ALGAE SYMBIOSIS IN OXIDATION PONDS *

III. Photosynthetic Oxygenation

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Organic matter decomposes rapidly in water because of its ready availability to microorganisms and their enzymes when in solution. Saprophytic microorganisms convert complex suspended or dissolved organic compounds to simple substances such as carbon dioxide and ammonia. Because oxygen is the ultimate hydrogen acceptor in the oxidations that accompany these rapid conversions, it frequently becomes so depleted that aerobic oxidation is halted.

Secondary sewage treatment processes involve the oxidation of dissolved or colloidal organic matter in settled sewage by microorganisms under conditions wherein oxygen does not become depleted. Most of these, such as the activated sludge process or the trickling filter process, are so designed that oxygen from the atmosphere is forced to enter the liquid phase at an accelerated rate.

Photosynthetic oxygenation is a completely different method of supplying the needed oxygen. Photosynthetic oxygenation may be defined as the production of oxygen through the action of light on the chloroplastic tissue of microscopic green plants, growing dispersed in the aqueous medium. This oxygen is produced by photosynthesis and derived from the decomposition of water. Its availability is independent of the physical laws normally governing oxygenation from atmospheric sources. Bodies of water containing green algae may attain supersaturation with dissolved oxygen up to three or four times the normal saturation value of water when in equilibrium with the atmosphere.

Sewage Oxidation Ponds

Sewage lagoons or oxidation ponds have been employed by sanitary engineers in the United States, mostly in the West and Southwest, in an effort to make use of photosynthetic oxygenation together with natural reaeration. Figure 1 is a schematic representation of such a pond.

When sewage enters the lagoon, bacteria are already at work. Decomposition of the sewage organic matter progresses swiftly. Carbon dioxide and ammonia are produced, some of which escapes to the atmosphere. Oxygen is consumed at a rapid rate, and when pond loadings exceed certain limits, complete oxygen depletion occurs, particularly in the initial sections of the pond. These various actions make up the bacterial phase. Field observations indicate that it is normally completed in 10 days, provided continuous aerobic conditions are maintained.

Following the period of bacterial action, and when light and temperature are satisfactory, algal populations appear in significant numbers. Photosynthetic oxygenation occurs, and should the water become supersaturated with oxygen, gaseous oxygen will escape to the atmosphere. The algae fix available carbon (that is, carbon
dioxide made available from the alka-
linity of the water, by bacterial
metabolism, or by solution from the
atmosphere). Available nitrogen is
synthesized into cell protein. Finally,
the mixed liquor, comprised of spent
sewage and a large amount of algal
cells including their excretory prod-
ucts, is discharged.

The efficiency of such a system leaves
much to be desired. Oxygen is pro-
duced and wasted at a time and in a
place where it is not needed. Also,
from the point of view of algae yield,
carbon dioxide is produced and lost
that might be utilized to promote
growth of additional algae. Moreover,
valuable organic and inorganic sub-
stances are finally discharged and
wasted.

This picture is typical of many ac-
tual pond installations. Wind currents
and wave action tend to promote some
mixing of the bacterial and algal
phases. In warm and sunny weather
the algae frequently invade the bac-
terial phase, effecting oxygenation that
accomplishes sewage treatment more
quickly. However, due to the long
over-all detention period of the system,
such algae become old and fat (3),
frequently dying and contributing
their cell material to the pond load,
cancelling out their earlier beneficial
effect.

As indicated by the upper arrow in
Figure 1, ideal pond treatment would
involve an overlapping of the bacterial
and algal phases, forcing a symbiosis
wherein the algae supply the oxygen
demand of the bacteria through photo-
synthetic oxygenation, and bacteria
supply the algae with needed carbon,
nitrogen, and other products of sew-
age decomposition. Recirculation has
been used as a practical means for
partial accomplishment of this objec-
tive.

