Carbon Monoxide in the Biosphere:
CO Emission by Fresh-Water Algae

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FINAL REPORT TO:

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Carbon Monoxide in the Biosphere: CO Emission by Fresh-Water Algae*

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SUMMARY

The chemical processes associated with the biosynthesis and degradation of the photosynthetic pigments in algae produce large amounts of CO. Senescent cells are found to produce CO in excess of that that can be accounted for only from degradation of chlorophyll indicating the existence of additional plant sources of CO. Our results indicate that plants may be the source of 10^8 tons or more of CO per year.

An accurate assessment of the role of carbon monoxide in the atmosphere requires that all large natural sources of carbon monoxide be detected and the magnitude of their CO contributions to the atmosphere be determined. The discovery of large and previously unknown natural sources of carbon monoxide would obviously have considerable impact on the generally held opinion that the carbon monoxide of the atmosphere is largely man-made (1). We believe, on the basis of experimental work described here, that the biogenesis, and, particulary the degradation, of photosynthetic pigments are such large and previously unsuspected contributors to CO in the atmosphere. The older literature contains intimations to this effect. Thus, there are reports that higher plants (2) and marine algae (3) evolve CO. Ocean waters appear to act as a natural source of CO (4,5). An atypical fresh-water blue-green alga has also been shown to produce CO (6). The relationship between these observations and the generality of the phenomenon have, however, not been made explicit.

There are at least two pathways by which CO can be produced by algae and higher plants. Blue-green algae have two characteristic photosynthetic pigments called phycocyanin and phycoerythrin, whose prosthetic groups, phycocyanobilin and phycoerythrobilin, result from the oxidation of a tetrapyrrole macronucleus such as chlorophyll (6). (See Figure 1 for the structural formula of

these tetrapyrrole compounds.) The chemistry of this process probably is similar to the conversion of the heme of hemoglobin in animals to the bile pigments, a process which, as is well known (7), produces one molecule of CO for each molecule of heme transformed to bile pigment. The macrocycle ring is opened by oxidation of the carbon atom at the α bridge position to yield a linear tetrapyrrole and a molecule of carbon monoxide. The chemical relationship between chlorophyll and algal bilins is very similar to that of heme and bile pigment, and each molecule of tetrapyrrole converted to bilin would produce one molecule of CO (6). Thus, as the blue-green algae grow, they evolve CO. It would be expected that as all blue-green algae contain phycocyanin and/or phycoerythrin, all would produce CO. However, under our culture conditions, production of CO by blue-green algae by this process turns out to be strongly species specific, and for no very obvious reason.

The degradation of chlorophyll in dying plant material is a second way for plants to produce CO, for the degradation of chlorophyll might also follow a course analogous to the degradation of heme. All photosynthetic organisms that fix carbon dioxide and produce oxygen, without exception, contain chlorophyll a. Thus, it might be supposed that the degradation of chlorophyll in algae and in higher green plants could produce large amounts of CO.

The work described here shows that the catabolism of chlorophyll in algae does indeed make a large contribution to the carbon monoxide content of the atmosphere. This is a previously unrecognized large natural source of CO that will have to be taken into account in describing the inventory of CO in the atmosphere. Our observations furnish an initial basis for the interpretation of the CO isotope studies of Stevens (8), which appear to require the existence of a large, unknown natural source of CO.

Techniques of Algae Culture

Both green and blue-green algae were cultured by the techniques described by DaBoll *et al.* (9) and Crespi *et al.* (10). The nutrient solutions contain a standard mix of salts (9) and carbon dioxide is fed to the cultures as a mixture of 5% CO_2-95% N_2. The feed gas is passed over Hopcalite (a manganese-copper oxide catalyst) to remove all traces of CO. The light intensity is controlled by means of neutral density screens. As a culture grows from a small inoculum, the light intensity is increased in steps of from 12.5 to 25 to 50 to 100 percent of the maximum light intensity. All cultures are thermostated for temperature control. Figure 2 is a photographic view of the "algae farm".

Analysis of CO, Chl, Bilin

An F and M Model 700 gas chromatograph with a flame ionization detector was modified for the detection of low concentrations of carbon monoxide (11). A gas sampling system was added so that the sample can be swept directly from a collection tube into the chromatograph gas carrier stream. A catalyst tube with furnace and hydrogen supply was placed bewtween the chromatographic column and the detector to convert CO to methane (CO may be determined directly with a thermal conductivity detector, but the sensitivity is low). A nickel catalyst is used for this conversion. Carbon dioxide is removed by a CO_2 absorant positioned before the sample collecting tube, and Linde 5A molecular sieve is used for the GC column packing to retain any residual trace of carbon dioxide and to separate methane, if any, from CO. An absolute sensitivity of 0.005 microliters (1 ppm CO in a 5 ml sample) is readily attained, with a precision of ± 10%. Our standard gas mixtures (Matheson) have also been analyzed manometrically. Chlorophyll was extracted and analyzed according to the methods of Strain and Svec (12) and phycocyanobilin was determined as phycocyanin (13).

Results and Discussion

The results of a survey for CO evolution of algae under routine culture in our laboratory are shown in Table 1.

Table 1. CO Production by Various Algae

Species	Type	Co Evolved
Phormidium luridum	Blue-green	+
Fremyella diplosiphon	Blue-green	-
Synechococcus lividus	Blue-green	-
Chlorella vulgaris	Green	+
Scenedesmus obliquus	Green	+

The blue-green alga Phormidium luridum produces CO at the rate of 100-300 μg per gram of new growth, a CO production rate that correlates with the rate of biosynthesis of the photosynthetic pigment phycocyanin. However, the blue-green algae Synechococcus lividus and Fremyella diplosiphon evolve CO at very much lower levels or perhaps not even at all. As all these blue-green algae contain phycocyanin, it would appear that either different biosynthetic pathways are used for the production of phycocyanin, or that the algae differ from each other in their ability to utilize endogenously produced CO. It is entirely possible that CO in these organisms is being fixed, as recent reports (14) indicated that many soil organisms are able to fix CO. The environmental impact of blue-green algae may thus involve both large-scale emission and fixation of CO.

Figure 3 is a plot that shows CO evolution by a culture of the blue-green algae Phormidium luridum. CO was evolved throughout the culture cycle at a rate dependent on light intensity and at a level consistent with the amount of CO expected from the extent of phycocyanobilin biosynthesis. An average of about 50 x 10^{-3} µl/min of CO were evolved during the approximately 160-hour lifetime of the culture. Integration over this time period gives a total of 480 µl or 600 µg total CO evolved by this culture. At harvest, our Phormidium cultures contain 6.0 grams of newly formed algae of which about 8 percent is phycocyanin. Since phycocyanin is 4 percent bilin by weight, the culture will have generated 19.2 milligrams of bilin from which 925 µg of CO is expected to be evolved. This number is somewhat higher than the measured value of 600 µg, but the CO that was evolved is probably due to bilin biosynthesis, as the rate of CO evolution appears related to light intensity is shown by Figure 3, and therefore to biosynthetic activity.

