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The papers presented here are based on addresses given in the symposium sponsored by the Ecological Society of America at the Western Division meetings of the AAAS *in Los Angeles last June. Concerning the authors, Dr. Ludwig and Mr. Paul are sanitary engineers, Dr. Mohr is a protozoologist, Dr. Newcombe and Dr. Phinney are botanists, and Mr. Oswald is a research engineer.*

Role of Algae in Sewage Oxidation Ponds

HARVEY F. LUDWIG and WILLIAM J. OSWALD
Sanitary Engineering Laboratories, University of California, Berkeley

A SEWAGE oxidation pond is an economical biological device employed for the treatment of settled sewage, which is widely used in regions where the climate is sunny and dry. The sewage treated has undergone previous primary clarification, which removes the readily settleable solids, or about a third of the sewage organic material. The remaining soluble and nonsettleable suspended materials may be removed economically only by biological methods.

An oxidation pond is usually an artificially constructed shallow basin from 3 to 5 feet in depth, big enough to hold the flow for a period of 20–30 days. Through atmospheric re-aeration, oxygen is adsorbed into the pond, and aerobic bacteria convert much of the organic carbon contained in the sewage to carbon dioxide. The resulting environment is favorable for the growth of algae. Through photosynthesis these convert the CO_2 to algal cell material and simultaneously produce a surplus of oxygen beyond their own needs. The algae tend to predominate in the latter sections of the pond, where the water may become highly supersaturated with oxygen, and sometimes some of the pond effluent is recirculated back to the inlet. This transfers some of the excess oxygen to where it is most critically needed and also furnishes seed for promoting algal growth in the incoming sewage.

The precise role of the algae in proper pond functioning has not been completely evaluated. Such factors as the types and numbers of algae occurring, their nutritional requirements, the amounts of excess oxygen produced, the effects of antibiotics produced by them on bacteria and other microorganisms, etc., need to be studied, in order that pond designs may be most efficiently adapted to treatment requirements.

Laboratory Studies

The Sanitary Engineering Laboratories of the University of California are studying the growth characteristics of a single species of algae, *Euglena gracilis,* cultured in a sewage environment. *E. gracilis* frequently occurs in oxidation ponds and also is commonly reported as an indicator of pollution in fresh-water streams and lakes. The Euglena are grown in the laboratory at constant temperature (25° C) in especially designed culture tubes (Fig. 1), which are subjected to continuous abundant light, and through which air is bubbled upwards to maintain a uniform suspension. Each day a fraction of the culture (the "yield") is removed from the tube, the amount depending upon the retention period desired, and an equal amount of settled sewage "food" is introduced into the tube. To begin a test for any given retention period, the sewage initially in the tube is heavily seeded with Euglena, after which the population of algae increases to an equilibrium, at which condition the test data are obtained.

The intense lighting on the tubes (averaging 1,200 ft-c throughout the tube) causes an acceleration of algae growth, as compared to actual pond conditions where lighting is not continuous and moreover does not penetrate throughout the pond depth. The cell population, or number of Euglena per ml of tube culture, is from five to ten times the

FIG. 1. Algae culture tubes.

total population of all types of algae observed in many pond effluent samples.

The retention periods studied were varied from two to twenty days, with the result that the environment to which the algae were exposed varied progressively from very favorable (abundant nutrients) to unfavorable (shortage of one or more nutrients).

Cell Types and Properties

Microscopic examinations of the cells produced at varying retention periods show that *E. gracilis* can exist in many forms, corresponding to different environmental conditions. Figure 2 illustrates the changes in cell forms for retention periods varying from two days ($R = 2$) to twenty days ($R = 20$). At short retention periods, $R < 7$, the cells exist in an environment of abundant food, and reproduce and grow as rapidly as their rate of reproduction (about 24 hours) permits. The predominant forms are the mitotic (dividing) and the typical motile ("textbook") Euglena. At $R = 7$ the percentage and population of motile forms are at maximum, corresponding to a "most vigorous" condition, wherein the cell population is as great as possible for the nutrients available. As the retention period increases and the environment becomes progressively more unfavorable, the population of cells decreases, although their concentration, or weight per unit volume of culture, increases. The cells increase in size, reproduce more slowly, and accumulate fat. Some of them are forced into a "palmellar," or passive, state in which they have little motility, or into a "swarming" state, which is a highly motile form that may evolve from the palmellar state, and seems to be a "last-gasp" effort by the organism to find necessary nutrients or die.

