Arch. Microbiol. 122, 77–84 (1979)

Archives of
Microbiology

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Anaerobic Heterotrophic Dark Metabolism in the Cyanobacterium *Oscillatoria limnetica*: Sulfur Respiration and Lactate Fermentation

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Abstract. The cyanobacterium *Oscillatoria limnetica*, capable of anoxygenic photosynthesis in the light with sulfide as electron donor can anaerobically break down its intracellular polyglucose in the dark. In the absence of elemental sulfur, the organism carries out lactate fermentation; in its presence, anaerobic respiration occurs in which sulfur is reduced to sulfide. Induction of anoxygenic photosynthesis or synthesis of new proteins is not necessary for either process. Cells adapted in the dark to sulfur reduction are capable of anoxygenic photosynthesis during a subsequent light period, unless protein synthesis has been inhibited during the dark incubation period.

Key words: *Oscillatoria limnetica* – Sulfur respiration – Lactate fermentation – Anoxygenic photosynthesis.

The cyanobacteria, though possessing prokaryotic cell structure, display a plant-type oxygenic photosynthesis in which CO_2 is fixed and oxygen evolved. Part of the fixed carbon can be stored as polyglucose in the form of α-granules. In the dark, many cyanobacteria can utilize this reserve for generation of the energy essential for maintenance purposes. The polyglucose is broken down to CO_2 via the pentosephosphate pathway, and the liberated reducing power used for the generation of energy by transfer of electrons via a chain of electron carriers to oxygen, the terminal electron acceptor (Fogg et al., 1973). A number of species are even able to use exogenous organic substrates for dark energy generation and grow chemoheterotrophically under dark aerobic conditions (Pan, 1972; Raboy et al., 1976; White and Shilo, 1975).

A great number of cyanobacteria, of different typological groups, have been shown to use sulfide instead of water as the electron donor in photosynthesis (Castenholz, 1976, 1977; Cohen et al., 1975b; Garlick et al., 1977). In this anoxygenic type photosynthesis, sulfide is oxidized to elemental sulfur which is excreted from the cells (Cohen et al., 1975a; Garlick et al., 1977). Photosystem II does not participate in the process (Oren et al., 1977). Anoxygenic photosynthesis has been demonstrated at least in one cyanobacterium, *Oscillatoria limnetica*, to permit growth in the light in the absence of molecular oxygen both in culture (Oren and Padan, 1978) as well as in its natural habitat, the Solar Lake near Elat, Israel (Cohen et al., 1977).

O. limnetica readily shifts from aerobic to anaerobic metabolism, and in fact may thrive for months under anaerobic conditions (Cohen et al., 1977). This raises the question whether the organism can generate energy under anaerobic conditions in the dark, thereby allowing its maintenance and survival during the night or when temporarily shaded. Until now such a mechanism has never been demonstrated in the cyanobacteria.

The present study reveals that under anaerobic conditions, endogenous polysaccharides, formed in the light, are broken down in the dark to CO_2 using elemental sulfur (the product of sulfide oxidation in the light) as the terminal electron acceptor, thereby reconverting it to sulfide. Furthermore, in the absence of elementar sulfur, or even concomitantly with sulfur respiration, *O. limnetica* is able to generate energy under anaerobic conditions by fermenting endogenous polysaccharides to lactic acid. In addition we demonstrate the existence of an oxygen-dependent dark respiratory mechanism, similar to that known in many cyanobacteria (Fogg et al., 1973; Raboy et al., 1976).

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Abbreviations. DCMU = 3-(3,4-dichlorophenyl)-1,1-dimethylurea; FCCP = Carbonylcyanide p-trifluoromethoxyphenylhydrazone; mgat = milligramatom; OD = optical density

0302-8933/79/0122/0077/$01.60

Methods

Cyanobacterial Strains and Culture Conditions. The *Oscillatoria limnetica* strain used (Cohen et al., 1975b) was grown aerobically in Erlenmeyer flasks of 2 l capacity filled with 500 ml growth medium as described previously (Oren and Padan, 1978). Growth in the presence of sulfide was carried out as before (Oren et al., 1977) in 150 ml glass-stoppered bottles, filled to capacity with the above medium, enriched with 3 mM $Na_2S \cdot 9H_2O$. *Plectonema boryanum* 594, obtained from the Indiana University Culture Collection (Bloomington, Ind.) was grown photoautotrophically as already described (Raboy et al., 1976).