Figures 4 and 5 illustrate the growth and CO emission of Phormidium luridum under conditions similar to the cultures shown in Figure 3, but over a longer time period. As the culture became very dense, the rate of CO evolution increased to levels much higher than those expected from bilin biosynthesis only. A total of 7-10 x 10^{-5} moles of CO were emitted during the course of each of these experiments. About 2-3 x 10^{-5} moles of CO is

expected from bilin synthesis, which leaves an excess of 5-7 x 10^{-5} moles of CO. While this number corresponds clearly to the total amount of chlorophyll in these cultures, there is no evidence of chlorophyll degradation during the course of the experiment. These results indicate a source of CO other than bilin synthesis or chlorophyll degradation. It is possible that Ph. luridum, a filamentous organism, some form of cell senescence occurs throughout the growth cycle. It has been observed in this Laboratory (J. Norris, personal communication) that Ph. luridum cells often generate a very weak electron spin resonance photosignal, an indication of possible cellular degradation. We have also found that the "typical" results depicted in Figures 3-5 are not consistently reproducible, so that one must postulate processes involving carbon monoxide other than those associated with the simple scheme outlined for tetrapyrrole metabolism.

Figures 6, 7, and 8 show the results of monitoring the CO evolution by cultures of the green alga, Scenedesmus obliquus. Because green algae contain no bilinoid pigments, no CO is expected from this source, and no CO evolution is, in fact, observed until after about 450 hours of growth, when a dramatic output of CO begins. CO emission begins as soon as the algae go into the stationary phase of growth and begin to die. Table 2 shows the results of analysis of various portions of the curves shown in Figures 6, 7, and 8. During the initial phase of CO

Table 2. Correlation of CO Emission with Chlorophyll Disappearance in S. obliquus

Experiment	Culture Days	Total CO Emitted (moles x 10^5)	Decrease in Chlorophyll (moles x 10^5)
Fig. 5	3 (Days 23-26)	5.4	9.9
Fig. 6	6 (Days 23-29)	11.0	13.7
Fig. 6	7 (Days 29-36)	24	6.7
Fig. 7	2 (Days 23-25)	2.3	10.0
Fig. 7	3 (Days 32-35)	8.5	9.0

evolution and chlorophyll disappearance, the amount of CO evolved is equal to or lower than the amount of chlorophyll that has disappeared (on a molar basis). In the later stages of growth, however, considerably more CO is evolved than can be accounted for by chlorophyll degradation. The data of Figure 8 indicate

that CO evolution from S. obliquus is related to plant senescence. At day 26 the entire algal culture was centrifuged, washed and resuspended in fresh nutrient medium. Total chlorophyll content then increased and CO evolution decreased as the cells became more viable and at day 32 CO evolution abruptly increased as chlorophyll content went sharply down.

Figure 9 shows the CO evolved from a culture of Chlorella vulgaris as a function of growth and chlorophyll content. Unlike the other green alga, S. obliquus, the Chlorella culture emitted CO at a low level through most of its life cycle and there is no rapid emission of CO as chlorophyll content drops at senescence.

These data are consistent with the following interpretation: (1) the biosynthesis of bilinoid pigments in blue-green algae yields CO; (2) the degradation of chlorophyll in both blue-green and green algae yields CO; (3) an additional unknown source of CO exists in plants. It is possible that additional CO is evolved from the oxidation of phenolic types of compounds (15,16) as it has been shown that flavonoid degradation by molds yields CO (17). Figure 10 summarizes our results. The fact that some of the algae examined here do not emit CO may be due to the fact that CO can be fixed by many organisms (14). Thus, the algae may fix the CO that they generate, or the particular heterotrophic flora supported by these cultures may fix CO. Our results complement those of C. M. Stevens and co-workers (8) who have observed CO

emissions from trees. These workers have observed, in the northern hemisphere, an autumnal burst of CO that corresponds to an emission of the order of 5×10^8 tons of CO over a 1.5 month period. Fogg (18) estimates that the total annual world yield of photosynthesis to be 25×10^{10} tons of organic matter. If it is assumed that 0.5% of this organic matter is chlorophyll, chlorophyll degradation could yield 6×10^7 tons of CO per year. Bilin biosynthesis could account for about the same amount of CO per year, so these two sources could emit a total of 1×10^8 tons of CO per year. The data of Stevens et al. (8) indicate natural sources of CO in addition to bilin biosynthesis and chlorophyll degradation, and our data indicate that algae and other plants are contributing in further unknown ways to the large natural CO emissions.

REFERENCES

1. L. S. Jaffe, Ann. N. Y. Acad. Sci. 174, 76 (1970).
2. S. Wilks, Science 129, 964 (1959).
3. C. C. Delwiche, Ann. N. Y. Acad. Sci. 174, 116 (1970).
4. J. W. Swinnerton, V. J. Linnenbom and R. A. Lamontagne, Science 167, 984 (1970).
5. D. F. Wilson, J. W. Swinnerton and R. A. Lamontagne, Science 168, 1577 (1970).
6. R. F. Troxler, A. Brown, R. Lester and P. White, Science 167, 192 (1970).
7. T. Sjostran, Ann. N. Y. Acad. Sci. 174, 5 (1970).
8. C. M. Stevens, L. Krout, D. Walling, A. Venters, A. Engelkemeir and L. E. Ross, Earth and Planetary Sciences Letters, In press.
9. H. F. DaBoll, H. L. Crespi and J. J. Katz, Biotech. Bioeng. 4, 281 (1962).
10. H. L. Crespi, H. F. DaBoll and J. J. Katz, Biochem. Biophys. Acta 200, 26 (1970).
11. J. W. Swinnerton and V. J. Linnenbom, J. Gas Chromatog. 5, 570 (1967).
12. H. H. Strain and W. A. Svec, in The Chlorophylls, L. P. Vernon and G. R. Seely, Eds., Academic Press, N. Y., 1966.
13. H. L. Crespi, U. Smith and J. J. Katz, Biochemistry 7, 2232 (1968).

14. R. E. Inman, R. B. Ingersoll and E. A. Levy, Science 172, 1229 (1971).

15. S. Miyahara and H. Takahashi, J. Biochem. 69, 231 (1971).

16. T. Matsuura, H. Matsushima and H. Sakamoto, J. Am. Chem. Soc. 89, 6370 (1967).

17. D. W. S. Westlake, J. M. Roxburgh and G. Talbot, Nature 189, 510 (1961).

18. G. E. Fogg, Photosynthesis, The English Universities Press, Ltd., London, 1968.

FIGURE LEGENDS

Figure 1 -- Structural formulas of heme, bilirubin, chlorophyll *a*, phycocyerobilin, and phycoerythrobilin.

