AD 680972

This document has been approved for public release and sale; its distribution is unlimited

AD______________

TECHNICAL REPORT
69-45-FL

SPECTRAL LIGHT REQUIREMENTS OF ALGAE

by

Thomas E. Brown

Charles F. Kettering Research Laboratory
Yellow Springs, Ohio

Contract No. DA 19-129-AMC-565(N)

Project reference:
1C014501A71C

Series: FL-82

October 1968

Food Laboratory
U. S. ARMY NATICK LABORATORIES
Natick, Massachusetts 01760

FOREWORD

These studies are a continuation of a project designed to investigate the physiological reflection of environmental change on a group of algae of diverse characteristics and taxonomic position. This is part of an overall program concerning the relationship of structure to function with specific reference to locations and roles of photoactive pigments.

Many different photosynthetic organisms successfully coexist in nature and the demand for these organisms as research tools has greatly increased in the past few years. This expanded interest has made it important to understand physiological adaptation of the living unit to environmental change. Yet, although the diversity of algae has led to many specialized investigations, only a limited number of algal types have been studied.

With reference to the specific area covered by this report, there are few standards on which to base experimental designs. Results are often biased by inadequate control of the test organism since in many cases algae are only "treated" with illumination of various wavelengths. Little is known of the actual effect of light quality on the potential levels of processes involved in algal physiology.

The work covered in this report was performed by the Charles F. Kettering Research Laboratory, Yellow Springs, Ohio under Contract Number DA 19-129-AMC-565(N). Dr. Thomas E. Brown was the Official Investigator.

The U. S. Army Natick Laboratories' Project Officer was Mr. Robert O. Matthern. The Alternate Project Officer was Dr. Mary Mandels.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF TABLES

LIST OF TABLES (Continued)

LIST OF TABLES (Continued)

LIST OF TABLES (Continued)

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

ABSTRACT

Seventeen species of algae representing ten taxonomic divisions were individually grown in white light and light of nine separate 10 nm bandwidths corresponding to the major absorption peaks of known photoactive pigments. Energy levels of the incident light were equalized through the entire series, approximating 15,000 ergs cm^{-2} sec^{-1}. Measurements of growth, pigmentation, photosynthesis, respiration, and where possible, morphology and structure were made following seven to ten days continuous exposure to the light regimes. The rates of photosynthesis and subsequent respiration were determined using the same full light regime as for growth. Light enhancement characteristics and wavelength requirements are shown for these parameters and compositions of specific illumination sources are suggested.

Introduction

The problem, as advanced in the Forward, is that of a lack of information surrounding physiological effects of environmental change. Light(duration, intensity, and quality), being the most important factor in the success of photosynthetic plants, is of primary concern. Earlier (10) we showed the influence of light intensity on the physiology of algae and a logical sequal was a study of the effects of light quality. The final results of such a study are reported here.

The objectives of this program are to provide information which can be used to standardize handling techniques for algae and to serve as the substrate on which experimental designs can be based. The information gained through light quality effect studies can be used in many ways:

1. to determine specific light requirements of measured parameters for individual algae.
2. to denote the biological significance of light absorbed by the different pigments.
3. to control algal response in specified areas.
4. to act as a guide in developing efficient illumination sources for use with specific pigment types or groups of algae.

The algal species chosen (Tab. I) were picked both for ease in handling and to represent differing pigment compositions since changes due to light must come about via light absorbing molecules. Specific absorption maxima for given pigments will vary between algal species and with culture conditions. The interference filters chosen for this work (Tab. II) were a best compromise anticipating these fluctuations.

Although published studies indicate considerable interest in effects of light quality on algal physiology, few direct comparisons have been made between species. The current study attempts to fill this gap.