Figure 2 shows also that as the organisms become "older" their color changes. In this case, where the bubbled air was not enriched with CO_2 (i.e., % CO_2 in air = 0.03), the average color varied from dark-green at $R = 2$ to yellow at $R = 20$. In other, earlier tests, employing air enriched with CO_2, the color varied from very dark-green at $R = 2$, to yellow at $R = 10$, to brown at $R = 20$. Comparison of the two sets of data indicates that at low retention periods the greater availability of CO_2 results in a greater rate of growth, but at high retention periods the organisms are overexposed to CO_2 and suffer injury.

Besides causing changes in the form and color of cells, changing the retention period affects the cell shape, size, and density. The younger motile cells are "slimmer" or more elongated. The individual cell size and weight decrease from $R = 2$ to

$R = 7$, as the environmental conditions remain favorable and more time for reproduction is allowed, but beyond $R = 7$ the cells become progressively larger. Thus at $R = 7$, the "most vigorous" condition, where the population of cells is at maximum, the cells are smallest and have accumulated little stored material. Beyond $R = 14$, when some nutrient becomes limiting, the individual cells become more dense, indicating that the very old cells store up products containing less water.

Oxygen Produced by Photosynthesis

Since the production of oxygen is related to synthesis of cellular material, the younger or more rapidly growing cells produce oxygen at a higher rate. In Figure 3 are shown curves illustrating (*a*) the rate of oxygen production by the algal cells in cultures at varying retention periods, (*b*) the rate of utilization of oxygen by algal cell respiration, and (*c*) the rate at which the same cells would exert a biochemical oxygen demand if dead. Two oxygen production curves are given, one representing photosynthesis 100 per cent of the time, and the other representing field conditions or photosynthesis during the daytime (12 hours per day).

The curves show that young cells, or those growing in a favorable environment, photosynthesize much more oxygen than they respire, whereas old cells respire more oxygen than produced; hence young but not old Euglena cells could be responsible for the supersaturation of oxygen found in many pond effluents. Examination of the types of algae occurring in actual ponds in California[1,2] has shown that *E. gracilis* is present in large numbers in many ponds, and when present occurs in the typical motile forms. This indicates that the environment obtaining under field conditions is favorable, and produces cell types similar to those produced in the laboratory culture tubes at low retention periods.

There is no direct relationship between the laboratory tube retention period and pond retention periods. The Euglena in ponds are not uniformly distributed but occur in zones of favorable environment. On the fringes of such zones some of the cells might be transformed into palmellar types and, losing motility, would tend to sink to the bottom.

A subject of great practical interest is what happens to young algal cells discharged from ponds into receiving streams. Whether the cells remain young, become old, or die will be determined by the new environment. If the stream at the place of discharge is polluted, the new environment should

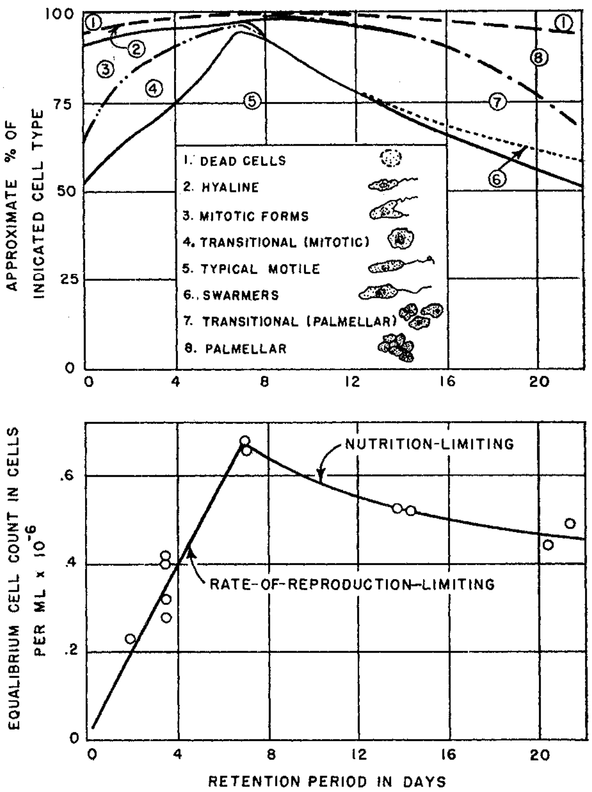

FIG. 2. Number and distribution by types of Euglena cells grown at varying retention periods.

be a favorable one, and the algae should be beneficial in producing a surplus of oxygen, which will assist in minimizing the oxygen sag. Farther down the stream, where after recovery the waters are clean and contain much dissolved oxygen, the environment becomes unfavorable; algal respiration may exceed algal oxygen production, resulting in a small oxygen demand. Thus the algae are beneficial where conditions are critical and oxygen is scarce, and will create an oxygen demand only when such demand has no significant deleterious effect. Similarly, death of the algae and an exertion of BOD by the dead cells should not occur to any significant extent except in very clean streams.