Figure 2 -- View of the algae farm showing Lucite rocking trays and associated equipment.

Figure 3 -- The production of carbon monoxide by the blue-green alga *Phormidium luridum*. Culture conditions are as described in the text, with 5% CO_2-95% N_2 being fed at a rate of 18 ml/min. As the light is stepped up in intensity, CO emission increases, a reflection of an increased rate of bilin synthesis. At about 95 hours, the lights were turned off, and at 100 hours, turned on again.

Figure 4 -- Growth and CO production by the blue-green alga *Phormidium luridum* under standard culture conditions, but with a 5% CO_2-95% N_2 feed rate of 9 ml/min. As compared to the culture described by Figure 1, there is a large CO output as the culture ages.

Figure 5 -- Growth, CO production, chlorophyll and bilin levels during the course of culture of *Ph. luridum* under culture conditions as for the culture of Figure 3. There are large CO emissions without concommitant disappearance of chlorophyll.

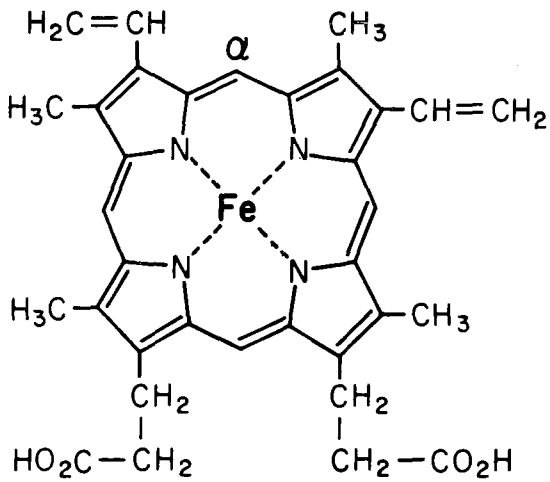
Figure 6 -- Growth, CO and chlorophyll production by the green alga Scenedesmus obliquus. CO production correlates with cessation of growth and a decrease in chlorophyll content of the cells.

Figure 7 -- Growth, CO and chlorophyll production by S. obliquus followed far into the stationary phase of growth. Even after disappearance of most of the chlorophyll, CO is being emitted at a rapid rate.

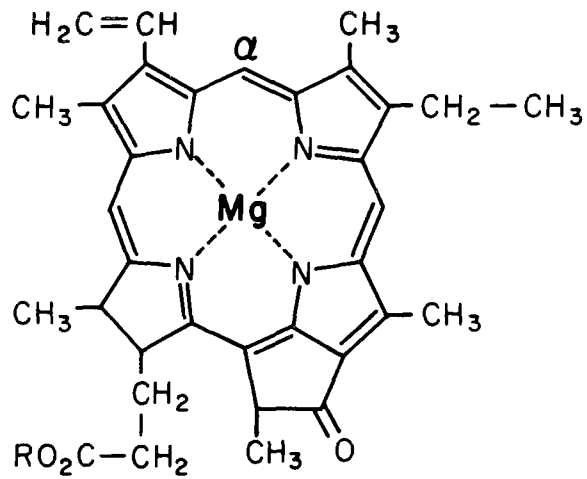
Figure 8 -- Growth, CO and chlorophyll production by a culture of S. obliquus thas was centrifuged, washed, and resuspended in fresh nutrient medium at day 26. Although the culture was in the stationary phase of growth at the time of resuspension, cell density increased further in the fresh medium, as did the chlorophyll content, and CO emission dropped. After a few days, the normal processes of senescence resumed.

Figure 9 -- Growth, CO and chlorophyll production by the green alga Chlorella vulgaris. Small amounts of CO are emitted throughout the life cycle.

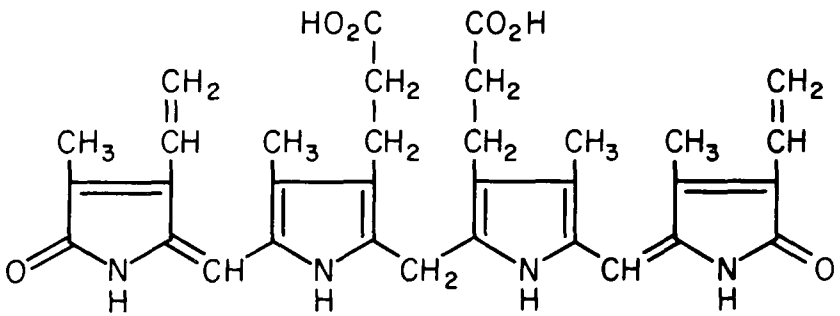
Figure 10 - A schematic picture of CO emission by algae.



