that a local temperature rise of some several degrees is acceptable for the small dimensions used. Some feeling for the hydrodynamics of the situation can be obtained from the use of the Grashof number, Gr_D.

$$Gr_D = g\beta(\Delta T)D^3/(\mu/\rho_0)^2,$$

where g = gravitational constant, β = volume coefficient of expansion, D = diameter, μ = viscosity, and ρ_0 = density. Using $g = 9.81$ m/sec^2, $\beta = 0.0002$ per °C, $D = 0.05$ cm, $\mu/\rho_0 = 10^{-6}$ (mks)

$$Gr_D \cong \frac{10 \times 2 \times 10^{-4} \times (5 \times 10^{-4}\ \text{m})^3}{(10^{-6})^2} \Delta T \cong \Delta T/4\,.$$

Since the Prandtl number for water is 7 at these temperatures,

$$Gr_D \cdot Pr \sim \frac{7}{4} \Delta T \sim 2\Delta T,$$

which implies even for $\Delta T \sim 10$°C a very low Nusselt number ~0.4, and therefore little turbulence.

Dependence upon Physical Parameters

Voltage. Fig. 3 shows the variation of yield with applied voltage at two different frequencies, 10^5 Hz, and 2.55×10^6 Hz. The conductivity was 6×10^{-4} mho/m, and the elapsed time for each measurement was 1 min. It is seen that the response is approximately linear with moderate voltages. It deviates from linearity at high voltages primarily because of strong stirring that results. When the stirring is moderate it brings more cells close to the pins where they can be more rapidly attracted by the strong field gradient there, thus causing an increase in collection. At the highest voltages, though, this stirring becomes turbulent, rips cells from the pin, and hence reduces the yield. This is illustrated by the fact that the deviation from linearity begins at lower voltage for 100 kHz which also has more attendant stirring than at 2.55 MHz. We conclude that for low and moderate voltages, the yield is seen to vary linearly with voltage.

Cell Concentration. The yield as function of cell concentration is shown in Fig. 4. An original concentration, C_o, of about 10^7 cells/ml was prepared, diluted, and yield measurements made. The conductivity was about 2×10^{-4} mho/m, the the voltage was 10 v at 10 kHz, and the measurement elapsed time was 1 min. The results indicate that the yield is generally linear with cell concentration.

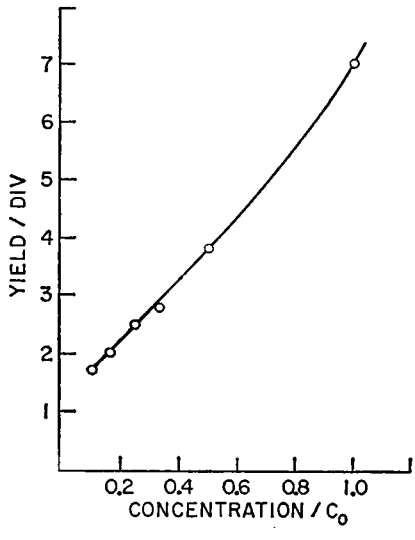

FIGURE 4

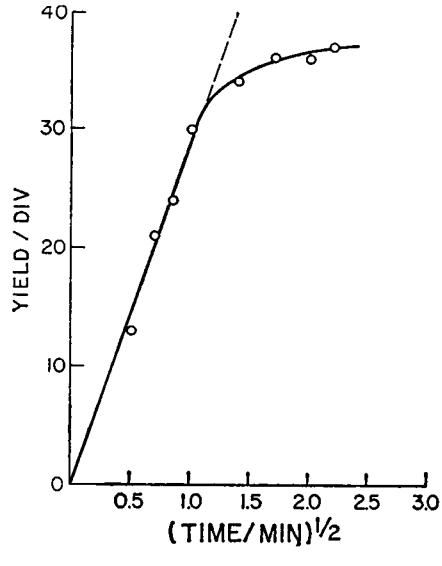

FIGURE 5

FIGURE 4 Dependence of yield on concentration. $V = 10$ v, rms, $f = 10^4$ Hz, $\sigma = 3.10^{-4}$ mho/m.

FIGURE 5 Variation of collection with the square root of the length of time the field is applied. $V = 120$ v, rms, $f = 2.55 \times 10^6$ Hz, $\sigma = 4.10^{-4}$ mho/m.