Table I

Algal Species Studied

Division	Name	Color and Form
Bacillariophyta	Nitzschia closterium	golden, unicellular
Chlorophyta	Chlorella pyrenoidosa	green, unicellular
	Chlorella 7-11-05 (C. sorokiniana)	same
	Chlorococcum wimmeri	same
	Chlamydomonas reinhardi	same, motile
Chrysophyta	Ochromonas danica	golden, unicellular, motile
Cryptophyta	Cryptomonas ovata	brown, unicellular, motile
Cyanophyta	Gloeocapsa alpicola	blue, unicellular
	Phormidium luridum	blue, filamentous
	Phormidium persicinum	red, filamentous
Euglenophyta	Euglena gracilis	green, unicellular, motile
Phaeophyta	Sphacelaria sp.	dk. brown, branched filamentous
Pyrrophyta	Amphidinium sp.	graybrown, unicellular, motile
Rhodophyta	Porphyridium aerugineum	blue, unicellular
	Porphyridium cruentum	red, unicellular
Xanthophyta	Botrydiopsis alpina	green, unicellular
	Tribonema aequale	yellow green, filamentous

Table II

Transmission of interference filters* and corresponding major absorbing pigments

Transmission peak nm	Major absorbing pigments
405	Carotenoids
440	Chlorophyll a
** 520	Astaxanthin
540	Fucoxanthin-Peridinin-Phycoerythrin
560	Phycoerythrin
620	Phycocyanin
*** 630	Phycocyanin-Chlorophyll c
640	Chlorophyll c
650	Chlorophyll b
670	Chlorophyll a
*** 710	Far red chlorophyll et.al. forms
White	

* 2" x 2" Baird-Atomic, Inc. blocked against infra-red

** Used with Chlorococcum wimmeri only

*** One or the other used depending upon algal species

The following paragraphs cite and briefly describe the work of others pertinent to the subject area of this paper.

The influence of light quality on growth.

Many studies of individual algae have been made which sought the effects of wavelength on growth or cell division. Two studies, typical of these, spanning thirty years of investigation are Meier with Stichococcus bacillaris (31) and Kowallik with Chlorella (27).

The influence of light quality on pigments and pigment composition.

The visible tendency of some algae to chromatically adapt to differing qualities of light has been the cause of many studies concerned with pigmentation as light.

The works of Brody and Emerson (6) and Brody and Brody (7) show the changes in phycoerythrin/chlorophyll a ratio of the red alga Porphyridium cruentum when grown in various wavelengths of light. They suggest that the thalli of red algae, freshly harvested from their natural habitat, are relatively equivalent to green-light grown material (7). Although the resulting change in pigment composition speaks against classical chromatic adaptation, intensity of illumination must be considered (6).

Karlander and Krauss (24,25) studied Chlorella vulgaris grown in monochromatic light and found stimulation of pigment formation but not of growth, in comparison with dark grown controls. Apparently no comparison was made with cells grown in white illumination. Based on nutritional requirements, there is considerable difference between Chlorella species (36). This can be seen, in the present paper, to extend to wavelength response even though pigment compositions are essentially the same.

Although their test material was wheatroots, it is of interest to mention the work of Bjorn, et al (4) with wavelength response. Formation of chlorophylls a & b as well as cell length and number are correlated with wavelengths of irradiance. This is coupled with controls exposed to white illumination and dark. The effects appear to be exaggerated *Chlorella* responses.

With respect to blue-green algae, Hattori and Fujita (18) and Fujita and Hattori (15) have shown that, following strong white pre-illumination, chromatic light can direct the composition of pigments in *Tolypothrix tenuis*. They also show the order of wavelength effectiveness. Although the positive evidence presented here for chromatic adaptation is in opposition to the findings of Brody and Brody with *Porphyridium cruentum* (7), the methods used were significantly different. In addition, Jones and Myers (23) show that large changes in the Chlorophyll *a*/phycocyanin ratio of *Anacystis nidulans* can be produced by quality control of illumination.

The influence of light quality on photosynthesis and C^{14} fixation.

The most general phenomena with respect to wavelength influence on photosynthesis is that of the inefficient use of light absorbed by the red maxima of chlorophyll *a*. This anomoly has been discussed by Franck (14). Discussions of pigment-photosynthesis response to wavelength of illumination together with action spectra of photosynthesis by individual algal species, are scattered throughout the literature. Typical of the former is a discussion of *Cyanidium caldarium* response (M.B. Allen, 1) and of the latter, the photosynthesis action spectra of *Ulva lactuca* and *Trailliella intricata* (Halldal, 17) and of several marine species (Haxo and Blinks, 21).