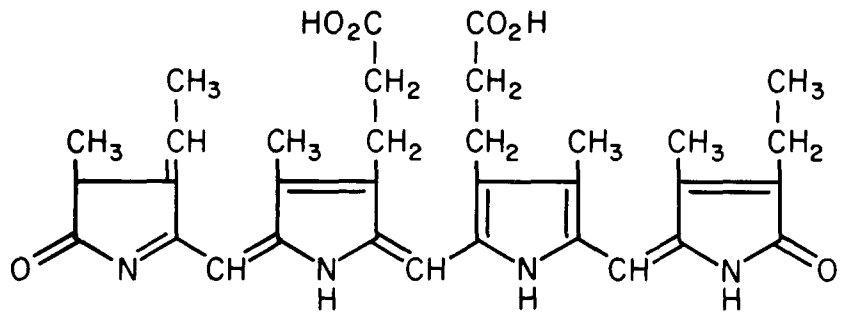
Heme



Chlorophyll



Bilirubin



Phycocyanobilin

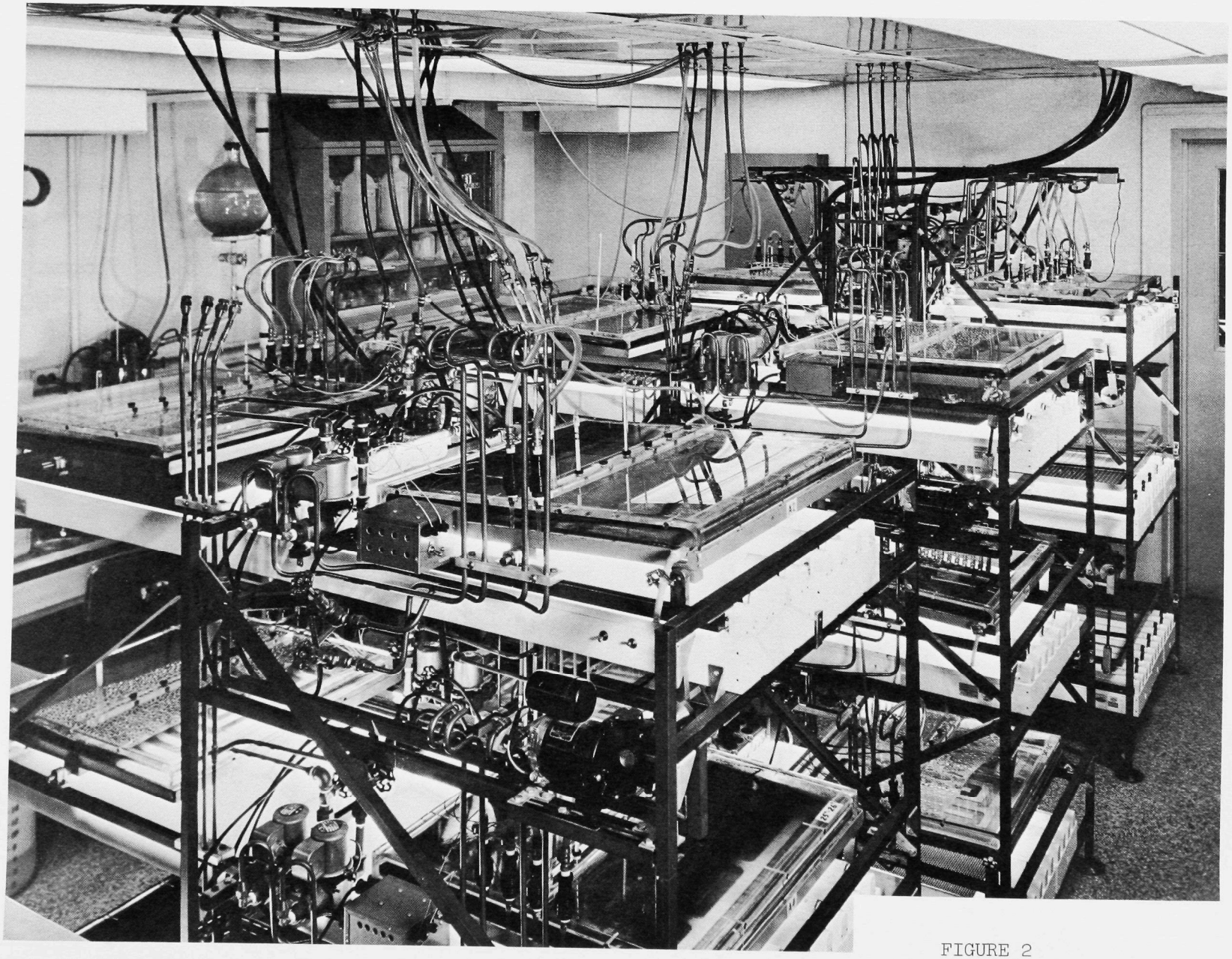
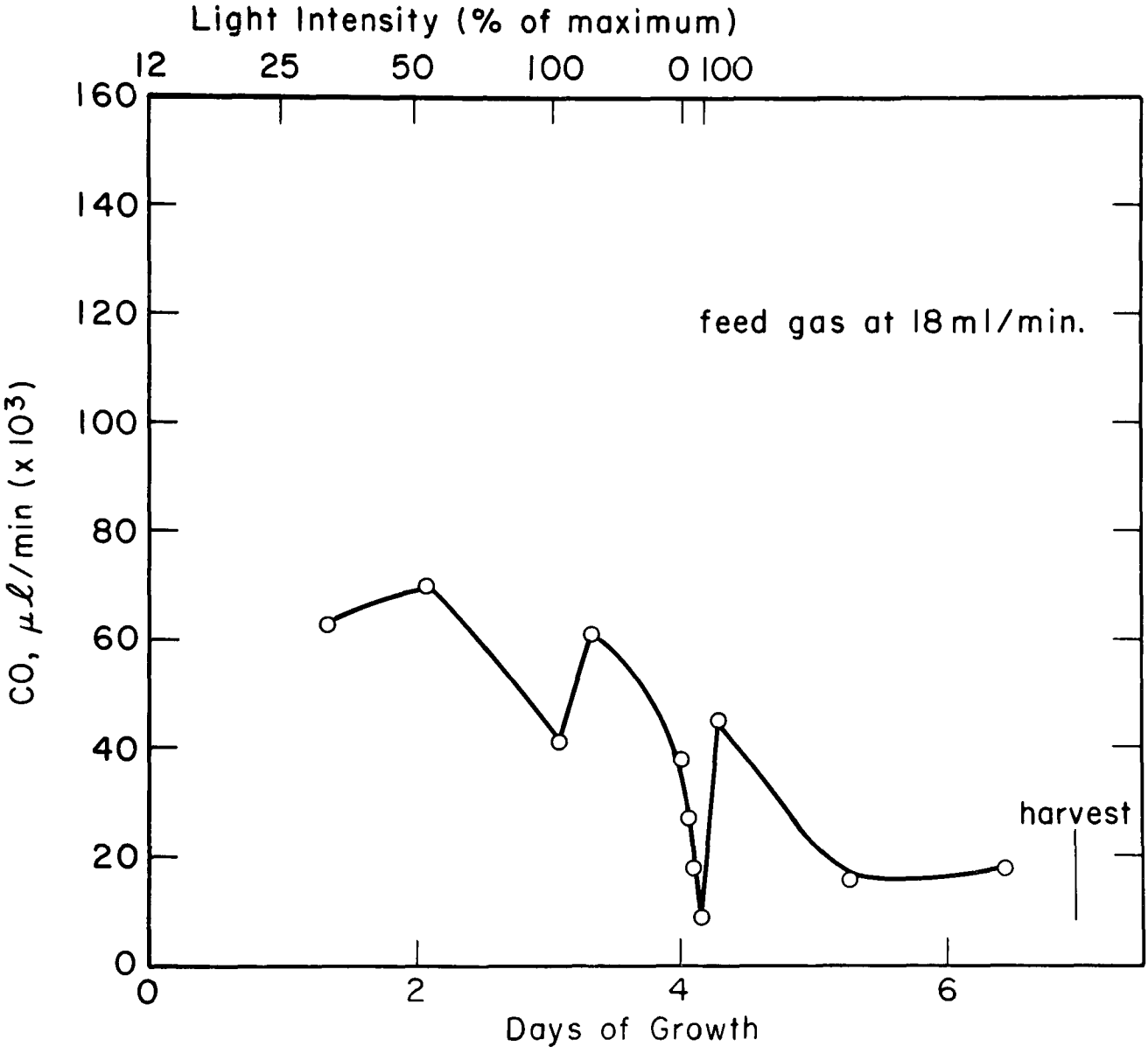
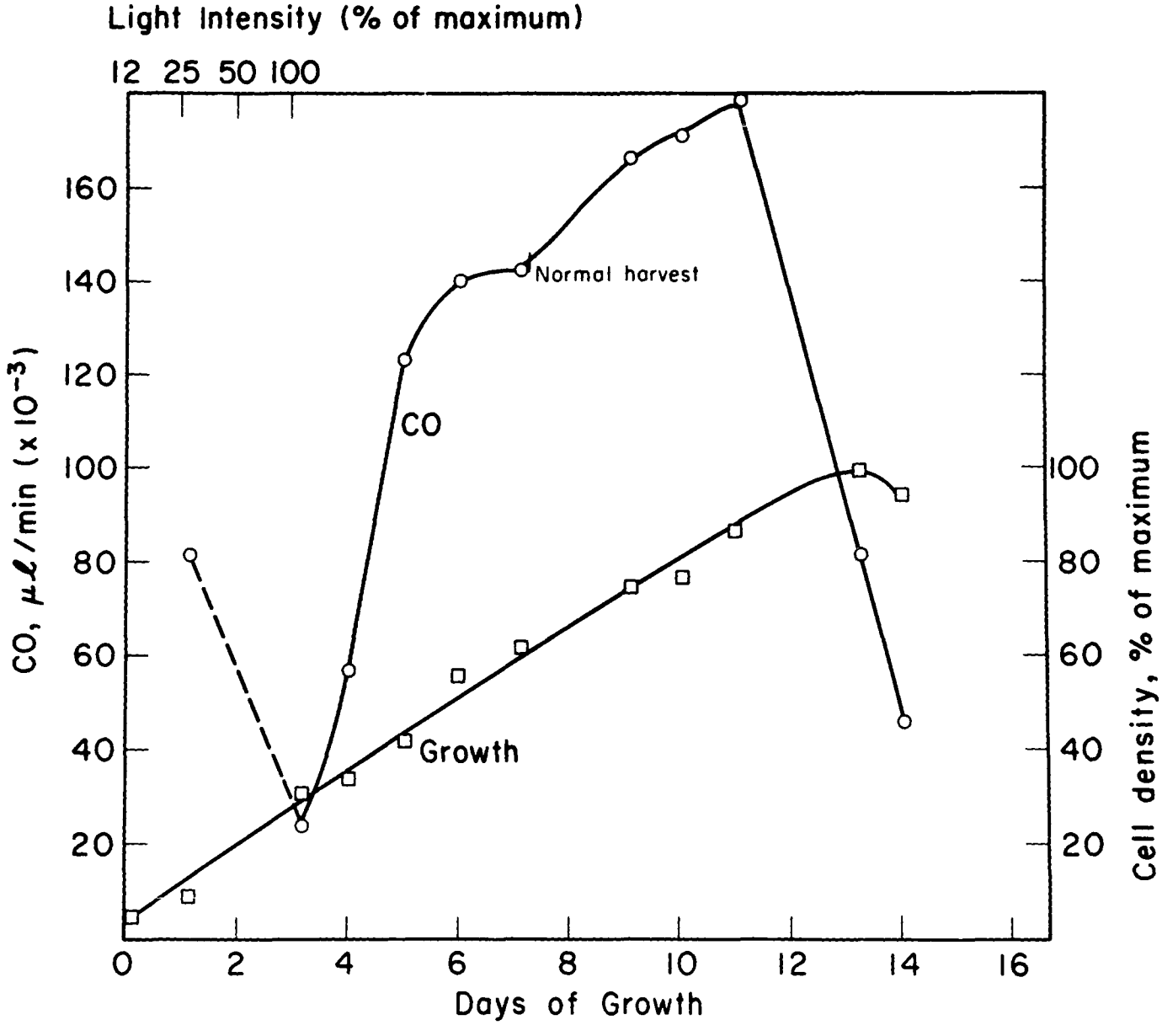