Integration of Bacterial and
Algal Phases

A complete overlapping or integra-
tion of bacterial and algal phases is
seldom encountered in nature. This is
probably due to competition for nu-
trients, and in some cases even to
antibiotic activity (1).

Closed Continuous Systems

Figure 2 presents two sets of data
from 20-day laboratory tests conducted
with a specially designed culture tube
called a "symbiocon." More com-
pletely described elsewhere (2), the
symbiocon is a closed system simulat-
ing a "balanced aquarium" for bac-
teria and algae, in which the atmos-
phere is eliminated as a factor in gas
exchange.
Figure 2 shows that a symbiosis of algae and bacteria can be maintained in a closed system, to which nutrient sewage is being continuously added at a constant rate, and from which an equivalent volume of the culture is continuously withdrawn.

Both diagrams show that as algae grow, oxygen is produced, and that after completion of the log phase of algal growth oxygen depletion occurs. The higher rate of loading in Figure 2B produced higher bacterial and algal populations, and more “gain” in dissolved oxygen at the peak of oxygen production.

A state of dynamic equilibrium is exceedingly difficult to obtain in a symbiocon, indicating the complex interdependence of the variables affecting overlapping algal-bacterial growth. The symbiocon technique has just been developed, and further studies are underway to determine the best operating conditions. It is presently believed that an equilibrium that can be maintained indefinitely in the symbiocon can obtain only when each of a number of major variables is held within close but as yet unknown limits.

**Open Continuous Systems**

Equilibrium is readily obtained in open continuous systems termed “growth units.” These are large culture tubes having facilities for light, temperature, feed rate, and sterility control (3). They differ from symbiocons in that instead of being sealed from the atmosphere, bubbling air is utilized to maintain mixing in the tubes. Open units such as these are more simply operated than symbiocons. Moreover, they attain an equilibrium that may be continued indefinitely (4). Because of this, growth units are more versatile than the symbiocon for studying such physical variables as light, temperature, and retention periods.

**Variables Affecting Symbiosis**

The major variables influencing symbiotic growth of bacteria and algae are retention period (determined by
the rate of feed), sewage strength (including characteristics), light intensity and periodicity, temperature, and the bacterial and algal species and activity. All of these in one way or another influence (a) the growth of bacteria; (b) populations and general physiology of the algae, including their age and physical appearance, composition of cell material, chlorophyll formation, rate of oxygen production, rate of respiration, rate of uptake of carbon dioxide and nitrogen and inorganic ions, and the total density and yield of algal cell material; and (c) the overall sewage treatment accomplished in such systems.

Effects of Varying Retention Periods

Because bacteria and the algae studied multiply by binary fission, similar rules govern the populations of each produced in batch cultures as a function of time. As shown in a previous paper (2), the population attained in a batch culture at a given age corresponds roughly to the equilibrium population in a continuous culture having a retention period equal to that age. To obtain precisely the same populations, the retention period for the continuous culture must be slightly greater than the age of the corresponding batch culture. The population in the continuous culture is maintained at its equilibrium value, whereas the batch culture population falls off as multiplication ceases.

Bacterial Activity

In Figure 3 the data obtained from a batch culture of bacteria, and from batch and continuous cultures of algae, grown in the test sewage are utilized.

FIGURE 3.—Maximum population of algae and bacteria cultured in sewage.
to estimate the apex population for a continuous culture of comparable bacteria. The result indicates the maximum bacterial population to be approximately $10^8$ occurring at $R = 1.5$ days.

In continuous cultures, the maximum yield of algae occurs at a retention period about one-sixth of that for maximum population (1) (3). Assuming this relationship applies also to bacterial cultures, the maximum bacterial activity (that is, maximum oxygen uptake) should occur at $R = 0.25$ days. This value agrees with the time required to obtain maximum rate of oxygen uptake in the Warburg test made with sterile domestic sewage at 25°C, as previously noted (3). It also checks closely with the detention times of 4 to 8 hr. used in the operation of activated sludge plants.