Time. From the theory of dielectrophoresis (Pohl, 1951) one expects the amount of collection to vary directly with the square root of the time that the field is applied. Experimental results for yeast are shown in Fig. 5, and this prediction is shown to be fulfilled up to the time range when exhaustion of the chamber contents by dielectrophoresis and by settling causes the collection rate to drop.

Frequency and Conductivity. The yield is a complex function of both these parameters. The variation of the yield as a function of the frequency of the applied voltage is shown in Fig. 6. The voltage was 20 v, rms, the conductivity 10^{-2} mho/m, the concentration $2 \cdot 10^6$ cells/ml, and the time interval was 2 min. One sees a rather complicated dependence with frequency, the yield being a minimum at about 10^4 Hz, a maximum around 10^6 Hz, and falling back to zero about 10^8 Hz.

If the experimental conditions are changed by altering the conductivity of the suspension, then the resulting frequency dependence is changed also. This is shown in Fig. 7, where the conductivity is varied from 3×10^{-4} to 9×10^{-2} mho/m. As can be seen, not only do the magnitudes of the maxima and minima change, but the frequencies at which these extremes occur are shifted also. This would imply that the effects of frequency and conductivity upon the polarizability are interrelated, not independent.

We conclude that for the five physical parameters considered, three of them, the voltage, the concentration, and the elapsed time, are rather independent quantities

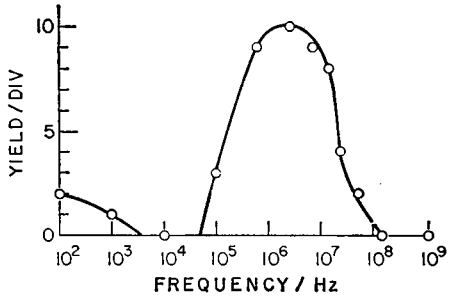

FIGURE 6

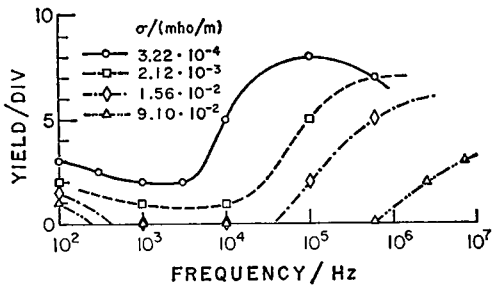

FIGURE 7

FIGURE 6 Frequency dependence of yield. $V = 20$ v, rms, $\sigma = 10^{-4}$ mho/m.
FIGURE 7 Variation of yield with frequency and suspension conductivity. $V = 20$ v, rms.

and affect the yield in particular manners more or less independently of the other variables. The other two parameters, frequency and conductivity, are strongly interrelated. One must be specified before the effect of the other can be given. It will be the goal of subsequent papers to present a theoretical account of these effects.

Dependence on Biological Parameters

At present it is not yet possible to designate various cell constituents, structures, or processes as the seat of various dielectrophoretic effects. It is possible to subject the organisms to a variety of physical and chemical circumstances which may affect the various cellular components in different ways, and thereby to hope to isolate the important mechanisms. Among the more readily tested variations are those due to colony age, heat, radiation, and chemicals.

Colony Age. A yeast cell changes considerably in its physical and chemical makeup as it ages. As the age of a colony increases, the average age of the constituent cells also increases, especially once the reproduction rate has slowed. The effect of the age of the cell can therefore be implied from the effect of the colony age. It usually takes about 2 days after innoculation before significant numbers of cells appear in the growing tubes. Rapid growth continues until about 5 days, after which the number of cells in the colony increases very slowly. Cells taken from colonies of ages 2, 5, and 9 days can then be assumed to represent young, old, and very old cells respectively.

Samples from 2-, 5-, and 9-day old colonies were studied under similar external conditions. The frequency dependences at low conductivity are shown in Fig. 8. There is little difference in the three at the lower frequencies, except for the lack of a minimum for the 5-day cells. At higher frequencies, the 5- and 9-day old cells respond about the same, about half that of the 2-day old cells.