Measurement of Dark Sulfide Production and Sugar Consumption. O. limnetica cells in the logarithmic phase of growth were collected by centrifugation (2 min, $2{,}000 \times g$) and resuspended (30—40 µg cell protein per ml) in growth medium containing or lacking sulfide, in completely filled stoppered 250 ml Erlenmeyer flasks. They were then incubated at 35° C for the periods indicated in light provided by 60 W tungsten lamps [incident light intensity 2×10^{-3} J cm^{-2} s^{-1} (2×10^4 ergs cm^{-2} s^{-1})]. *Plectonema* cells were incubated under similar light conditions at 26° C in a medium similar to that used for *O. limnetica*, but distilled water was used for medium suspension. When indicated, $NaH^{14}CO_3$ (Radiochemical Center, Amersham, England) (final specific activity, 0.26 µCi per µmol) was added at the onset of incubation.

Subsequently, cells (and free and adhering sulfur granules, if present) were collected by centrifugation (2 min, $2{,}000 \times g$), and resuspended (250 — 500 µg cell protein per ml) in aerobic or anaerobic medium in completely filled, rubber stoppered Erlenmeyer flasks of 10, 25 or 50 ml capacity. Alternatively, when indicated, cell suspensions (5 ml) in 15 ml vials sealed with sleeve-type rubber stoppers. were flushed with pure argon for 15 min in the dark in order to expel molecular oxygen. The cell suspensions were incubated further in the dark at the appropriate temperature. The absence of oxygen in the anaerobic systems was verified by the Winkler oxygen determination as modified for the presence of sulfide (Ingvorsen and Jørgensen, in press). When indicated, chloramphenicol (10 µg/ml), KCN (20 µM), D-glucose (55 mM), elemental sulfur (prepared by acidification of sodium thiosulfate (Roy and Trudinger, 1970) (about 20 mgat/l) or sodium thiosulfate (10 mM) were added to the cell suspensions, or magnesium sulfate omitted from the medium and replaced by an equivalent osmolarity of magnesium chloride. At different intervals, samples of cells and medium were assayed for sulfide, elemental sulfur, sugar content, lactate, cell protein and chlorophyll *a* (see below).

Analysis of Metabolites Excreted into the Medium in the Dark. Cells preincubated in the light for 6 h with sulfide and $NaH^{14}CO_3$ as described above were washed free of labeled bicarbonate, and resuspended in sulfide-containing growth medium. After 18 h dark incubation, the suspension was centrifuged and the supernatant medium was collected. A portion of the medium was acidified with concentrated HCl, followed by bubbling of air through the solution for 15 min. Other portions were treated with solid $BaCl_2$, added to a final concentration of 0.2 M and the resulting precipitate was removed by filtration through a Millipore filter (0.22 µ average pore size). Radioactivity was determined in 0.5 ml aliquots of medium, before and after these treatments, to which 4 ml Insta-gel scintillation coctail (Packard) was added. The samples were counted in a Packard Tricarb Scintillation Counter (efficiency 70 %). Cell radioactivity was counted in cell samples collected by filtration onto Whatman GF/C filters, washed with 20 ml cold trichloroacetic acid (10 %). After drying at room temperature, the filters were counted in a gas flow counter (Nuclear Chicago, model C-110B) (efficiency 20 %).

CO_2 photoassimilation measurements were performed as described earlier (Oren and Padan, 1978).

Sulfide was determined colorimetrically by the methylene blue method (American Public Health Association, 1971).