**Algal Activity**

Other factors being constant, the most favorable environment for algal growth in continuous cultures is obtained at short retention periods. Under these conditions algal growth is maintained in the logarithmic phase. The cells are large, rich in chlorophyll, low in carbohydrate and fat, and produce algal cell material rapidly. Oxygen production per cell is high. Even though the cell population may be low, the total net oxygen production and the total algal yield both attain their highest values at a retention period approaching the minimum regeneration time of the specific algal species. Under such conditions the daily yield of algae is an easily measured and quite accurate criterion of daily oxygen production.

As the retention period increases beyond this point, the algae enter a negative acceleration phase of growth. Multiplication is slower and may cease entirely for many of the cells. Other cells continue to multiply, but their daughter cells have diminished size. Chlorophyll content per cell is reduced and, therefore, photosynthetic oxygen production is retarded. The total respiratory requirement is increased because the mass of cells has increased (although the individual cell respiratory requirement may have remained low). The net oxygen yield falls off rapidly as retention period continues to increase.

At very long retention periods, beyond the end of the negative acceleration phase, the total cell population has not decreased, but the size of the individual cells has increased to maximum. In the case of both *Euglena* and *Chlorella*, further increase in retention period causes a final dropoff in population due to autolysis, cell senescence, and death. Algae existing in this phase have a high respiratory requirement per cell. Although autolysis is occurring, the cell population remains high and chlorophyll content is very low; hence, the total oxygen uptake of the algae may exceed the amount of oxygen produced in photosynthesis.

With both *Euglena* and *Chlorella* it was found that the cell-free supernatant effluent from culture units had relatively low 5-day Warburg B.O.D. values almost independent of retention period. Chemical analyses of these effluents showed that they contain large amounts of carbon, hydrogen, and nitrogen. The C/H ratios or C/N ratios of this material give little indication as to its nature, but the B.O.D. tests indicate that it is highly stable and hence probably highly oxidized.

**Effects of Sewage Strength and Composition**

In all of the studies it has been consistently observed that natural sewages with higher 5-day B.O.D. values support greater populations of *Euglena gracilis*, *Chlorella pyrenoidosa*, *Schneideriopsis obliquus*, and *Chlamydomonas* sp. than lower strength sewages. The presence of more carbon, nitrogen, and other critical elements seems to produce this effect, but there are indications
that the ratios of these substances may also be important. Preliminary to the light studies reported hereinafter, repeated attempts to grow *E. gracilis* on a synthetic sewage containing higher concentrations of all known critical elements than natural sewage, and having a higher B.O.D., resulted in only marginal growths. On the other hand, in similar tests, *Chlorella* growths in this synthetic sewage were more abundant than in normal sewage. Also, magnesium may be a critical ion in certain cases, because it is an essential part of the chlorophyll molecule.

**Study of Varying Light Intensities**

These experiments were made with *E. gracilis*, employing the open-type continuous growth units. A constant and sterile sewage of low B.O.D. was used as the substrate,* so that culture densities would remain relatively low and thus minimize light absorption. The tests were made at a fixed retention period of 7 days, which was selected after preliminary work had shown that *Euglena* populations cannot be readily maintained at low light intensities at shorter retention periods. Temperature in all of the series ranged between 24° and 26° C., and individual series varied ± 0.5° C. from the mean.

Light intensities were estimated on the basis of the number of new 30-watt daylight fluorescent lights utilized. With a single light the interpolated in-

tensity within the growth units is 400 ft.-c. (foot-candles). Interpolation is made between the measured intensities at the front and rear surface of the culture tube with respect to the light source. This intensity is measured with a photoelectric galvanometer, and calibrated variable light source. Intensities of 100 and 200 ft.-c. were obtained by blacking out proportionate strips of the fluorescent light. Duplicate bulbs were alternated to cancel minor differences in intensity among different bulbs.