FIGURE 2



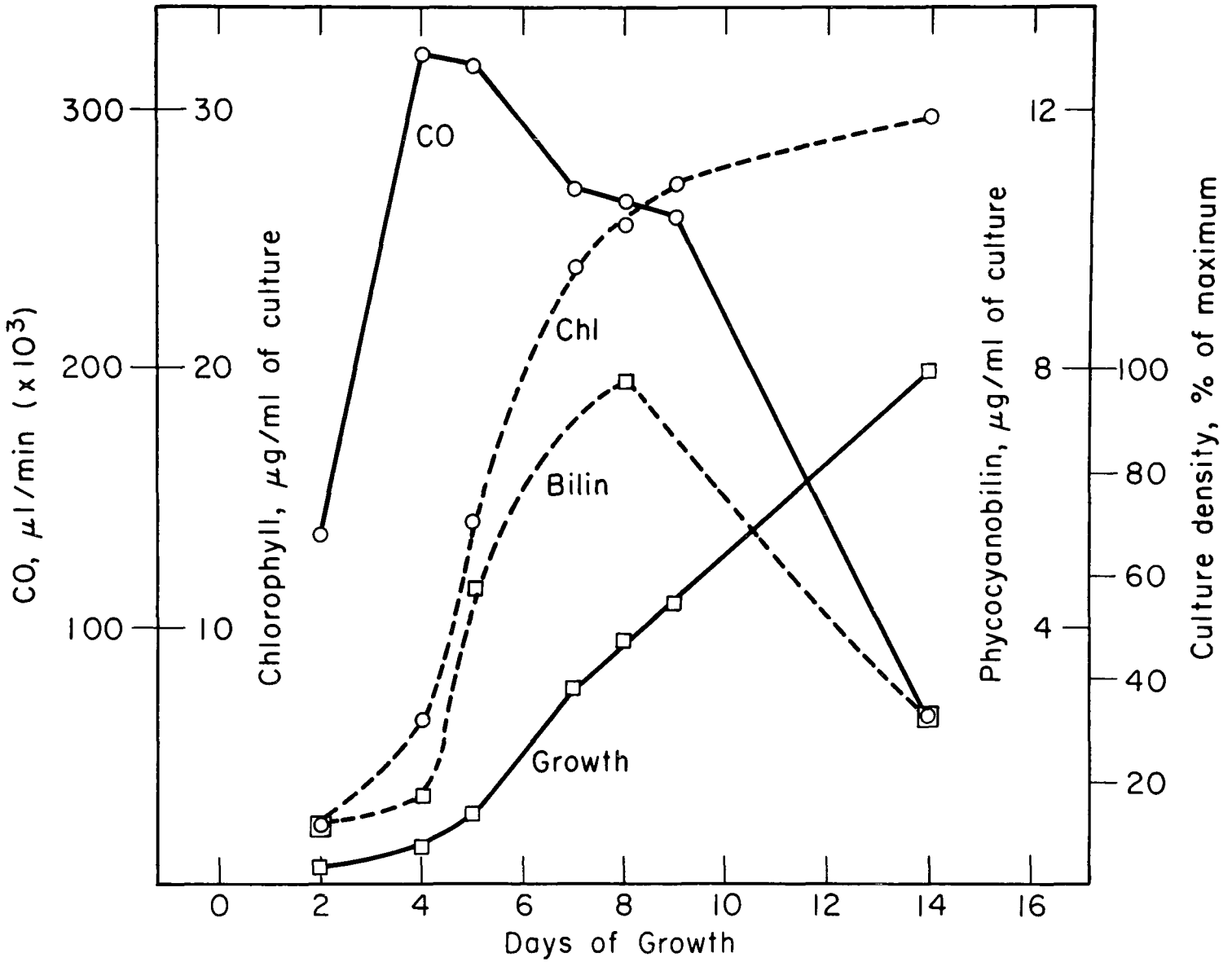
CO Production by Phoridium luridum

FIGURE 3



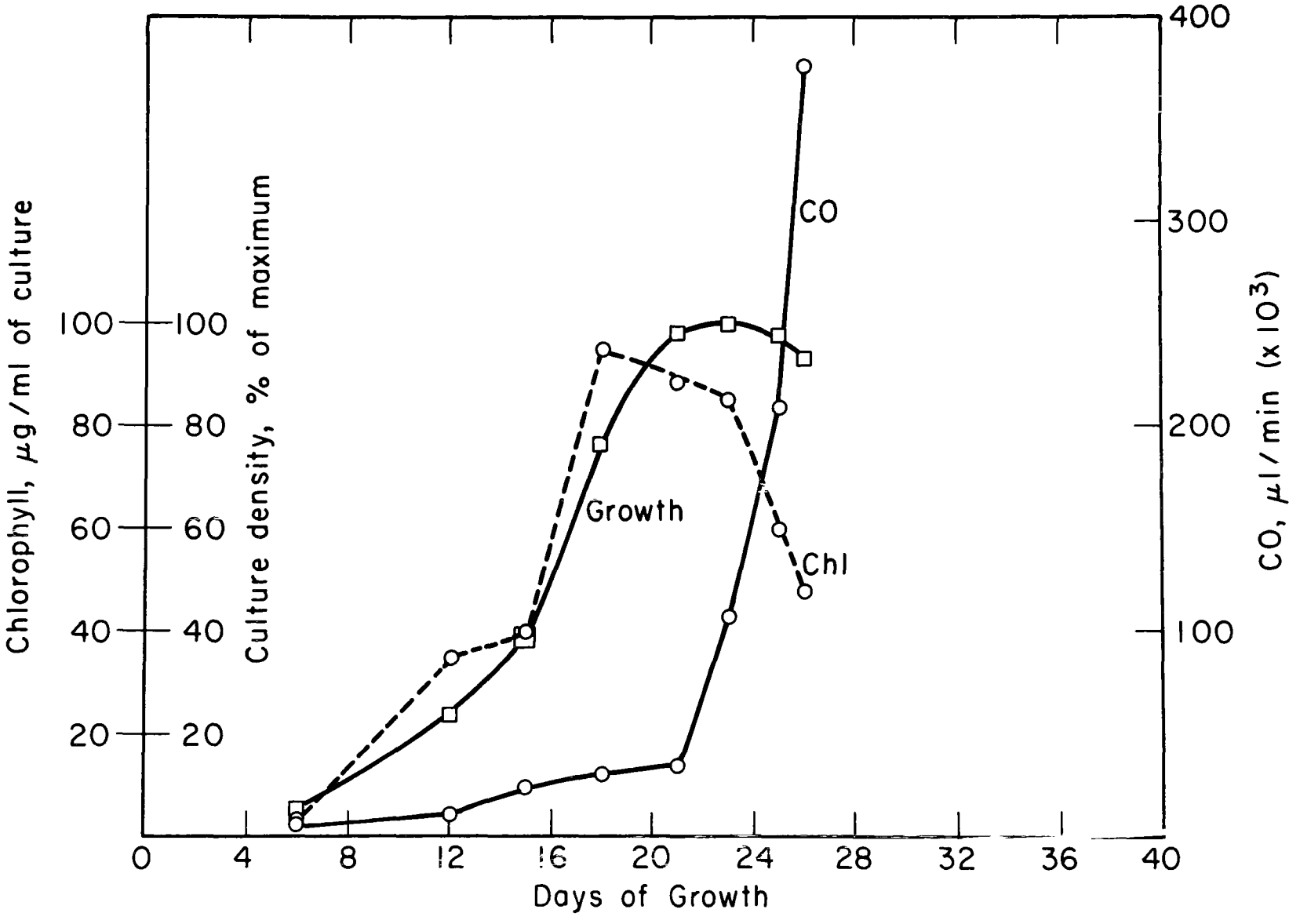
Growth and CO Production by Phormidium luridum