Elemental sulfur was determined according to a modification of the procedure described by van Gemerden (1968a): cells and sulfur granules, collected by centrifugation (10 min, $10{,}000 \times g$) were extracted for 20 min in 10 ml boiling absolute ethanol. After cooling to room temperature, readjustment of the volume to 10 ml and removal of cell debris by centrifugation, the extinction of the supernatant at 260 nm was measured and compared with that of standard solutions of sulfur in ethanol. The OD_{260} was corrected for extinction by chlorophyll *a*, using the ratio OD_{260}/OD_{665} determined for extracts of cells grown aerobically in the absence of sulfide. The pellet remaining after ethanol extraction and centrifugation was used for protein determination (Lowry et al., 1951).

Total reducing cellular sugar was determined with the anthrone reagent (Trevelyan and Harrison, 1952). If the medium was enriched with glucose, the cells were washed twice with glucose-free medium prior to assaying.

Polyglucose in the cells was determined with the glucostat reagent (Worthington, Freehold, N.J.) (Worthington Enzyme Manual, 1972) after hydrolysis of the cells for 120 min with 1 N HCl at 100° C, followed by neutralization with 1 N NaOH and removal of cell debris by centrifugation.

Poly-β-hydroxybutyrate was assayed according to Law and Slepecki (1961).

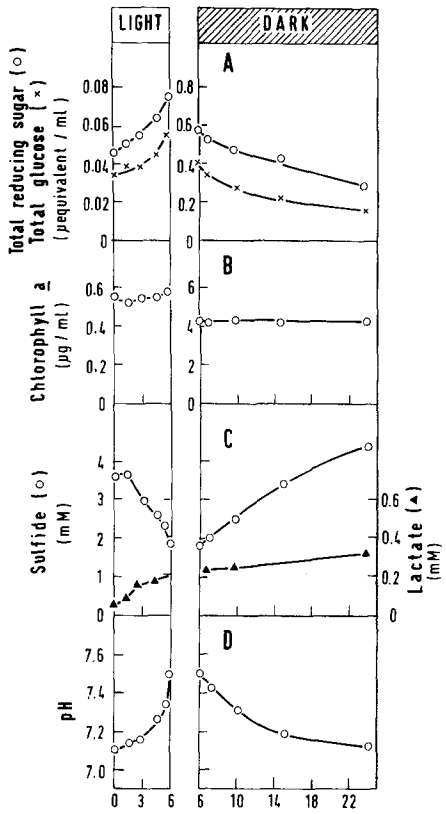
Chlorophyll a was determined in cells collected on glass filters (Whatman GF/C), extracted overnight with 5 ml methanol (4° C), then centrifuged (10 min, $10{,}000 \times g$) and the supernatant assayed (Mackinney, 1941).

Lactate was determined colorimetrically according to Nanni and Baldini (1964).

Results

In the light, under aerobic (water as electron donor) as well as anaerobic conditions (sulfide as electron donor), *Oscillatoria limnetica* accumulates large amounts of polysaccharides, which consist entirely of D-glucose units (Fig. 1). When cells loaded with polysaccharides during 6 h of anoxygenic photosynthesis were transferred to dark conditions together with the sulfur globules formed during the light period, the polysaccharides nearly disappeared from the cells within 20 h (Fig. 1). Microscopically, the cells appeared completely normal after 20 h dark incubation, i.e. the decrease cannot be accounted for by cell lysis. It may be calculated that under these conditions (anaerobic, dark) polyglucose is utilized by the cells at a rate of about 0.005 milliequivalent glucose per mg chlorophyll *a* per h. As the polyglucose disappeared, the concentration of sulfide in the medium increased; the number of sulfide molecules produced per net glucose unit varied in different experiments between 5 and 9. As Table 1 shows the sulfide formed is derived from elemental sulfur. In the dark, the pH dropped slightly (about 0.2 units per mM sulfide formed).

Poly-β-hydroxybutyric acid could not be demonstrated in the cells, whether light-grown, or after dark incubation.