*Euglena* cultures were started on sterile sewage in Erlenmeyer flasks, and after 10 days were transferred to the growth units and inoculated with sewage bacteria as previously described (2). Sterile sewage was then added to the culture at a rate corresponding to the selected retention period until full culture volume was attained. Air was bubbled through the cultures continuously at the rate of 500 ml. per minute. After full volume was reached, one-seventh of the culture was removed daily and replaced by sterile sewage. Evaporation losses were made up with sterile distilled water before withdrawal.

Daily tests for cell population, cell volume, pH, and alkalinity were performed on effluents until changes were negligible, indicating a state of equilibrium. The entire culture was then sacrificed in order to have sufficient experimental material for complete testing.

In addition to the tests made with the natural sewage, certain comparable tests were made with a stronger synthetic sewage *at a retention period of

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* This sewage was different from that for the retention period studies. After processing as described elsewhere (3), it had the following characteristics: pH 9.5, total alkalinity 175 p.p.m., bicarbonate alkalinity 115 p.p.m., manometric B.O.D. (5-day, 25° C.) 90 p.p.m., suspended solids 41 p.p.m. (including 28 p.p.m. volatile suspended solids), dissolved solids 369 p.p.m. (including 155 p.p.m. volatile dissolved solids), total solids 410 p.p.m., total ash 227 p.p.m., total volatile solids 183 p.p.m., organic carbon 56 p.p.m., organic hydrogen 16.6 p.p.m., organic nitrogen 17 p.p.m., ammonia nitrogen 14 p.p.m., nitrate nitrogen 1 p.p.m., Ca 9.5 p.p.m., Mg 6.5 p.p.m., P 4.1 p.p.m., K 11.8 p.p.m., and Na 71.5 p.p.m.

* This synthetic sewage was prepared as described by Weinberger (6). After sterilization at 115° C. for 20 min. in sealed containers, it has these characteristics: pH 9.5, total alkalinity 160 p.p.m., bicarbonate alkalinity 117 p.p.m., manometric B.O.D. (5-day, 25° C.) 105 p.p.m., suspended solids 175 p.p.m. (including 147 p.p.m. volatile suspended solids), dissolved solids 377 p.p.m. (including 130 p.p.m. volatile dissolved solids), total solids 652 p.p.m., total ash 107 p.p.m.,
produced each day are shown in Figure 5. Both curves follow the trends of the comparable population curves in Figure 4.

There was no systematic variation in the morphology of *Euglena* with varying light intensities. From 90 to 95 per cent of the cells remained motile, as was the case in earlier studies (3). Great variations in the size of cells, perhaps resulting in variation in yield, are noted at all light intensities; however, cells produced at low light intensities tend to be larger than those from highly illuminated cultures.

Bacterial populations attained a maximum at $10^6$ c.p.m.l. (colonies per ml.) at 100 ft.-c. The minimum, $10^6$ c.p.m.l., occurred at 2,400 ft.-c. Intermediate populations were obtained at intermediate light intensities, but without consistent variation.

**Chlorophyll Production**

Figure 6 shows the variation of chlorophyll content per million algal cells as a function of light intensity. Chlorophyll per cell is low in the range where highest yield is obtained, 400 to 1,200 ft.-c. The decrease in chlorophyll is very rapid in the range from 100 to 400 ft.-c., indicating that there is no direct relationship between chlorophyll content and yield under varying

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**Populations and Yields**

Effect of light intensity on population of *E. gracilis* is shown in Figure 4. From 100 to 400 ft.-c., the population in the natural sewage (upper curve) increases to a maximum at or near 400 ft.-c. From 1,200 to 2,400 ft.-c. the population falls off slightly. The lower curve shows similar data obtained with the relatively strong synthetic sewage. The lower curve indicates that the *Euglena* did not develop as well as with the greater amounts of the stronger nutrient, except at a high light intensity. The yields of algae total volatile solids 445 p.p.m., organic carbon 112 p.p.m., organic hydrogen 20 p.p.m., organic nitrogen 28 p.p.m., ammonia nitrogen 14 p.p.m., nitrate nitrogen 0 p.p.m., Ca 18.6 p.p.m., Mg 8.5 p.p.m., P 5.5 p.p.m., K 3.7 p.p.m., and Na 66 p.p.m.
light intensity. Photo-bleaching may explain the slower rate of chlorophyll decrease beyond 400 ft.-c. The measured chlorophyll values for cultures in the natural sewage indicate a slight increase in the range from 1.200 to 2.400 ft.-c., but these measurements may be in error since the macroscopic observations showed increased yellowing through this range.