FIGURE 4



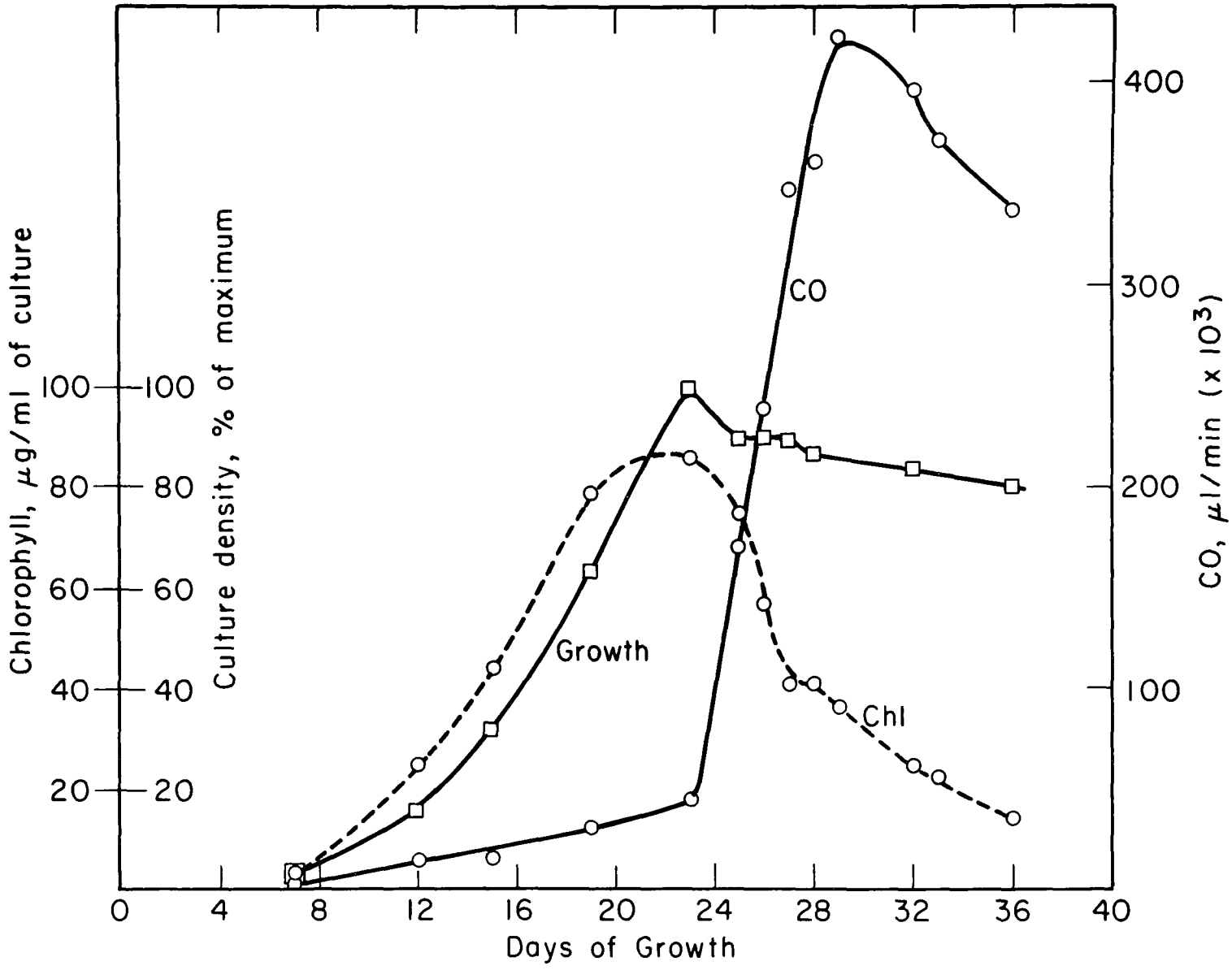
Growth, CO, Bilin and Chlorophyll Production by Phoridium luridum

FIGURE 5



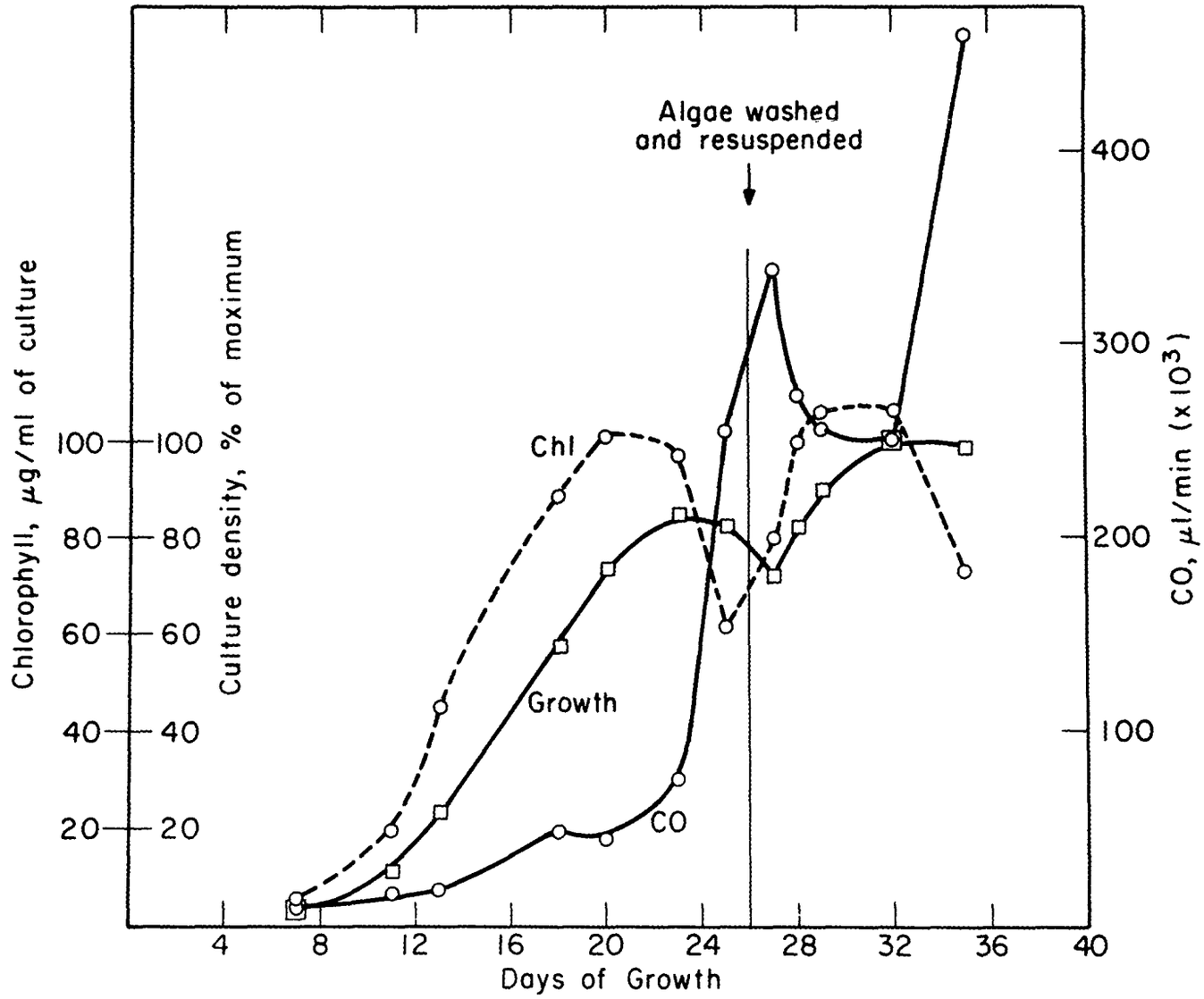
Growth, CO and Chlorophyll Production by Scenedesmus obliquus