Concerning C^{14} fixation, Cayle and Emerson (11), using *Chlorella*, found that greater specific activities in amino acids occurred with blue illumination. Hauschild, Nelson and Krotkov more thoroughly investigated this effect (19) and found that it is specific for blue light affecting both the rate of photosynthesis and the path of carbon whether it is given before or during the period of Ps measurement (20).

The influence of light quality on the enhancement of photosynthesis.

Thus far, all algal species studied for enhancement of photosynthesis, have not shown this phenomenon. Failures are unexplained except for the suggestion by McLeod (34) that it is due to adverse cell prehistory. Nevertheless with three different growth stages of Chlorella pyrenoidosa and C. vulgaris, he found enhancement but not with Amphora exigua, Navicula minma, or Ochromonas sp. Govindjee and Rabinowitch did find that Navicula minma exhibited enhancement (16). At low light intensities, McLeod (33) was able to show enhancement for Phormidium persicineum and Botrydiopsis alpina. The list of species exhibiting Ps enhancement has been increased by Fork (13) to include Cryptopleura crispa, Drouetia rotata, Endarachne binghamiae, Porphyra perforata, and Ulva sp. With some of these, enhancement is slight caused probably by the thick thalli of the multicellular marine forms.

The general mechanism of enhancement (as exemplified by Chlorella) has been elaborated by Myers and Graham (32) following the earlier observations of Emerson and Chalmors (12).

The influence of light quality on metabolic products.

That metabolism and the resulting products can be significantly altered by growth in different wavelengths is shown in the case of protein production by Chlorella pyrenoidosa (Kowallik, 28). In this instance, blue light (450-490 nm) provides the greatest enhancement of protein production.

The influence of light quality on respiration.

It was stated earlier, based on work with Chlorella, that: "No evidence for photostinclation of oxygen uptake was obtained in any experiment where photosynthesis was uninhibited", (A.H. Brown, 8). Since then light stimulated respiration of algae has been shown by several workers. Kowallik (29,30) recently produced action spectra for light enhancement of respiration of a yellow mutant of Chlorella vulgaris indicating blue illumination the most effective. Karlander and Krauss (26) have shown a light requirement for heterotrophic growth of the same species. In addition, Vidaver (37) has shown a similar action spectrum for *Ulva lobata* which he relates to pigment system I.

On the other hand, Hoch, et al (22) have shown an inhibition of oxygen uptake in *Anacystis nidulans* which is wavelength dependent.

Apparatus and Methods

The experimental design included duplicate measurements in addition to complete duplication of each growth series. Therefore a four-fold duplication of individual measurements were made for each alga.

A. Maintenance culturing:

Agar slants and liter liquid cultures of each alga were continuously maintained (Fig. 1,2). If supplementary gassing was necessary, algae were grown in 2800 ml low-form Pyrex culture flasks with built in side arm and gassing tubes, otherwise either 2800 ml Fernbach or two liter wide-mouth Erlenmeyer flasks were used (Fig. 3). Cultures were agitated on platform shakers illuminated from below by Westinghouse daylight fluorescent lamps with intensity controlled by neutral plastic screening. The temperature was held at 25°C. by baffled room air conditioning units. Growth atmospheres of either 1 or 5 percent CO_2 in air were provided from high pressure tanks premixed to an accuracy of > 0.1 percent. Culture media, conditions of culturing and sampling data are indicated on the culture sheets for each alga. "Soda bar" arrangement for media preparation is shown in Fig. 4.

B. Monochromatic light culturing:

No single, practical source of illumination was found which could allow continuous operation (7-14 days) at high intensities through the entire visible spectrum. Therefore, two sources and two polychromators were designed:

1. Source for blue and green wavelengths: GE A-H6 high pressure mercury lamp, water cooled.
2. Source for red wavelengths and white light: GE DGH 750 W projection lamp, air cooled.

