chromatography may also be very useful when a continuous radiometric analysis of effluent gases is desired (*3*), as in the quantitative determination of both C^{14} and H^{3} in doubly labeled compounds (*4*).

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25 April 1960

Esters from Bacterial Oxidation of Olefins

Abstract. Identification of esters isolated from culture fluids of bacteria growing upon terminal olefins indicates that bacteria oxidize olefins at the saturated methyl group, leaving the double bond intact. The yeast *Candida lipolytica* produces α-glycols from olefins, presumably by attacking the double bond.

Utilization of straight chain olefins for energy and carbon sources among microorganisms is recognized (*1*), but few data are available to indicate the mechanism involved in the oxidation of these compounds. Bruyn (*2*) isolated *n*-hexadecanediol-1,2 from cultures of *Candida lipolytica* growing at the expense of hexadecene-1. Recent reports (*3–5*) have indicated that paraffins are oxidized by bacteria at the terminal carbon, probably via 1-alkyl hydroperoxide formation. Strains of Gram-negative coccoidal bacteria have been used in the laboratory of the department of bacteriology, State University of Iowa, to study the oxidation of alkanes containing an even number of carbon atoms, from C_{10} to C_{18} (*5*). The organisms grew well when a variety of olefins constituted the sole carbon source, and search was instituted for materials arising from bacterial oxidation of olefins in the liquid culture fluids.

Under conditions similar to those used in prior work (*4*), two strains of the hydrocarbon bacteria grew profusely in minerals–hexadecene-1 media, and a solid material of low melting point was isolated from each of the culture fluids. The material did not respond to any glycol tests but yielded a strongly positive hydroxamic acid test for esters. Growth and ester production were followed (Fig. 1); isolations were carried out at periods of peak ester formation, and the isolated material was subjected to mass spectrometric and infrared analysis (*4*). Mass analysis indicated the material to be an ester of molecular weight 478, with characteristic fragment ions:

$$m/q = 257 \quad (C_{15}H_{31}CO_2H_2^+)$$
$$= 256 \quad (C_{15}H_{31}CO_2H^+)$$
$$= 222 \quad (C_{16}H_{30}^+)$$

The infrared spectrum showed an ester carbonyl group and a sharp absorption peak at 11.0 μ in CCl_4 characteristic of $-CH{=}CH_2$. Thus, the data suggest an ester $C_{15}H_{31}CO_2CH_2(CH_2)_{13}CH{=}CH_2$ (15-hexadecenylpalmitate).

Analysis of material isolated from octadecene-1 cultures proved more difficult to evaluate and indicated the situation with respect to ester formation from the C_{18} olefin to be more complex. Mass spectra of such material showed ions of m/q = 285, 271, and 257, with relative intensities 1–2, 7–8, and 2–3, respectively, and of m/q = 250, with relative intensity 16. Since m/q=250 would correspond to $C_{18}H_{34}^+$ and m/q= 285, 271, and 257 would be $C_{17}H_{35}CO_2H_2^+$, $C_{16}H_{33}CO_2H_2^+$, and $C_{15}H_{31}CO_2H_2^+$, respectively, the mass spectra suggest the product to be a mixture of octadecenyl stearate, octadecenyl margarate, and octadecenyl palmitate in the ratios 1–2 to 7–8 to 2–3. It appears that bacteria attack 1-olefins at the saturated terminal carbon rather than at the double bond and, in the case of this organism, form esters in which the acid moiety is reduced (or totally synthesized via conventional fatty acid synthesizing pathways). These findings are in accord with those of Stewart and Kallio, who found esters (predominantly palmitate) formed from bacterial action on normal alkanes in the even-numbered series from C_{12} to C_{18} (*4*).

The data appear to contradict the observations of Bruyn (*2*), and a reassessment of the action of *Candida lipolytica* on hexadecene-1 was undertaken. After growth of *C. lipolytica* (ATCC 8661) for 5 days on minerals—hexadecene-1 media, ether extraction of culture fluids yielded a white crystalline material identical to that described by Bruyn. Infrared spectra of the isolated material and authentic hexadecanediol-1, 2 were indistinguishable. The isolated compound showed no carbonyl function in its spectrum and was negative to the hydroxamic acid test, but was cleaved by periodic acid. The physical constants of the material isolated were identical with those of authentic hexadecanediol-1,2. Total "diol," as determined by periodate titration, and C, H, and O content were also consistent with the conclusion that the material was essentially pure hexadecanediol-1,2.