FIGURE 6



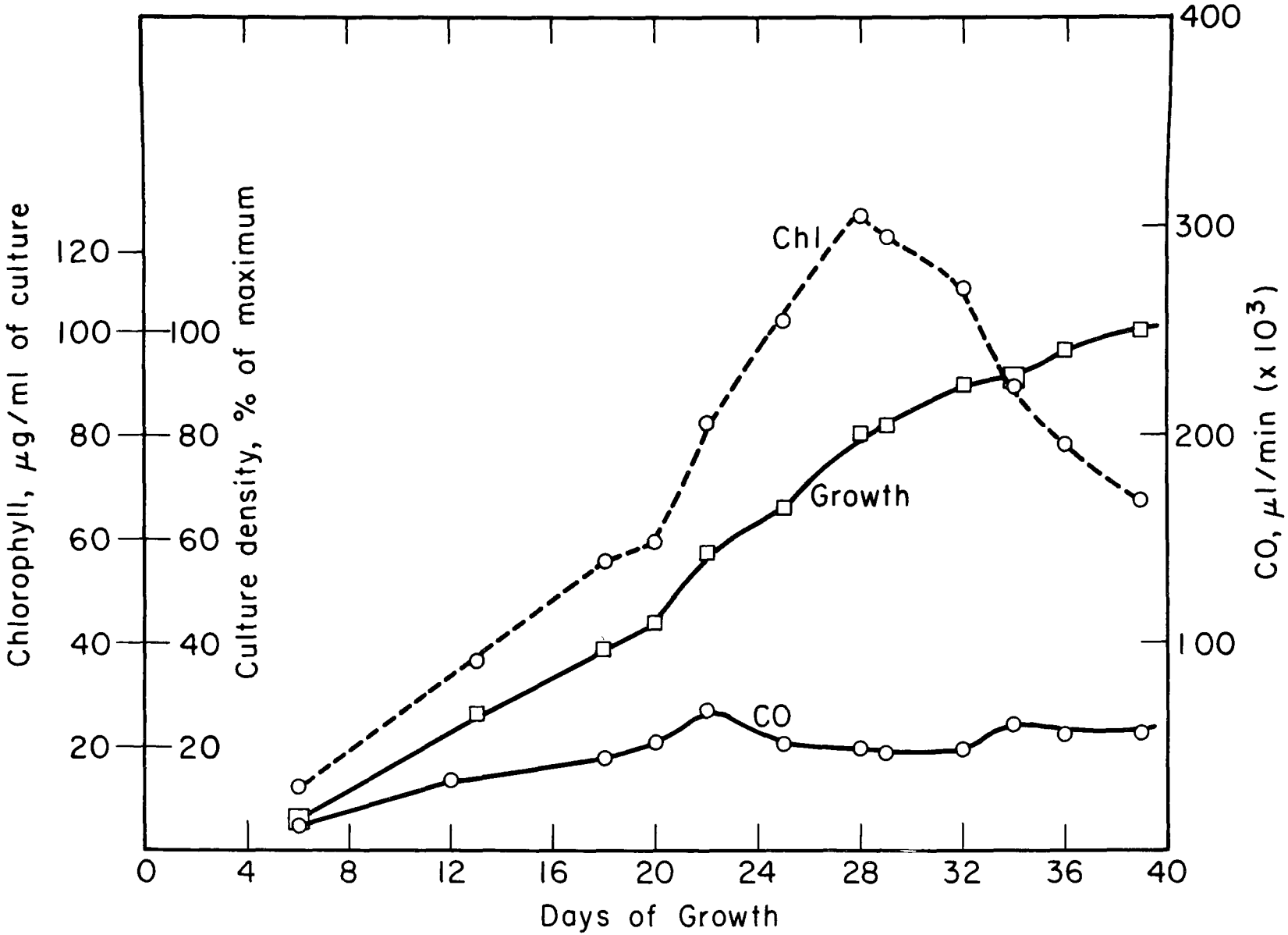
Growth, CO and Chlorophyll Production by Scenedesmus obliquus

FIGURE 7



Growth, CO and Chlorophyll Production by Scenedesmus obliquus

FIGURE 8



Growth, CO and Chlorophyll Production by C. vulgaris

FIGURE 9

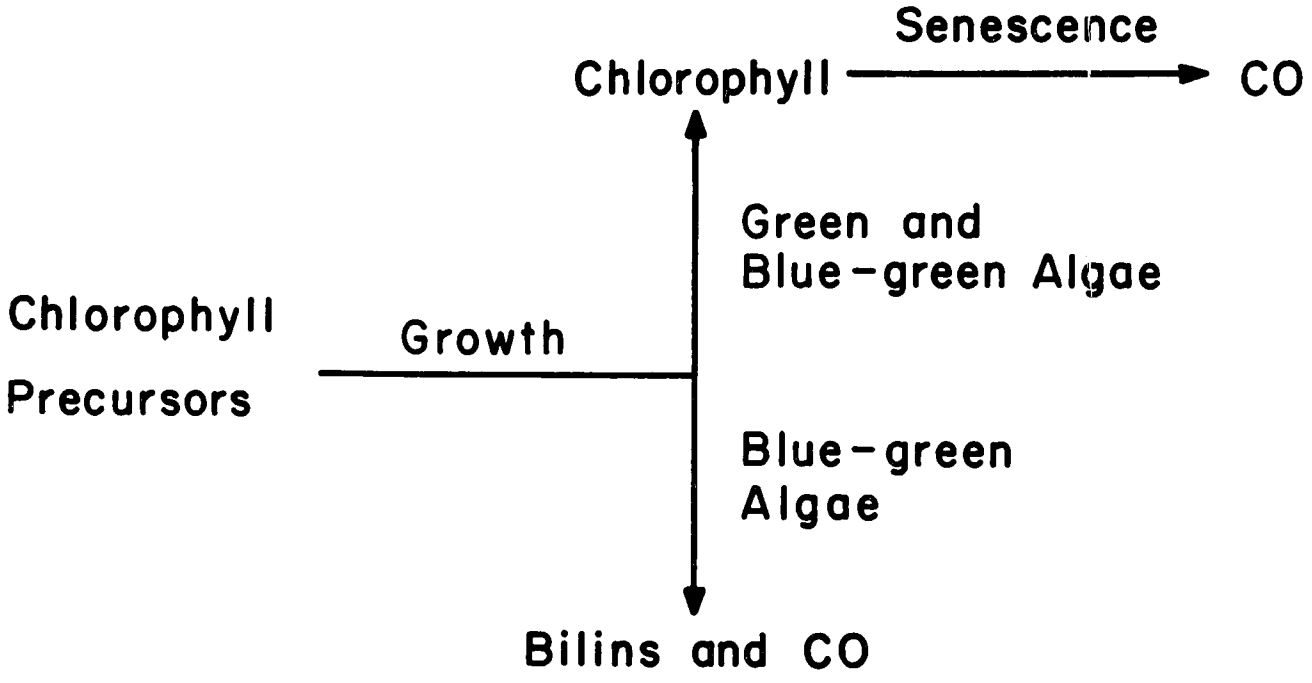

Senescence
Chlorophyll
CO
Green and
Blue-green Algae
Chlorophyll
Precursors
Growth
Blue-green
Algae
Bilins and CO

FIGURE 10