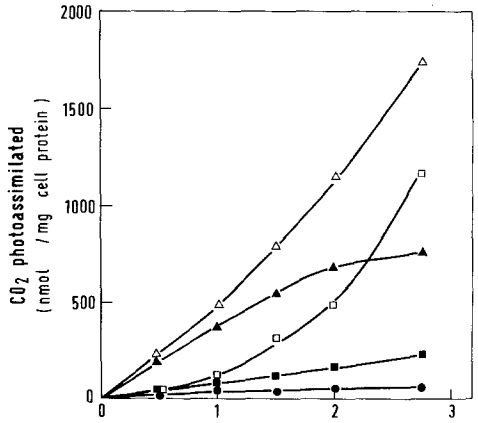


Fig. 1A—D. Light and dark metabolism of *Oscillatoria limnetica* in the presence of sulfide and elemental sulfur. Cells grown for 5 days in the presence of sulfide in the light were resuspended (0.5 μg chlorophyll *a* per ml) in sulfide-containing medium, and incubated in completely filled 250 ml stoppered Erlenmeyer flasks for 6 h in the light [light intensity 2×10^{-3} J $cm^{-2} s^{-1}$ (2×10^4 ergs $cm^{-2} s^{-1}$)]. The cells were then reconcentrated in their medium (4 μg chlorphyll *a* per ml) and further incubated in the dark in 10 ml aliquots contained in 10 ml stoppered vials. A different vial was used for each set of determinations. After different intervals of light or dark incubation, the parameters were determined as shown: total reducing cell sugar (**A**, ○), total cell glucose (**A**, ×); chlorophyll *a* content of cells (**B**), sulfide (**C**, ○) and lactate content of the medium (**C**, ▲), and pH (**D**) of medium

Fig. 2. Anoxygenic photosynthetic activity in *O. limnetica* cells incubated in the dark under various conditions. Aerobically grown cells were further incubated under aerobic conditions in the light for 6 h when they were heavily loaded with polyglucose. The cells were reconcentrated in their medium to a density of 4.2 μg chlorophyll *a* per ml, and incubated for 18 h in the dark: aerobically (□), after argon flushing and the addition of either Na_2S (1 mM), and elemental sulfur (20 mgat/l) (△), or Na_2S (1 mM), elemental sulfur (20 mgat/l), and chloramphenicol (10 μg/ml) (●). Subsequently the cells were suspended (cell density: 0.17 μg chlorophyll *a* per ml) in sulfide-containing medium (3.5 mM sulfide) in the presence of DCMU (5 μM) and $NaH^{14}CO_3$ (0.12 μCi per μmol) and incubated in the light in the absence (*open symbols*) or presence of chloramphenicol (10 μg/ml) (*closed symbols*). After different intervals, cell radioactivity was determined in 5 ml samples

Table 1. Elemental sulfur as the source of sulfide excreted in the dark by *Oscillatoria limnetica*. Cells grown on sulfide were incubated in the light (cell density 0.41 μg chlorophyll *a* per ml) in the presence of sulfide in sulfate-free or sulfate-containing medium. After reconcentration (to 5 μg chlorophyll *a* per ml) the cells were incubated in the dark. At the onset of dark incubation and after 18 h, sulfide, elemental sulfur and total reducing cell sugar were determined

Medium	Sulfate absent	Sulfate present
Decrease in S^0 (mgat/l)	4.1	4.1
Increase in S^{2-} (mM)	3.7	4.1
Decrease in cell sugar (mequivalent glucose per l)	0.72	0.72
Sulfide molecules formed per glucose units degraded	5.1	5.7

The fate of the disappearing polyglucose was determined as follows: cells were incubated for 6 h under anaerobic conditions (with sulfide) in the light (enabling cell growth as well as synthesis of storage sugars) and $NaH^{14}CO_3$ added. When the cells were subsequently washed and resuspended in similar medium but lacking radioactive bicarbonate, and incubated in the dark, 60% of the cell radioactivity was discharged into the medium. Apparently this radioactivity was derived from the storage polysaccharides: when cells grown in the presence of sulfide for 5 days at low light intensity 5×10^{-4} J cm^{-2} s^{-1} (5×10^3 ergs cm^{-2} s^{-1}) in the presence of labeled bicarbonate, and then in the absence of label for 6 h at high light intensity 2×10^{-3} J cm^{-2} s^{-1} (2×10^4 ergs cm^{-2} s^{-1}) (enabling accumulation of polysaccharides) a subsequent dark incubation of 18 h evoked only a 7% loss of cell radioactivity. As seen in Table 2, about 75% of the carbon atoms excreted into the medium was identified as CO_2 (precipitable by Ba^{2+} ions, disappearing from the solution upon acidification). The remaining fraction was unidentified, but might have been lactate: small amounts of lactate are often found upon dark incubation (Table 3). No cell growth was detected during the dark period, neither according to chlorophyll *a* content, nor by cell protein determination.