At high conductivities, Fig. 9, the 5- and 9-day old cell yields were nearly identical at the middle frequencies. The very old cells had a slightly higher yield at low frequencies and a slightly lower yield at the high frequencies. The young cells differed

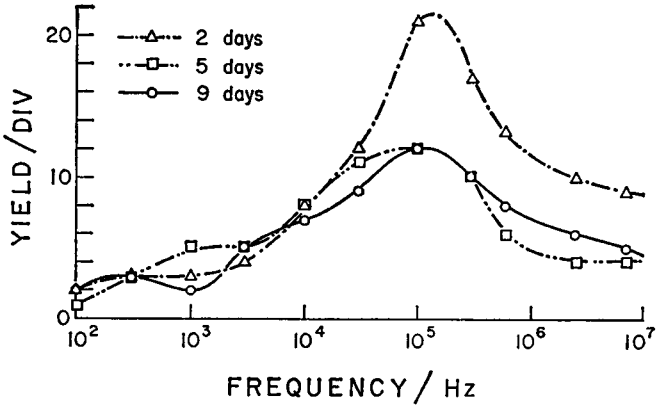

FIGURE 8 Low conductivity collection for cells from colonies of different ages. $V = 20$ v, $\sigma = 2.2 \times 10^{-4}$–$8.3 \times 10^{-4}$ mho/m.

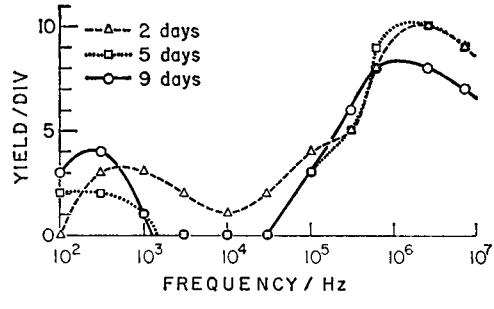

FIGURE 9 High conductivity collection for cells from colonies of different ages. $V = 20$ v, $\sigma = 1.1 \times 10^{-3}$ mho/m.

from the others by showing no collection at 100 Hz and a nonzero minimum in the middle frequencies.

Heat Treatment. According to Pohl and Hawk (1966), yeast cells killed by treatment at 60°C with crystal violet will not produce a yield at 2.55 MHz whereas generally live ones will. This suggests the treatment by other killing methods to determine the generality of that result. One of the methods chosen for killing the cells is the use of heat. In this case, the dead cells were produced by autoclaving for at least 15 min in a test tube containing a growing culture. They were then prepared for observation in the same manner as live cells. The concentration chosen was that which gave the same optical density as the live concentration of $2 \cdot 10^6$ cells/cm^3.

The yield for dead cells was measured as a function of voltage, time, and concentration and found to be similar to the results for live cells. That is, the yield was linear with applied voltage and concentration, and proportional to the square root of the elapsed time. This again implies that these relations are independent of the sample body.

The relations which are dependent on the body, the variation with frequency and conductivity, and seem to reflect the salt content, were not the same as for the live cells. It was found surprisingly difficult to get reproducible results with killed cells even in terms of general trends. Living cells gave much more reproducible behaviors. The striking differences between these results for killed cells and those for live cells were the lack of a minimum in the mid-frequency range and the occurrence of the high-frequency cutoff at a much lower frequency. The shift with increasing conductivity was toward lower frequencies in some cases and toward higher frequencies in others indicating the lack of reproducibility. This may be because of as yet uncontrolled differences in killing preparation such as the age of the cells to be autoclaved, the washing procedure before autoclaving, and the length of time in the autoclave. This needs more detailed and controlled study.

Exposure to Ultraviolet Radiation. Another effort was made to check the response of a cell which might be defined as dead. In cooperation with Dr. K. Haefner of the Southwest Center for Advanced Studies in Dallas, Texas, yeast cells were studied which had been irradiated with selected ultraviolet light. The light at the wavelength chosen ($\lambda = 2537$ A) inflicted nuclear damage, causing the nucleus to appear granular, and resulted in the cells being unable to reproduce. In the strictest sense, the cells were not living, although they did continue to metabolize. The cells were studied at $f = 3$ MHz, $V = 50$ v, and $\sigma = 10^{-2}$ mho/m. The cells collected quite readily at this frequency-conductivity condition, appearing to be normal. No detailed studies were made for these particular cells.