A study of the components of the chlorophyll (that is, chlorophyll A and chlorophyll B) showed from 90 to 95 per cent chlorophyll A at all light intensities when the natural sewage was employed. With synthetic sewage, at $R = 5$, however, chlorophyll A averaged only 60 per cent. Strain (7) suggests that the ratio of chlorophyll A to B is indicative of the character of the nutritional environment, pointing out that higher ratios of B to A result when conditions are more favorable to chlorophyll formation.

FIGURE 6.—Effect of light on chlorophyll production.

FIGURE 7.—Variations in pH and alkalinity with varying light intensities.
Character of Whole Effluents

Carbon—Figure 7 shows the results of pH and alkalinity data as a function of light intensity. From these curves it can be seen that E. gracilis consistently reduces pH, checking results previously reported (3). Probably the algae exchange H+ ions for NH4+ ions. As shown by Figure 8A, which illustrates the carbon balance at equilibrium conditions, at all light intensities greater than 100 ft.-c, the total effluent carbon exceeds the total carbon contained in the influent sewage. The difference must be attributed to carbon supplied from the bubbling air, in accordance with the reaction

\[ \text{HCO}_3^- \rightarrow \text{CO}_2 + \text{OH}^- \]

This reaction does not change the alkalinity of the system, but does increase the pH. Evidently, however, the production of acid already noted is sufficient to neutralize any OH formed and also to reduce the alkalinity initially present. In the process the HCO3- concentration is increased at the expense of CO3--. The net changes in pH, HCO3- and CO3-- in any system depend upon the relationship between acid production and CO2 absorption.

A large portion of the carbon utilized by the algae is not incorporated into the cells. As shown by Figure 8A, much of this carbon appears in the effluent supernatant, perhaps as a highly oxidized excretory product of the Euglena.

Nitrogen—Figure 8B shows there is very little loss of total nitrogen in the system in spite of the high influent pH. The small residual ammonia in the effluent indicates that the principal source of nitrogen for Euglena is NH4+. The large amount of effluent organic nitrogen in the supernatant may again be an indication of some excretory product by Euglena.

Hydrogen, Phosphorus, and Other Ions—The synthesis of an excretory product by Euglena is further indicated by the relatively large amount of organic hydrogen in the supernatant
Total effluent organic hydrogen exceeds total influent organic hydrogen, presumably because of photochemical decomposition of water. The phosphorus balance shown in Figure 8D indicates that this element may have been a limiting nutritional factor at the higher light intensities. Similar balances (not shown) for magnesium, potassium, and calcium show that these elements were not limiting to growth. More calcium was taken up by the Euglena cells at low light intensities than at high light intensities.

**Oxygen Production**

A basic problem in studying photosynthetic oxygenation is the determination of the oxygen produced. In earlier studies the oxygen-producing capacities of the algae were directly measured by transferring them to the Warburg apparatus. However, the environment in the Warburg flask, including light intensity, is different than in the growth units. For this reason, in the present studies the oxygen production has been calculated utilizing the method introduced by Myers (8). This method employs the analyses of the algal cells to determine the "assimilatory quotient." As an example, an analysis of Euglena may show 59.3 per cent C, 5.24 per cent H, 26.3 per cent O, and 9.1 per cent N on an ash-free dry-weight basis. Division by the atomic weights of these elements converts these percentages to the formula

\[ C_{7.62}H_{8.08}O_{2.32}N_{1.0}. \]