Though in the dark *O. limnetica* was able to utilize its internal polyglucose, externally added glucose (55 mM) proved ineffective: it did not enhance dark sulfide production, nor did it have any sparing effect for the 24 h duration of the experiment.

Dark sulfide production and polyglucose consumption were not inhibited by the photosynthesis inhibitor DCMU (5 µM), the phosphorylation uncoupler FCCP (10 µM) or the cytochrome oxidase inhibitor KCN (200 µM).

In the absence of elemental sulfur *O. limnetica* possesses two additional pathways for breakdown of its storage polysaccharides in the dark: aerobic respiration and lactate fermentation. In the absence of elemental sulfur and in the presence of oxygen cells light-loaded with polysaccharides were able to utilize them by aerobic respiration in the dark (Table 3). However, when cytochrome oxidase was inhibited by cyanide,

Table 2. Fate of the radioactive label from ^{14}C-labeled polyglucose in *O. limnetica* during dark incubation. Sulfide-grown cells were resuspended in sulfide-containing medium (at a density of 0.47 µg chlorophyll *a* per ml) and incubated for 6 h in the presence of $NaH^{14}CO_3$ (0.29 µCi per µmol) in the light. Subsequently, the cells were washed practically free of labeled medium, and resuspended at the same cell density in similar medium lacking labeled bicarbonate. The radioactivity of the cells and of the medium were measured at the onset of dark incubation ($t = 0$ h) and after 18 h

Cell radioactivity (per ml suspension)	
$t = 0$ h	264,500 dpm
$t = 18$ h	105,500 dpm
decrease	159,000 dpm
Medium radioactivity (per ml)	
$t = 0$ h	76,900 dpm (= 100%)
$t = 0$ h, acidified	430 dpm (= 0.6%)
$t = 0$ h, $BaCl_2$-treated	1,630 dpm (= 2%)
$t = 18$ h	212,200 dpm
increase ($t = 18$ h) − ($t = 0$ h)	135,300 dpm (= 100%)
idem, acidified	28,400 dpm (= 21%)
idem, $BaCl_2$-treated	37,300 dpm (= 26%)

Table 3. Dark metabolism of *O. limnetica* grown in the absence of sulfide. Aerobically grown cells were resuspended (0.3 − 0.5 µg chlorophyll *a* per ml) in growth medium, and incubated for 6 h in the light [2×10^{-3} J cm^{-1} s^{-1} (2×10^4 ergs cm^{-2} s^{-1})]. Subsequently, cells were concentrated to a final density of 3 − 4 µg chlorophyll *a* per ml, and 5 ml aliquots added to 100 ml Erlenmeyer flasks provided with a cottonwool plug (aerobic systems) or in 15 ml glass vials sealed with rubber stoppers (argon-flushed systems). When indicated, cell suspensions were flushed with argon in the dark for 15 min, and the various compounds added at the following concentrations: KCN (200 µM) chloramphenicol (10 µg/ml), $Na_2S \cdot 9\,H_2O$ (1 mM), sodium thiosulfate (10 mM) and elemental sulfur (about 20 mgat/l). The cell suspensions were incubated in the dark for 18 h. At onset and termination of dark incubation, total reducing sugar, lactate and sulfide were determined. Results presented are mean values from two or three experiments