The discharged algae also constitute a living food, which may be of much value in sustaining fish and other wildlife.

Materials Balance

The amount of algal cell material produced at any given environmental condition is limited either by reproduction rates or by some nutrient. In oxidation ponds the limiting nutrient is probably CO_2. This is being produced continuously by bacterial action, so that the CO_2 concentration in the pond

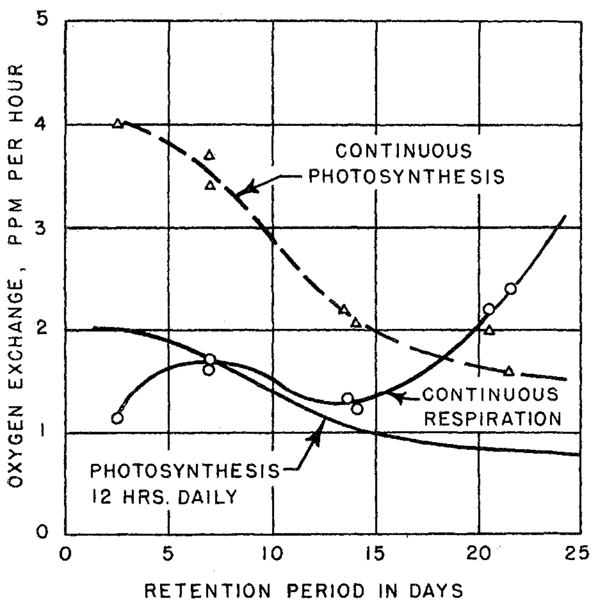

FIG. 3. Oxygen exchange capacities of algae cultures.

water is one of supersaturation with respect to the atmosphere.

The algae abstract much of this CO_2, and also abstract CO_2 from the alkalinity of the sewage, in accordance with the equation:

$$HCO_3^- \longrightarrow CO_2 + OH^-.$$

This reaction causes an increase in pH but no change in alkalinity. However, both laboratory and pond data show a reduction in alkalinity. This can only be due to the production by the algae of some acid substance. The laboratory data show that when *E. gracilis* is grown under conditions where CO_2 is not present in abundance the production of acid is increased and may become great enough to destroy all the alkalinity and depress the pH.

The laboratory data show also that the Euglena pick up and incorporate nitrogen, phosphorus, manganese, potassium, magnesium, and other minerals. At $R = 7$, the amounts of these constituents in percentage of the dry cell weight are as follows: Total ash, 10.0; N, 5.5; PO_4, 4.5; Ca, 1.2; Mg, 0.35; K, 0.35; and Mn, 0.001.

Yield of Algae

In recent years much study has been given to the feasibility of producing high-protein food by photosynthesis of algae. Cook,[3] studying yields of *Chlorella pyrenoidosa* grown under continuous illumination in an ideal synthetic nutrient prepared from commercial CO_2, fixed nitrogen, and other minerals, obtained yields as high as 0.48 g/l/day. Since settled sewage contains appreciable amounts of waste nitrogen (principally as ammonia and organic nitrogen), the bacterial-algal symbiosis may be considered a means for reclaiming the waste nitrogen in the form of algal cell material. The algae absorb the ammonia initially present in the sewage (20–40 ppm) and also the ammonia or nitrate formed by bacterial action on the organic nitrogen.

The maximum yield of *E. gracilis* occurs at a very low retention period (from 1.5 to 2 days), representing the minimum period that allows time for the cells, growing in an envrionment of abundant food, to reproduce sufficiently to build up a population. The maximum yield of *E. gracilis* obtainable from sewage is close to 0.26 g/l/day, if illumination is continuous and if the sewage is fortified with CO_2 to permit maximum extraction of nitrogen. Using a more rapidly reproducing species of algae, such as *Chlorella* or *Scenedesmus,* which are also at home and grow vigorously in sewage, the yield obtainable from sewage can probably be appreciably increased.

As compared with the use of synthetic media, the growth of algae with sewage offers several important advantages: (1) Fixed nitrogen is already present, or becomes available through bacterial action; (2) other minor or trace nutrients, such as phosphorus, are already present; and (3) much of the needed CO_2 is made available through bacterial action.

The algae cells may be readily separated from the culture medium by simple alum flocculation followed by settling. The cell material is a high protein food which, following sterilization, should be suitable for use as cattle feed. From "sewage to beefsteak" may be a real economic possibility.

References

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