Treatment with Herbicides. Two well-known herbicides were chosen with which to treat the yeast cells. They were (2,4,5-trichlorophenoxy)-acetic acid (2,4,5-t.) and 2,2′-dipyridyl diquaternary bromide (2,2′-d.) They were used in concentrations of 10^{-3} M and $2 \cdot 10^{-5}$ M respectively for 2 hr. After treatment the cells were rinsed, prepared in the usual manner, and studied at 2.55 MHz. Those cells treated with 2,4,5-t. would not collect, just as expected for dead cells at this frequency. However the cells treated with 2,2′-d. collected readily, appearing very much like live cells. Longer treatment times gave the same results. Finally a medium consisting of dextrose, peptone, and 10^{-5} M 2,2′-d. was innoculated with yeast. A good culture developed. The detailed dependence of the yield upon the frequency and conductivity was not determined for these treated cells.

Phenomena Associated with Dielectrophoresis

During the course of the experiments, several phenomena were noticed concerning the movement of the cells. These were (*a*) stirring of the liquid, (*b*) repulsion of the cells from the pin, and (*c*) rotation of the cells about an axis through their centers.

Stirring. For almost all conditions there is some attendant stirring of the suspension. That is, the cells move in directions which are not coincident with the

field lines. It is probably connected with localized charge injection from "active spots" on the electrodes, and with thermal convection. At high frequencies and low conductivities, the stirring is very slight. The cells, in this case, move along the field lines and it is easy to tell the radius at which the force becomes significant. At this point the cells are given a rapid acceleration. At lower frequencies and higher conductivities the stirring becomes more pronounced and it is not obvious where the field becomes effective.

The stirring is usually in a pattern symmetrical about the pin-pin axis. Sometimes the flow is from left to right down the axis and at other times it is opposite to this. There do not seem to be any special conditions which determine the direction of flow.

On a few occasions at high conductivity and very high frequency ($\geq 7 \cdot 10^6$ Hz) the stirring was observed to come in pulses. There would be violent stirring for about a second and then calm for several seconds, after which the cycle would repeat. The length of the cycle was voltage dependent in that an increase in voltage shortened the time of least stirring.

At low frequencies with high conductivity, the stirring often became so violent that any collection was soon torn off by the swiftness of the current. Since the stirring effect is usually associated with high conductivity, or low frequency, its explanation probably involves a charging phenomenon, together with heating and its attendant convection.

Repulsion. Closely related to stirring is the repulsion effect. Occasionally particles which are moving in towards a pin to be collected suddenly move away in a direction normal to the surface. Usually this occurs before the cell has attached itself to the electrode, but sometimes a cell which has been in contact with the electrode for several seconds will suddenly shoot away. In a few cases, at a particular point on an electrode, a few cells will be in a cycle of touching the pin, shooting away a short distance, and moving back in to touch the pin again. This might continue for the duration of the experiment. Collected cells are never repelled from the ends of chains, only from contact or near-contact with the electrode. As in the case of stirring, repulsion seems to be a function of conductivity and so it too is probably due to charging effects associated with localized active spots on the electrode.

Rotation. Possibly the most puzzling phenomenon is that of cell rotation. Here the cells spin about an axis normal to the field lines at a rate of a few revolutions per second. This can be seen to occur with cells at almost any applied frequency and anywhere in the field; attached to an electrode, attached to another cell, or floating freely in the medium. It is not unusual to see several cells of a long attached chain rotating individually in their places. As the applied frequency is changed, the speed of rotation for a particular cell will also vary. It may speed up, slow down, or stop. A cell which has stopped as a result of a frequency change, many times will commence rotating again if the frequency is returned to its original value. Usually as the frequency is changed some cells will stop rotating and others will begin. The speed of

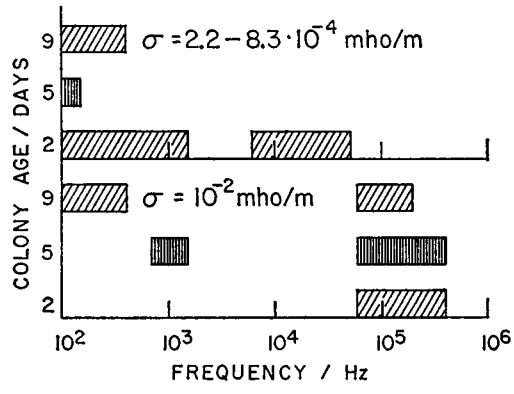

FIGURE 10 Occurrence regions of cell rotation as a function of frequency and conductivity for cells of different ages. $V = 20$ v.

rotation also seems to be voltage dependent in that an increase in applied voltage increases the revolving rate.