Incubation conditions and compounds added	Molecules of lactate formed per glucose metabolized	Molecules of sulfide formed per glucose metabolized
Air	0	0
Air, KCN	1.5	0
Air, KCN, chloramphenicol	1.9	0
Argon	1.6	0
Argon, KCN	1.4	0
Argon, Na_2S	1.4	0
Argon, Na_2S, $Na_2S_2O_3$	1.7	0
Argon, Na_2S, S^0	0.8	6.2
Argon, Na_2S, S^0, chloramphenicol	0.3	7.6

and/or under anaerobic conditions created by flushing cell suspensions with argon, breakdown of polyglucose in the dark still occurred, lactic acid being the product. On the average 1.4–1.8 molecules of lactate were formed per glucose unit metabolized. In this respect. *O. limnetica* is exceptional among the cyanobacteria; *Plectonema boryanum* which is incapable of anoxygenic photosynthesis with sulfide as electron donor (Garlick et al., 1977) did not significantly break down storage sugars under anaerobic conditions or in the presence of cyanide and did not excrete lactate or sulfide.

When aerobically grown *O. limnetica* cells were supplied with elemental sulfur under dark anaerobic conditions, the two anaerobic mechanisms of polyglucose breakdown operated. It was not possible to replace the elemental sulfur with thiosulfate as electron acceptor in anaerobic respiration. Synthesis of new proteins was not necessary for the operation of both processes, both mechanisms were not inhibited by chloramphenicol and their activity was constitutive even in aerobically grown cells which have never been in contact with sulfide.

Theoretically, it can well be postulated that the enzyme accepting electrons from sulfide during anoxygenic photosynthesis is the same as the one responsible for sulfur reduction in the dark. In view of our earlier observation that anoxygenic photosynthesis is an inducible property in *O. limnetica* (Oren and Padan, 1978) we tested whether aerobically grown, dark adapted sulfur-reducing cells would be able to photoassimilate CO_2 without previous induction. Figure 2 reveals that cells producing sulfide in the dark were simultaneously induced to anoxygenic photosynthesis. However, if chloramphenicol was present there was no induction of anoxygenic photosynthetic activity.

Discussion

The present study shows that in addition to aerobic dark respiration widely found in cyanobacteria, the cyanobacterium *Oscillatoria limnetica* possesses two mechanisms of anaerobic dark energy formation. Thereby, intracellular polyglucose can either be fermented to lactate or respired with elemental sulfur as electron acceptor. This extends our knowledge of the metabolic capacities of *O. limnetica* to include dark anaerobic heterotrophic metabolism.

O. limnetica has proved capable of anaerobic photoautotrophic growth with sulfide as electron donor of photosynthesis (Oren and Padan, 1978), and has developed ways of anaerobic energy generation in the dark. Such energy generation is common in other types of anaerobic phototrophs. Thus, some of the red nonsulfur bacteria (Rhodospirillaceae) are able to ferment intracellular polysaccharides, *Rhodospirillum rubrum*, for example, excreting formate and propionate (Schön and Voelskow, 1976). As this type of metabolism yields very little energy in comparison to that derived from the aerobic combustion of polysaccharides, cells starve relatively quickly under dark anaerobic conditions (Breznak et al., 1978). Other types, such as *Rhodopseudomonas capsulata* are also capable of fermenting sugars (fructose) to lactate and acetate, yet the cells are unable to cope with surfeit electrons. Growth will occur only if exogenous electron acceptors such as dimethylsulfoxide (Yen and Marrs, 1977) or trimethylamine-N-oxide (Madigan and Gest, 1978) are supplied. It is moot question, however, whether they or other suitable electron acceptors are available to these organisms in nature, and lend this process ecological significance. Some purple bacteria possess a similar fermentation pattern, e.g. *Ectothiorhodospira shaposhnikovii* has been shown to ferment fructose to pyruvate, lactate, formate and traces of acetate (Kondratieva, 1976). A very interesting mechanism for dark generation of ATP, and somewhat similar to the process described in this work, is present in *Chromatium* (van Gemerden, 1968b): endogenous polyglucose, accumulated during light periods is oxidized in the dark to poly-β-hydroxybutyric acid, while sulfur, in the form of intracellular globules, serves as electron acceptor, being reduced to sulfide. This is a relatively efficient process, as on the substrate level, 3 molecules of ATP can be generated per glucose unit via reactions of the glycolytic pathway. There is also a possibility that electron transport from NADH to sulfur may yield additional energy (see below). Moreover, a great quantity of the energy conserved in poly-β-hydroxybutyrate is available to the cells during subsequent light periods. *O. limnetica* can not carry out this process: we were unable to demonstrate the presence of poly-β-hydroxybutyrate in this organism: neither in light-grown cells, nor after a dark period, not even in cells grown in the light in the presence of acetate. This is consonant with the relative insignificance of this storage material in cyanobacteria; until now it has been found only in the unicellular strain *Chlorogloea fritschii* when grown in the presence of acetate (Carr, 1966; Jensen and Sicko, 1971). Dark sulfide production from extracellular and intracellular sulfur is also known in other phototrophic green and purple sulfur bacteria in the course of their anaerobic fermentative dark metabolism (Larsen, 1953; Trüper and Pfennig, 1966). Even in typical aerobic eukaryotic algae, anaerobic fermentative dark metabolism is known (Gibbs, 1962). Thus, the unicellular green algae *Chlorella* and *Scenedesmus* are able to ferment glucose and other sugars in the dark, forming (depending on the strain investigated) lactic