All evidence indicating that ammonia is the source of nitrogen, it may be assumed that the synthesis of this material follows the equation

\[ 1.0 \text{NH}_4^+ + 7.62 \text{CO}_2 + 2.53 \text{H}_2\text{O} \rightarrow C_{7.62}H_{8.08}O_{2.32}N_{1.0} + 7.62 \text{O}_2 + 1.0 \text{H}^+. \]

The assimilatory quotient \( A_q \) then is

\[ \frac{\text{CO}_2}{\text{O}_2} = \frac{7.62}{7.62} = -1.00. \]

These values have been determined for all light intensities investigated, and the results (Figure 9) show that oxygen yield per cell increases with intensity up to about 1,200 ft.-c. A numerically low assimilatory quotient corresponds to a high oxygen yield.

If the total daily carbon yield is known, the assimilatory quotient can then be used to calculate total oxygen yield by the formula

\[ \frac{\text{O}_2}{\text{C}} \times \frac{1}{A_q} \times Y_C = Y_{O_2}, \]

where \( \text{O}_2 \) and \( \text{C} \) are respective molecular weights, \( A_q \) is the assimilatory quotient and \( Y_C \) and \( Y_{O_2} \) are the yields of carbon and oxygen, respectively.

![Figure 9](image-url)
These calculated gross oxygen yields are plotted in Figure 10. Maximum gross yield occurs in the range from 400 to 1,200 ft.-c.

The oxygen consumed by the algae cells in the absence of light was measured with the Warburg respirometer. The bacterial respiration demand at equilibrium was assumed to be approximately B.O.D./R, where B.O.D. is the 5-day, 25° C., Warburg B.O.D. of the influent sewage and R is the retention period in days. By plotting these data with ordinates superimposed, the net oxygen yield is obtained (Figure 10).

The relatively high respiratory requirement of *Euglena* at 100 ft.-c. is indicative that little, if any, oxygen was produced at this low light intensity. *Euglena* are capable of growing saprophytically in the dark, a phenomenon commonly reported in the literature.

A comparison of the oxygen curve in Figure 10 and the cell yield curve in Figure 5 shows that maximum yield and maximum oxygen production coincide, at approximately 700 ft.-c.

As in the earlier studies (1)(3), the cell-free supernatants from continuous cultures grown under varying light intensities show consistently low values of B.O.D. These values ranged from 25 p.p.m. at 100 ft.-c. to approximately 10 p.p.m. at 2,400 ft.-c. Chemical analyses of the supernatant material (see Figure 8) show appreciable amounts of carbon, hydrogen, and nitrogen.

**Composition of Algal Cell Material**

As light intensity increases from 100 to 400 ft.-c., retention period being constant, large variations are found in...
the chemical composition of *E. gracilis* (Figure 11). Higher nitrogen content at lower light intensity might be attributed to the increased amount of chlorophyll contained in the individual cells. However, less total chlorophyll is formed at 100 ft.-c. than at intensities exceeding 100 ft.-c., because of the fewer cells present. More carbohydrate per cell is synthesized at higher light intensities, the maximum of carbon being contained in cells illuminated from 200 to 400 ft.-c. Ash content of the cells is at maximum, and hydrogen content at a minimum at 100 ft.-c.

Figure 11 shows that carbohydrate and fat tend to increase with increased light intensity, and that protein and ash tend to decrease. *Euglena* grown near optimum light intensities and at short retention periods may contain up to 50 per cent crude protein.

**Effect of Temperature**

In preliminary temperature studies to be reported later, the data indicate that algal population, chlorophyll content, assimilatory quotient, age of cells, and uptake of inorganic ions are comparatively independent of temperature near optimum temperature. However, the growth of bacteria, yield of algal cell material and oxygen, over-all sewage treatment accomplished, and uptake of organic ions may be appreciably influenced by slight changes of temperature. Jahn (11) has shown that the optimum temperature for *E. gracilis* when grown in acetate media in the dark is near 23° C.

