Dechlorination of DDT by Aerobacter aerogenes

Abstract. *Dechlorination of DDT to DDD in higher animals requires the presence of molecular oxygen, but in microorganisms the presence of oxygen hinders dechlorination. In cell-free preparations of* Aerobacter aerogenes, *the use of selected metabolic inhibitors indicated that reduced Fe(II) cytochrome oxidase was responsible for DDT dechlorination. This finding may possibly explain the persistence of DDT residues in soils and sediments.*

DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane] residues in soils strongly resist microbial degradation, although dechlorination of DDT to DDD [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane] can occur in higher organisms (*1*). In the rat, liver microsomal enzymes catalyze this reaction, which also requires molecular oxygen and reduced triphosphopyridine nucleotide (*2*). Dechlorination of DDT by microorganisms also is possible; *Proteus vulgaris* and baker's yeast reductively dechlorinate DDT to DDD (*3*). However, the efficacy of conversion by microorganisms is inversely proportional to the availability of oxygen in the system (*4*); and DDE [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene] is also produced. Castro (*5*) and Miskus *et al.* (*6*) found that dilute solutions of iron (II) porphyrin complexes were oxidized by DDT in vitro to the corresponding iron (III) halides (hemins) and DDD. Thus, it seemed likely that the microbial dechlorination of DDT might be explained as a process implicating the cytochromes of the respiratory chain, the absence of oxygen serving to keep the cytochromes in the reduced (Fe II) state.

To test this hypothesis, three facultatively anaerobic organisms not previously studied, *Escherichia coli, Aerobacter aerogenes*, and *Klebsiella pneumoniae*, were tested for ability to dechlorinate DDT (5 parts per million) to DDD. The organisms were grown in trypticase–soy broth or thioglycolate media (BBL). Anaerobic conditions markedly increased the yield of DDD. Although 2- to 3-percent conversion to DDE always occurred, this amount could be completely accounted for by the controls. There was no lag period in DDD production and DDE was not an intermediate, as shown by failure to metabolize it to DDD. Moreover, reduced glutathione, which is a required cofactor for DDT-dehydrochlorinase (*7*), did not promote DDE production even in mineral media. Because *A. aerogenes* effects up to 80-percent conversion to DDD, it was chosen for more detailed study. Because DDT is slowly absorbed by whole cells, cell-free systems were used in the remainder of the work so that incubation times could be shortened.

Cell suspensions of 10 mg/ml (dry wt) were sonically disrupted in 0.07*M* phosphate buffer (*p*H 7) at 15°C. Buffer (5 ml) containing 10 ppm DDT (added in 0.5 ml acetone) was combined with 5 ml of the sonorate in Thunberg tubes which were evacuated, flushed with nitrogen or carbon monoxide, and incubated overnight at 30°C. The controls were either boiled for 5 minutes or had 0.1 percent *p*-chloromercuribenzoate added. Endogenous metabolism was usually so rapid that it was not necessary to add Krebs cycle intermediates to maintain the cytochromes in the reduced state. After incubation, the suspensions were extracted with hexane and the extracts were dried with Na_2SO_4 and assayed for DDD, DDE, and DDT by use of a Wilkens-204 gas chromotograph with an electron-capture detector.

Under these conditions the average conversion to DDD was about 70 percent. Again the small amounts of DDE that always formed could be accounted for by the controls. Addition of 0.001*M* cyanide, which blocks the reoxidation of cytochromes but not their initial reduction by way of the dehydrogenase system (*8*), completely inhibited DDD production, as did an atmosphere of carbon monoxide. Significantly, the inhibition by carbon monoxide was completely reversible by 1614-mphot illumination and partially reversible by 484 mphot, but not by methylene blue, even though reduction of the methylene blue still occurred (that is, the dehydrogenase systems were still operative). Inhibition by CN^- was not reversible by either methylene blue or illumination. Also, 0.001*M* nitrate, ferricyanide, malonate, and antimycin A (2 μg/ml) all inhibited dechlorination of DDT, even though methylene blue could still be reduced. Inhibition by antimycin A was partially reversible by 0.01*M* ascorbate and completely reversible if 100 ppm cytochrome *c* also was added. However, addition of cytochrome *c* alone was ineffective.