A brief study of cell rotation as a function of frequency and conductivity was made for the cells of different ages used in the colony age study. For each sample, the frequencies were noted at which any cell could be seen to be rotating. The results are shown in Fig. 10 using bar graphs. The blackened portions represent regions of rotation. These should be compared with the yield measurements for these cells shown in Figs. 8 and 9. There are no obvious relations between the two phenomena.

One note of interest is that for young cells the existence of rotation at high frequencies and low conductivity and the lack of rotation at low frequencies and high conductivity distinguishes them from the older cells. It may turn out that in the future, the rotation of the cells may be as good a diagnostic tool as the yield.

Presently, there is no completely satisfactory explanation for the cause of the cell rotation. Since the field is not a rotating field, pure dielectrics should not experience a torque. The answer may again lie in a charge transfer process, although it must be on a smaller scale than that causing repulsion. In support of this suggestion is the fact that all rotation occurs in a direction such that the side of the cell nearest the pin-pin axis, and hence in the strongest field, moves away from the pin.

One explanation for the origin of the observed torque would appear to lie in the simultaneous deposition of opposite charge on opposite sides of the cell by ionic currents in the medium, creating a dipole on the cell surface which can be shown to be aligned such as to be unstable with respect to the field direction. Brownian motion in the rotational mode will then cause the dipole (cell) to begin to rotate. This puts the original current-deposited dipole at angle to the field lines and accelerates the rotation. Further deposition of charge by the current in the medium creates further renewal of the dipolar assymetry, and cancellation of the old charge as it reaches its angle of equilibrium. The total result will then be an imparting of rotational energy

by energy from the electric field and its loss to the medium in the form of viscous drag upon the rotating cell.

SUMMARY

We have seen that dielectrophoresis, the motion produced by the action of a nonuniform electric field upon a neutral object, is a useful technique for the study of cellular organisms. In the present detailed study of yeast cells using a simple pin-pin electrode system and high frequency alternating fields one observes that the collectability of cells at an electrode tip, i.e. in the region of the most intense field, depends upon physical parameters such as the field strength, field uniformity, frequency; upon the cell suspension concentration, its conductivity; and upon the time of collection. The yield of cells collected is also observed to depend upon biological factors such as the colony age, thermal treatment of the cells, irradiation with ultraviolet light, and chemical poisons.

The yield of collected cells increases linearly with increasing field strength until disruptive effects such as stirring and charge injection become prominent. These latter effects at first cause an increase in collection, and if too intense, can override normal dielectrophoresis and prevent or even undo collection. The yield of cells collected is observed to be directly proportional to the cell concentration as expected, and to depend upon the square root of the elapsed time. The yield of cells collected is observed to be highly dependent upon the frequency of the applied field, and upon the conductivity of the suspension. The two factors are interrelated. The spectrum of frequency response is found to be a useful interpretative guide in studying the electrical character of cells.

The yield of cell collection was found to depend upon colony age, with the widest differences between young and very old cells, as might be expected. Heat treatment produced drastic changes in the collectability, depressing the upper frequency range of collection, and altering the low frequency response. Ultraviolet radiation such as to cause severe damage to the nucleus did not, however, produce differences in dielectrophoretic response at the single (high) frequency tested. Chemical poisoning with 2,4,5-t. drastically reduced dielectrophoretic response; that with the relatively harmless 2,2′-d. did not.

Several interesting side effects of nonuniform field conditions were noted, including stirring as by jet effects from "hot" spots on electrodes, momentary repulsive actions by occasional cells, and cellular rotations. The rotations of cells were observed to be field and frequency dependent. Young and aged cells appear to have different rotative responses.

We conclude that dielectrophoresis is an interesting and informative technique for the study of cellular organisms.

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