acid or ethanol and CO_2. The rates of fermentation, inadequate for growth, may supply sufficient energy for maintenance purposes.

In the cyanobacteria, anaerobic respiration, yielding sulfide has been found earlier in the unicellular strain *Synechococcus lividus* Y52-s (Sheridan and Castenholz, 1968; Sheridan, 1973). In the dark, under anaerobic conditions, endogenous organic substrates are oxidized to CO_2 while sulfate or thiosulfate are used as terminal electron acceptors in respiration – sulfate being reduced to sulfide, and thiosulfate to sulfite and sulfide. Exogenously added substrates (glucose, sucrose) did not support anaerobic growth or sulfide production. The same organism was also able to generate sulfide in the light: under anaerobic conditions in the absence of CO_2, sulfate of thiosulfate could replace CO_2 as terminal electron acceptor of electrons extracted from water during photosynthesis. Dark sulfide production in *O. limnetica* differs from that in *Synechococcus lividus* in substrate specificity: *O. limnetica*, unable to utilize sulfate or thiosulfate as electron acceptors, uses only elemental sulfur for that purpose.

Until now the only organism known to obtain its energy from anaerobic respiration with sulfur as electron acceptor is the recently described *Desulfuromonas acetoxidans* (Pfennig and Biebl, 1976). This organism grows anaerobically, oxidizing acetate or ethanol to CO_2, and reducing elemental sulfur to sulfide. During the process one mol of ATP is formed per 6.86 mol electrons transferred from NADH to sulfur, the energy being inefficiently used for growth (a 5.56 g increase in cell dry weight per mol ATP consumed).

In contrast to *Desulfuromonas acetoxidans*, *O. limnetica* did not show measurable growth in the dark during sulfur reduction, i.e. neither cell protein nor chlorophyll *a* content increased (Fig. 1). This is understandable according to the following calculation: since in cyanobacteria the tricarboxylic acid cycle is incomplete, polyglucose can be broken down to CO_2 only via the oxidative pentose phosphate cycle (Fogg et al., 1973), no energy being afforded by phosphorylation at the substrate level. If all energy is derived from electron transport from NAD(P)H to elemental sulfur, but little energy can be generated as the $\Delta G'_0$ of the reaction $2[H] + S^0 \rightarrow H_2S$ is only -32.66 kJ per mol (-7.8 kcal per mol). The apparent inability of *O. limnetica* to utilize exogenously added glucose in the dark for growth makes it impossible to calculate growth yields under anaerobic conditions in the dark when the endogenous energy source is of necessity limited. If we assume that the efficiency of energy conservation is equal to that in *Desulfuromonas*, namely 6.86 electrons transferred per ATP formed (Pfennig and Biebl, 1976) we can expect the formation of 3.5 ATP molecules per glucose unit oxidized (24/6.86). This is very low compared to the theoretical yield of 36 ATP molecules formed per glucose unit broken down aerobically. If we assume that the energy generated is utilized for growth with the normal efficiency, $Y_{ATP} = 10.5$ g dry weight per mol ATP (in fact *Desulfuromonas* is much less efficient, $Y_{ATP} = 5.56$ g dry weight per mol ATP) (Pfennig and Biebl, 1976), we can expect an increase in dry weight of $3.5 \times 10.5 = 36.8$ g dry weight per mol glucose units oxidized. Assuming that the overall composition of structural cell material is $C_4H_7O_3$ (Harder and van Dijken, 1976), 41 g polyglucose can be assimilated per equivalent (= 162 g) of glucose units from the polyglucose oxidized (according to the equation $17\,C_6H_{10}O_5 \rightarrow 24\,C_4H_7O_3 + 6CO_2 + H_2O$), and 20.2% of the disappeared glucose units can be relocated as cell dry weight. In *O. limnetica*, cells loaded heavily with storage sugars contain about 2 mequivalent glucose units in the form of polyglucose per g of cell protein, or 1 mequivalent glucose units per g structural cell material dry weight (assuming that 50% of cell dry weight is protein, a common value in cyanobacteria) (Fogg et al., 1973). This can give rise to an increase of 33 mg dry weight, i.e. an increase of only 3.3%, undetectable by the methods of analysis used.