It is thus possible to confirm the findings of Bruyn with respect to olefin oxidation by yeast. Evidently there are at least two pathways by which microorganisms initiate oxidation of terminal olefins: by direct attack at the double bond, or via oxidation of the methyl carbon at the saturated end of the molecule (*6*).

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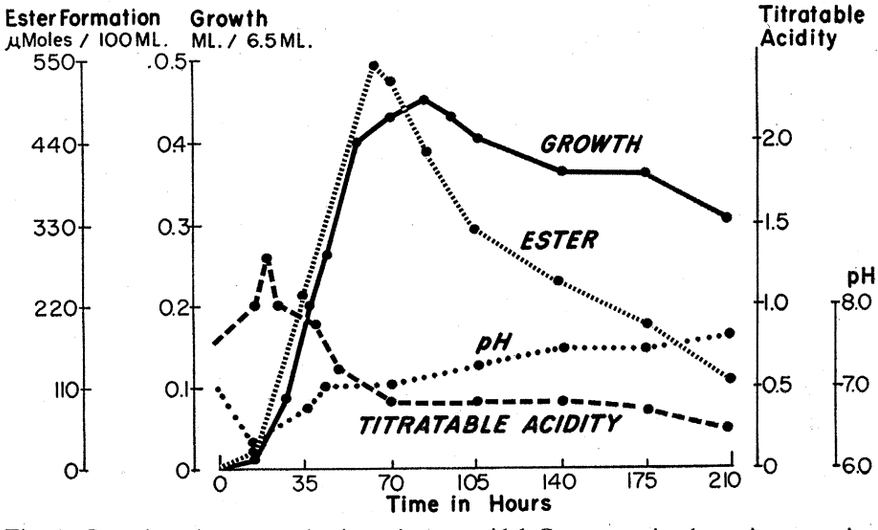

Fig. 1. Growth and ester production of a coccoidal Gram-negative bacterium growing aerobically in a minerals–hexadecene-1 medium. The ester was calculated as equivalents based on the ester group of cetyl palmitate (*4*).

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6 June 1960

Electrophoretic Interaction Studies by the Stable-Flow Free-Boundary Method

Abstract. A new method is described for rapidly mixing and unmixing (separating) components in free solution, enabling studies to be carried out on interactions of the components during their time of contact (presently as short as a few seconds). This method combines a multilayer, stable-flow fluid system with one (or more) transversely acting force fields, commonly an electric field, and is applicable to small molecules, large molecules, and cells.

The stabilized flow system recently reported (*1*) permits continuous electrophoretic separations and concentrations of large sample volumes in free solution without supporting medium. This achievement also permits other preparative and analytical investigations (*2*), including interaction studies, a type of which is the present subject. Several other interesting approaches to continuous solution separations have appeared recently. By isoelectric point immobilization and countercurrent flow, Bier separates the slowest or fastest component of a mixture (*3*). By hyperkinetic sample flow and high-viscosity polymer stabilization, Dobry and Finn fractionate dyes (*4*). [See also Bier's review (*5*)]. In none of these other methods, however, nor apparently in Philpot's early work (*6*), has true flow stability been realized in the sense described below.

A large number of independent, contiguous liquid strata can now be maintained in steady-state laminar flow through a separation (or analytical) apparatus and out into individual containers, with or without simultaneous transverse migration of components under the influence of applied forces. Flow rates from 0 to many milliliters per minute are practical. Flow stability is primarily due to the self-balancing nature of the system, the separate collection containers forming a single hydrodynamic unit with the flow cell. Significant inequities in flow rate (that is, in levels of identical bottles sharing a common horizontal) are thus precluded over a wide variety of operating conditions, that is, the laminar flows are stabilized by "hydrodynamic feedback."