The fact that the cytochrome oxidase–iron carbonyl complex is dissociable by light (*9*), together with the other data, leads to the conclusion that reduced cytochrome oxidase is probably the cellular agent in the reductive dechlorination of DDT to DDD. This idea may possibly explain the persistence of DDT residues in aerobic soils and sediments. Because DDT evidently does not keep the cytochromes in the oxidized state if oxygen is present, the suggestion by Castro (*5*) that the oxidation of Fe (II) in the respiratory chain is a mechanism of DDT toxicity is probably untenable.

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Separation of Living and Dead Cells by Dielectrophoresis

Abstract. *High-frequency non-uniform electric fields were used to cause selective dielectrophoresis of yeast cells in an aqueous medium. Living cells separated from admixed dead ones remained viable after the separation process.*

Differences in dielectric behavior in a non-uniform field can be used to produce selective migration of single-cell organisms, here represented by yeast cells.

The process described here is not electrophoresis, but dielectrophoresis. Electrophoresis is the motion of charged particles in an electric field. Dielectrophoresis is the motion resulting from the force set up by the action of a non-uniform electric field on a neutral

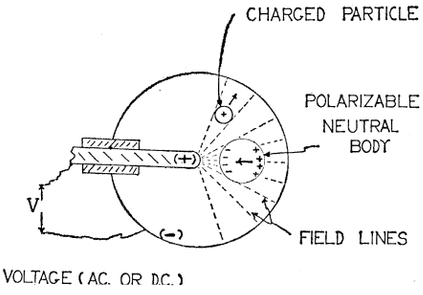

Fig. 1. Comparison of dielectrophoresis and electrophoresis. The direction of motion of the charged particle is dependent on the direction of the applied field. The plus-charged object is pulled toward whichever electrode is negative (electrophoresis). The neutral object (shown as a larger body) is pulled toward the region of highest field intensity. If the field is reversed, the direction of the induced dipole is also reversed but the net force is greater toward the more concentrated set of field charges (dielectrophoresis).

particle (*1–5*). It may be thought of as arising in the following way. Any dipole (induced or permanent) in a neutral body will have a finite separation of equal amounts of positive and negative charges in it. The electric field will cause some degree of alignment of the dipole with it. In a non-uniform (that is, divergent) field, one end of the dipole will be in a weaker field than the other. A net force will result, and the body will be pulled toward the region of greatest field intensity (Fig. 1). It can be easily seen that the direction of the field can be reversed and still give rise to the original direction of force on the polarizable body. As a consequence, one way motion takes place only toward the region of highest field intensity in either direct or alternating fields during dielectrophoresis. Not so in electrophoresis: reversing the field also reverses the force on the charged body (*1, 2, 4, 6*).

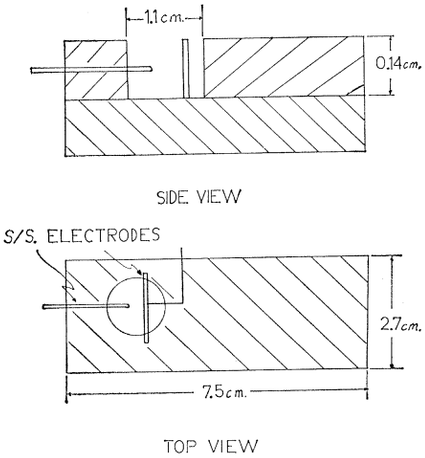

Fig. 2. Diagram of the dielectrophoresis cell.

The dielectrophoresis of particles in insulating liquids such as the hydrocarbon liquids has been described (*1, 3, 4*). Dielectrophoresis studies of particles in conducting liquids such as aqueous solutions present problems, however. Appreciable field strengths are generally required to produce ascertainable dielectrophoresis, and such strong fields are difficult to achieve in conducting fluids. We have examined the forces acting on solids of varied dielectric constant and conductivity in aqueous media of widely varied conductivity (*6*). The results confirmed the theoretical expectation that successful use of dielectrophoresis in aqueous media depends upon the simultaneous use of quite high frequencies, low ionic content of the liquid medium, and only moderate voltages. Accordingly, experiments were done with living cells to see whether living cells could be separated from dead ones by dielectrophoresis without appreciable harm to the cells.

The success of the dielectrophoresis method depends upon the existence of a difference in the volume polarizability between the particle and the medium. In a real dielectric the effective polarization is a complex function of the dielectric constant and the specific conductivity. We were encouraged that a significant difference would be found between the effective polarizabilities of certain aqueous solutions and those of living cells and tissue by the pioneering studies of Schwan and his co-workers (*7*). The dielectric constant of water and of most dilute aqueous solutions is about 80. The specific conductivity can range from about 3×10^{-6} ohm^{-1} m^{-1} to 2 ohm^{-1} cm^{-1}. The dielectric constant of living tissue is reported (*7*) to range from 10^2 to 10^4 and the specific conductivities range from about 10^{-2} to about 10 ohm^{-1} cm^{-1} (*8*).