**Summary and Conclusions**

In the laboratory, green algae have been employed to effect secondary sewage treatment through photosynthetic oxygenation. The physical and chemical environments act to determine the yield of algal cells and of oxygen. Under optimum conditions the algae cells remain young; that is, they are maintained in the logarithmic phase of re-production. Such cells have relatively high concentrations of chlorophyll and of protein, and low concentrations of carbohydrates and fats. Both algae yield and oxygen production are high, and the oxygen production exceeds the demands of all living organisms in the system.

Previous studies showed that, assuming favorable physical conditions, the availability of carbon is generally the limiting factor determining the yield of algae. As the loading rate decreases, and carbon becomes more limiting, cells are unable to multiply or multiply at a greatly decreased rate. Many of the cells become "old"; that is, they store up carbohydrates and fat from the limited amounts of carbon available. They contain lower percentages of chlorophyll, the yield of algae is reduced, and the oxygen produced may be less than the total demands.

The present studies show that optimum light intensity for the algal species *E. gracilis* depends upon such variables as strength of sewage and the retention period. For a natural sewage having a B.O.D. of 90 p.p.m. (Warburg, 5-day, 25° C.), the optimum light intensity is from 400 to 1,200 ft.-c. When light intensity increases beyond optimum, the cells undergo a transition from "young" to "old," which is somewhat comparable to that produced by a decreasing loading rate—that is, by a shortage in nutrients resulting from long retention periods. In both environments (constant light with varying retention period, or constant retention period with varying light intensity), the algal cells are subjected to comparable total light exposures; that is, the total exposure may be the principal factor determining the average cell "age," hence the yields of both algae and oxygen. For example, a low loading rate (long retention period) at a low lighting intensity can effect the same total exposure as a high lighting intensity at short retention periods. In natural bodies of wa-
ter, algae suffering from a shortage of nutrients take longer to reproduce. In becoming "older" they suffer prolonged exposure. High-intensity exposure could produce similar results, even when nutrients are abundant.

Light is both essential and inimical to photosynthetic oxygenation because of its effects on chlorophyll. It is essential to formation of chlorophyll, which in turn, under the influence of light, decomposes water, producing the hydrogen necessary to photosynthesis and, as an incidental result, the desired oxygen. However, the light intensity, the longer the period of exposure, and the greater the amount of chlorophyll present, the greater will be the rate at which the chlorophyll is broken down.

As light intensity becomes excessive, the rate of breakdown of chlorophyll approaches the rate of chlorophyll synthesis, resulting not only in bleaching and yellowing, but in reduced hydrogen supply and hence reduced photosynthesis. Reduced photosynthesis results, in turn, in a slower rate of multiplication and hence in "aging" of the cells. These results all tend to indicate that a period of intensive light followed by a dark period is beneficial if not vital to increasing the over-all rate of photosynthesis.

Variations in temperature appear to be principally important in their influence on rate of cell growth subsequent to initial photosynthesis, and hence upon total cell yield.

In summary, rates of cell multiplication, cell yield (including oxygen yield), chlorophyll synthesis, chlorophyll breakdown, and photochemical decomposition of water by chlorophyll may be considered as interdependent variables. Their particular interaction depends upon the physical and chemical environmental factors, especially availability of nutrients, light intensity, and temperature.

In actual oxidation ponds, all of these factors vary between wide limits every day. Moreover, at any given moment they are different at different places along the pond traverse. Because the algae are "ecologically versatile," they are able to adapt themselves to these changing conditions. However, if it proves feasible to control the environment of oxidation ponds to minimize unfavorable conditions, the efficiency of such ponds should be greatly improved.

Studies are under way, both in the laboratory and in a pilot-scale outdoor pond, to study the effects of light periodicity and of varying temperature. These will supplement the present work on light intensity and the earlier work, which investigated the nutritional factors.

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