Our results (Tables 2, 3) show that some of the polyglucose used for dissimilation processes can also be fermented to lactate. Accordingly, the number of sulfide molecules produced per glucose unit decreases, as seen in Table 3. Following the above calculation, if only anaerobic respiration is operative, 20% of the polyglucose can be incorporated into cell material. If a part of the glucose is broken down to lactate via glycolysis this percentage will not change significantly, since 3 ATP molecules will be formed per glucose unit, a yield no different than that obtained by anaerobic respiration.

It was shown (Table 3) that the potential for anaerobic sulfur respiration is also present in cells not previously induced to anoxygenic photosynthesis, and does not require further induction of special enzymes. Moreover, as revealed in Fig. 2, cells adapted to sulfur respiration in the dark at the same time acquired the ability for anoxygenic photosynthesis in the light, no further inductive processes being necessary. On the other hand, when chloramphenicol was added at the onset of the dark period, though sulfur respiration and lactate fermentation were not at all inhibited (Table 3) anoxygenic photosynthetic activity did not appear (Fig. 2). These facts may tell us something as to the nature of the inducible factor necessary for anoxygenic photosynthesis (Oren and Padan, 1978): if indeed the same enzyme extracts electrons from sulfide during anoxygenic photosynthesis and returns the electrons back to sulfur during dark respiration, it must be constitutive,

and the inducible factor might be more indirectly connected with the photosynthetic system.

Whether the properties of lactate fermentation and anaerobic sulfur respiration are limited to *O. limnetica* (or all cyanobacteria capable of anoxygenic photosynthesis with sulfide as electron donor) or are commonly found among the cyanobacteria remains to be determined. Our preliminary results have shown that the unicellular strain *Aphanothece halophytica*, capable of anoxygenic photosynthesis (Garlick et al., 1977) can also produce sulfide in the dark.

Anaerobic respiration seems to be of ecological importance in the Solar Lake. Preliminary results (B. B. Jørgensen, J. G. Kuenen and Y. Cohen, unpublished work) have shown both a nocturnal increase in the sulfide concentration of the lake's upper hypolimnion layer unaccountable by diffusion of sulfide from the lower layers, and a decrease in the concentration of elemental sulfur. As all attempts to isolate *Desulfuromonas* from this region have as yet proved negative, the processes might very well be attributed to the large masses of *O. limnetica*, respiring in the dark with sulfur as electron acceptor. Indeed, as shown in Fig. 1, the polysaccharides accumulated in the daytime may suffice as energy source during the night.

The great metabolic flexibility displayed by *O. limnetica* including oxygenic and anoxygenic photosynthesis, as well as different pathways for aerobic and anaerobic dark energy generation, allow this organism to thrive in ecosystems of greatly fluctuating conditions.

Acknowledgements. We thank Dr. Y. Cohen, the Hebrew University of Jerusalem, for stimulating discussions, and Alexandra Mahler for help in the preparation of the manuscript. This study was supported by the German Federal Ministry for Research and Technology (GKSS).

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Received November 24, 1978