Yeast cells were selected because they are rather large, are able to survive brief exposure to aqueous media of low ionic content (such as distilled or rain water), and are convenient to obtain. Cells grown in a liquid agar medium were harvested, centrifuged, and washed with water of high resistivity obtained by passage through a mixed-bed deionizer. Successive resuspensions in the high-resistivity water were repeated until the medium had a specific resistivity of 10,000 to 36,000 ohm cm.

The dielectrophoresis cell (Fig. 2) was constructed of polymethylmethacrylate plastic, with stainless steel electrodes. It had the overall shape of a microscope slide and had a cup-shaped cavity, 1.4 mm deep and 11 mm in diameter, into which the pin-and-plate electrodes projected. The pin electrode was 0.66 mm in diameter, with a carefully rounded end facing the flat plate electrode 1 mm away. The dielectrophoretic process could then be viewed from above on a microscope stage. The conductivity of the medium was measured separately with a set of immersible stainless steel electrodes in conjunction with a 1-kilocycle resistance-bridge. The field across the pin-and-plate electrodes was supplied by a 2.55-megacycle variable voltage capable of supplying up to about 200 volts (root mean square) across the electrodes and was monitored by a Hewlett-Packard type 410A a-c voltmeter.

In experiments with the yeast cells, the suspension was added to the dielectrophoretic cell and examined under the microscope. In the absence of an applied field the cells were distributed at random throughout the liquid volume. On application of 30 volts or more, the living cells rapidly clustered at the pin electrode. The gathering was essentially completed in 15 to 30 seconds. The behavior of dead cells was different. Yeast cells were killed by staining with crystal violet at 60° to 70°C for 3 minutes. Experiments on mixtures of the dead cells with live ones showed that the live cells congregated quickly at the pin electrode, and that the stained dead ones tended to remain behind in the liquid. A separation was thus achieved.

To ascertain whether the dielectrophoresis had grossly injured the living cells, portions of the cell suspension which had been subjected to dielectrophoresis for several minutes at 30 volts were removed and cultured. On both of two trials, the cells survived and grew again in the agar medium.

Thus key factors in the separation of living cells from stained dead ones by dielectrophoresis appear to be the use of (i) high-frequency alternating fields, (ii) media of very low conductivity, and (iii) non-uniform electric fields; and there should be an appreciable difference between the complex permittivities of the cells and the medium. The process is believed to be dielectrophoresis rather than electrophoresis for the experiment was conducted with a 2.55-

megacycle alternating current free of a d-c component.

It appears reasonable to attribute the high effective electrical permittivity of the living cells, in part at least, to the known existence of an easily distortable electric double layer across the cell membrane.

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Branched-Chain Fatty Acids in Sediments

Abstract. *Branched-chain (iso and anteiso) fatty acids were isolated from marine sediments from several environments. The relatively high ratio of branched-chain to straight-chain fatty acids for the even-numbered carbon molecules suggests a bacterial origin for the branched-chain isomers. The branched-chain fatty acids are present in the Green River shale. Possible geochemical implications are suggested.*

A small but significant fraction of the biochemicals produced by marine organisms is preserved in sediments. Most of this material quickly loses its biochemical identity and reappears as an insoluble material (kerogen) or as breakdown products. The fatty acids are among the chemical species best preserved in sediments. Cooper and Bray (*1*) reported straight-chain saturated acids, having even and odd numbers of carbon atoms, from recent and ancient sediments. Abelson *et al.* (*2*) and Parker and Leo (*3*) found even-numbered carbon unsaturated acids in addition to the saturated ones. We now report the finding of a homologous series of iso and anteiso methyl branched-chain acids in sediments from several environments.

Results of fatty acid analyses of samples from three different recent environments and the Green River shale are presented in Table 1. The Green River shale was collected near Rifle, Colorado, from the Mahogany Ledge outcrop (*4*). Baffin Bay is a hypersaline arm of the Laguna Madre located about 30 miles (48 km) south of Corpus Christi, Texas. The average results for nine samples of surface mud taken from the bay are reported. The Gulf of Mexico core was taken in 25 meters of water off Port Aransas, Texas. Since no significant variation of the acids was observed in the core, average values for seven samples are given. The British Honduras samples were from a 3.6-meter core, consisting mostly of carbonates, taken from a typical back-reef environment (*4*).