The current apparatus is of the symmetrical 12 inlet–12 outlet form (Fig. 1*A*). Flows are horizontal through the main migration chamber from right to left. Single or multiple samples and background fluids can be injected through a variety of inlet combinations; thus great flexibility of experimental design is obtained. For electrophoresis, a voltage is applied between the top and bottom electrodes (commonly platinum foil or mesh). Force fields other than electric also show promise, but will not be further discussed here. The electrode compartments are hydrodynamically (not electrically) isolated from the main chamber by membranes. Thus, flows through them can be independently varied without disturbing main chamber flows, for example, to prevent diffusion into the main chamber or to set up steady-state *p*H gradients. Pumping is usually by a motor-driven syringe rack, though a much simpler gravity feed system also appears feasible.

Fig. 1*B*, a photograph of a steady-state pattern without electric field (30-cm apparatus), attests to the excellent stability of the different flowing layers. (Spectral analyses on collected fractions also verify this stability.) Similar pictures have been taken with 12 alternating color streams. Cresyl violet enters via inlet No. 5; bromphenol blue, via inlet No. 7. Small density gradients assist in eliminating turbulence that might be caused by uneven pumping, shock, and so forth. Flows pictured are 1.2 ml/min per outlet, 14.4 ml/min over-all. (Sucrose concentrations in inlet streams are: Nos. 1 to 4, none; No. 5, 0.4 percent; No. 6, 0.6 percent; No. 7, 0.8 percent; No. 8, 1.0 percent; Nos. 9 to 12, 2 percent.)

If an electric field is applied to a two-sample system as in Fig. 1*B*, various migration principles can apply. Some discussion of these has already been given (*2*), and it is beyond the scope of the present report to consider them in detail. Suffice it to say that concentrations, *p*H values, densities, flow rates, and field strength can generally be chosen to cause the migration paths of the two components to cross. The time of contact will depend upon the flow rates and electrophoretic migration velocities, both of which can be varied. If during this time, reaction occurs which gives rise to a new component with different properties, it may be separated from the original components at the outlets. If desired, the migration paths after the crossover can be altered by conductivity discontinuities in solution. In the following example, inlet solutions 1 to 4 and 9 to 12 are of higher conductivity than 5 to 8, essentially eliminating further vertical migration of samples above the 4 to 5 and below the 8 to 9 free-boundary positions (*2*, *7*).

Figure 1*C* shows this situation for the dye system of Fig. 1*B*: 0.004 percent cresyl violet enters via inlet No. 5, and 0.001 percent bromphenol blue enters via No. 7 (sucrose concentrations as above). The solution in the

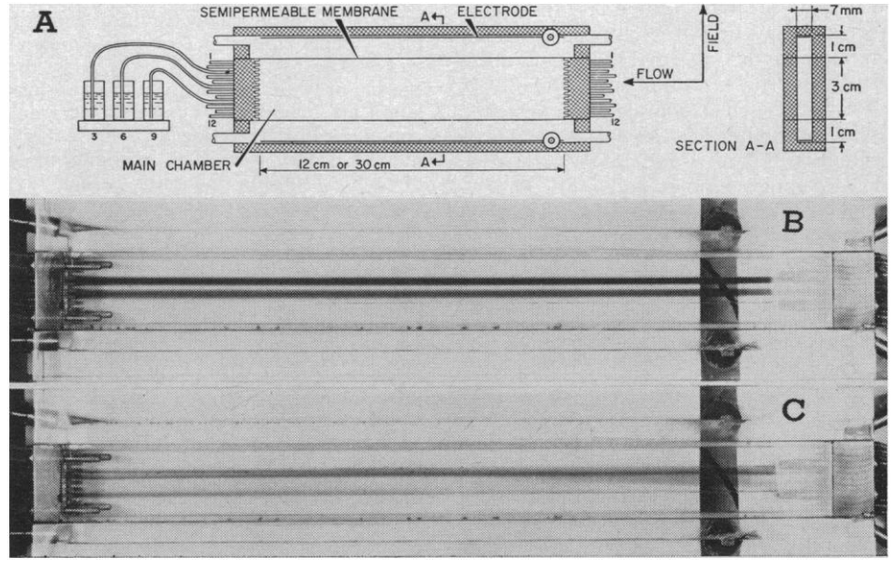

Fig. 1. (*A*) Free-flow apparatus. (*B*) Steady-state flow pattern without electric field. Dye samples admitted through inlets 5 and 7. (*C*) "Crossover" dye experiment with reaction product (middle component) separated at outlet.