All of the recent sediment samples were frozen soon after the time of collection and kept frozen until used, except the British Honduras samples which were preserved in alcohol during transportation.

The analytical procedure developed to avoid excluding any type of fatty acid has been described (*3*). The urea adduction used by Cooper and Bray was avoided because it excludes branched-chain acids. Since the branched-chain acids have not been reported to occur in sediments, special care was taken to confirm their identification. The methyl esters were identified and measured by gas chromatography on columns of diethylene glycol succinate (DEGS) and of Apiezon L. The gas-liquid chromatography peaks identified as methyl-branched chains were not removed by bromination, whereas the unsaturated peaks were removed. A linear-log plot of the DEGS retention values of the methyl-branched peaks yielded two straight lines, corresponding to the iso and anteiso acids (*5*). Confirming evidence was obtained from the infrared spectra of the combined iso and anteiso C_{15} peaks. The peaks were collected at the exit port of an Aerograph 202 in a capillary tube and redissolved in carbon tetrachloride. Although the collected peaks contained some normal C_{15}, the "isopropyl splitting" of the band at 1380 cm^{-1} was apparent (*6*).

All the recent sediments examined thus far contain appreciable amounts of branched-chain acids. Figure 1 is a typical gas chromatogram of the relative positions of the branched- and straight-chain acids. The iso and anteiso peaks are not well resolved from each other, but the curve of the logs of the retention values indicates that both series are present in many samples. The branched-chain isomers with odd numbers of carbon atoms are more abundant than those with even ones (Table 1). The 15 carbon branched-chain acids are especially abundant, often as abundant as the normal C_{14} acid. The C_{15}

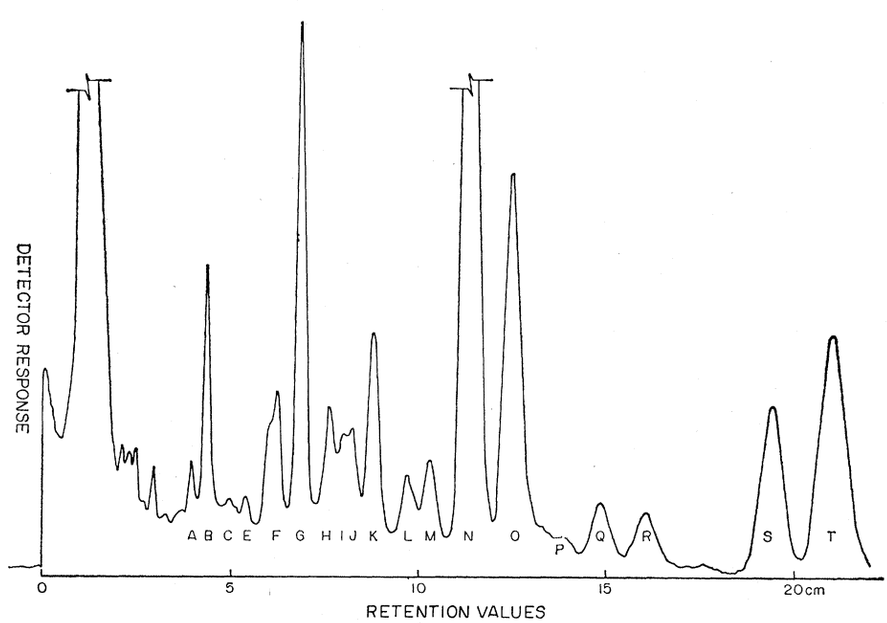

Fig. 1. Gas-liquid chromatogram of a fatty acid extract on a DEGS substrate. Key: A, iC_{12}; B, $C_{12}(0)$; C, iC_{13}; E, $C_{13}(0)$; F, iC_{14}, aC_{14}; G, $C_{14}(0)$; H, $C_{14}(1)$; I, iC_{15}; J, aC_{15}; K, $C_{15}(0)$; L, $C_{15}(1)$; M, iC_{16}; N, $C_{16}(0)$; O, $C_{16}(1)$; P, iC_{17}; Q, $C_{17}(0)$; R, $C_{17}(1)$; S, $C_{18}(0)$; T, $C_{18}(1)$. The numbers in parentheses indicate the number of double bonds; i, iso; a, anteiso.