Figure 1: Storage and Maintenance Facilities for Algae on Agar Slants

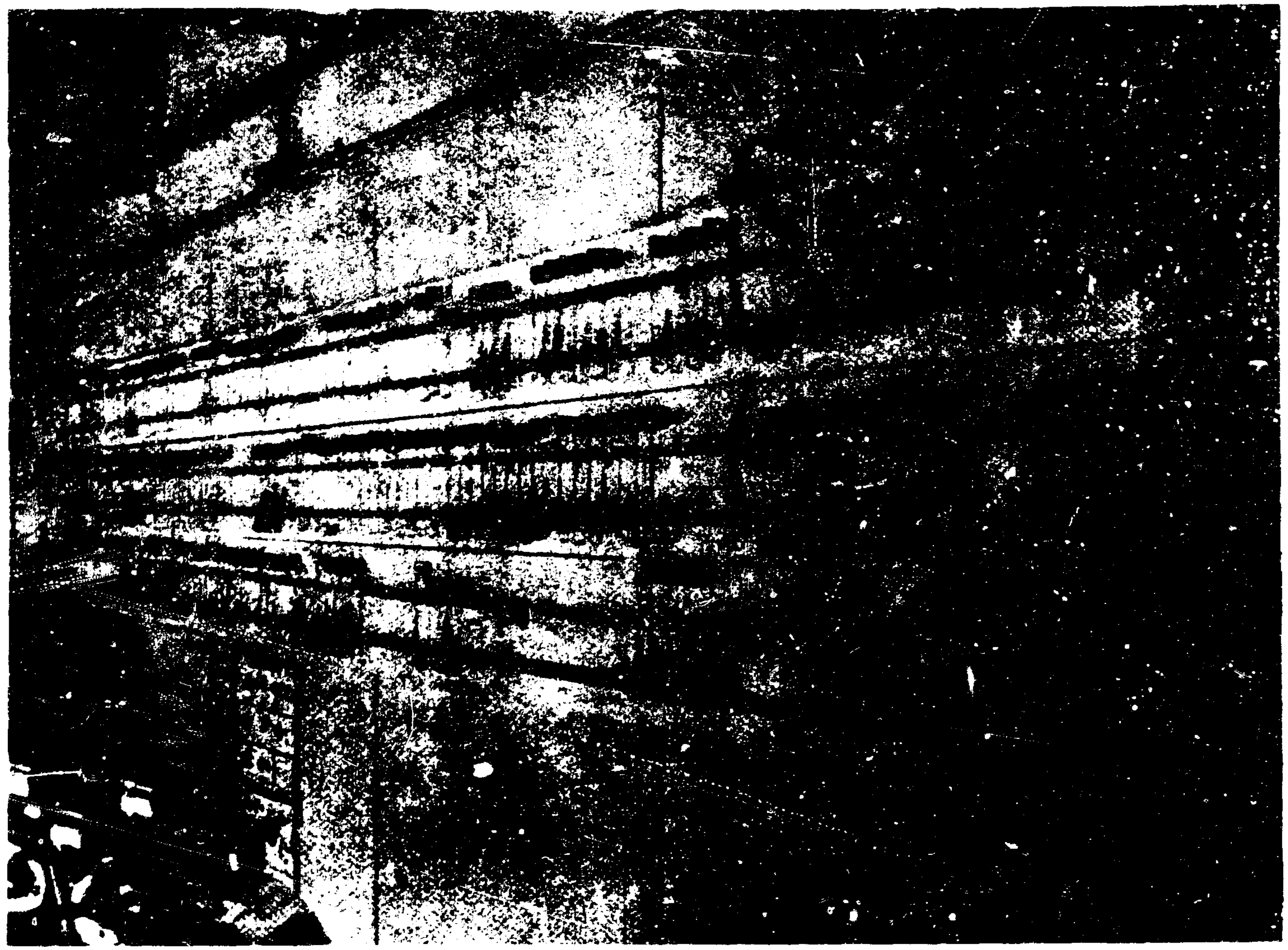


Figure 2: Maintenance and Experimental Facilities for Shake Cultures

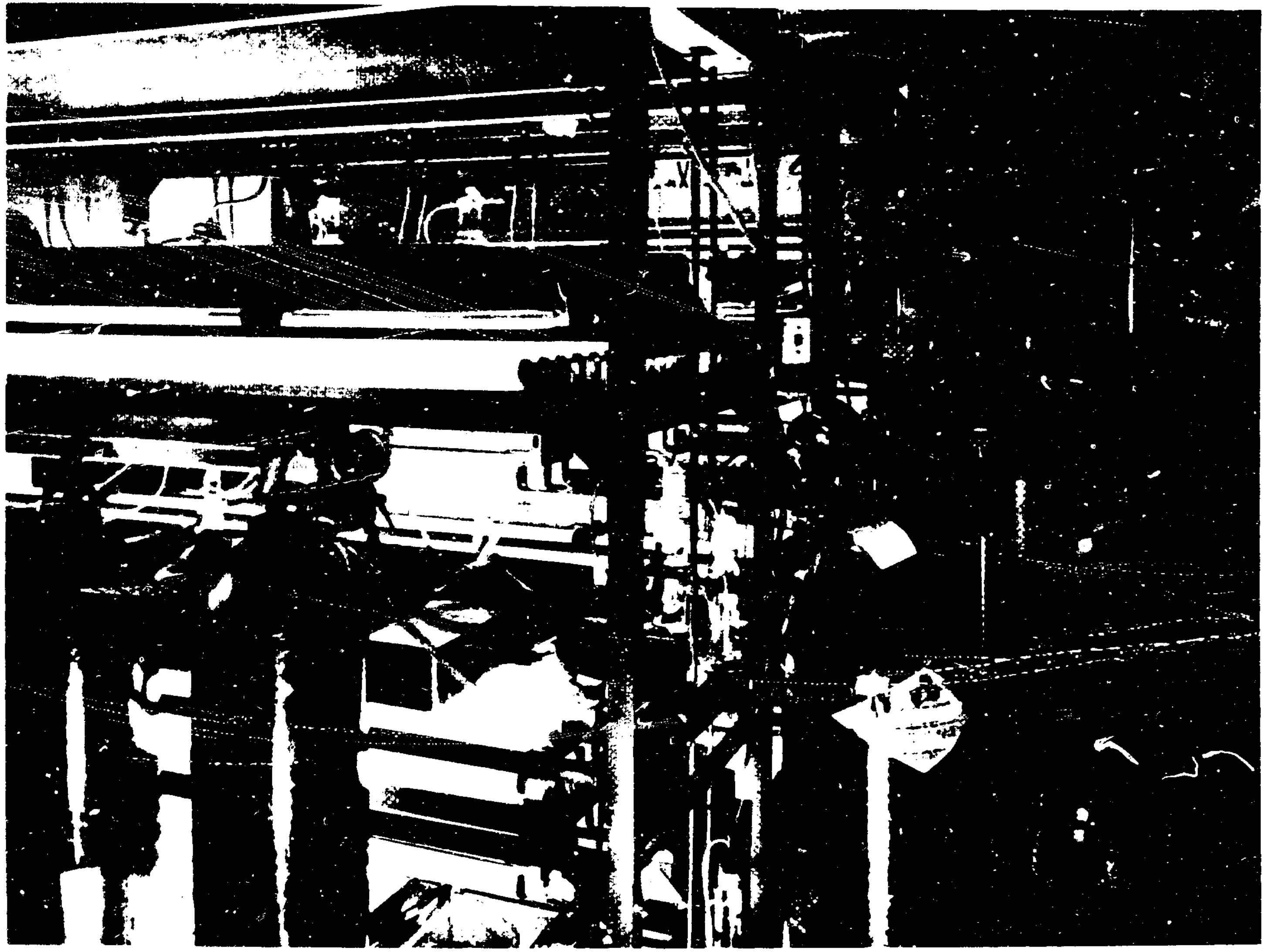


Figure 3: Culture and Harvesting Glassware

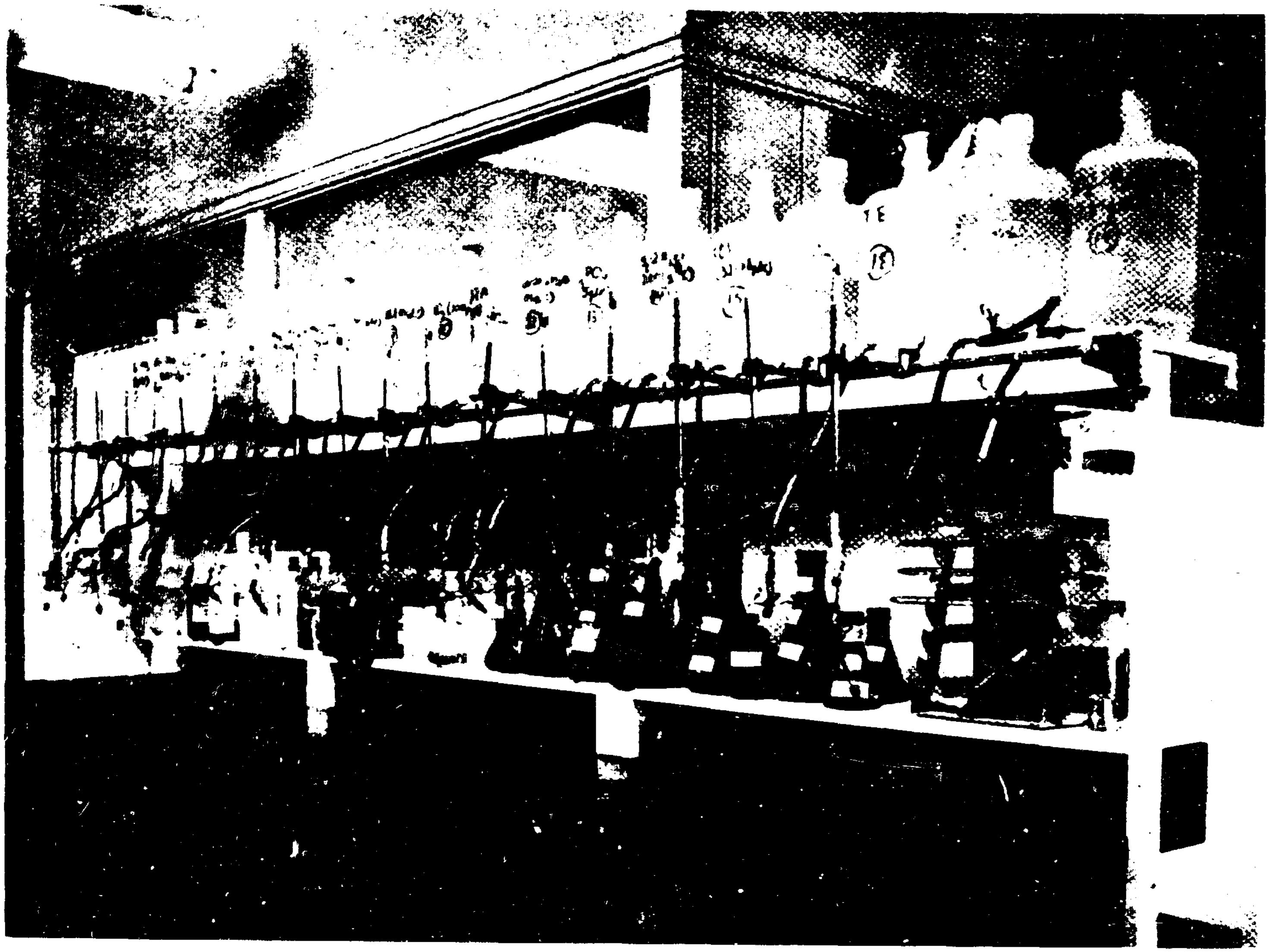
A. Fernbach Flask-Non Gassing

B. Gassing Flask

C. Experimental, Light Exposure Flask

D. Bauer and Schenk Tube and Holder

Figure 4: "Soda-Bar" Arrangement of Stock Solutions for Preparation of Culture Media



The polychromators and accessories (Fig. 5,6,7) allowed wavelength isolations and provided gassing facilities and aggitation with magnetic stirrers. Since decay of the mercury lamp was more rapid than the tungsten source, the latter polychromator was geared through an automatic variable resistance coupled with photosensors to allow artificial decay at the same rate thereby continuously equalizing the light intensities during the full experimental run. Initial intensities were equalized by use of neutral density filters and varying distance from the light source. Absolute intensities and intensity monitoring was accomplished by the use of a Y.S.I.-Kettering Radiometer (Y.S.I. Model 65). This instrument reads directly in $ergs/cm^{-2}\ sec.^{-1}$ independently of wavelength (see Fig. 11). Wavelengths were segregated by use of interference filters listed in Table II.

Custom made, (Kontes) 500 ml flasks were inoculated from maintenance cultures. These flasks were designed with flat sides for illumination, a well to house a magnetic stirring bar and gassing tubes separable for ease in cleaning (Fig. 3,8). The algae were grown with continuous stirring, and if needed, gassing in monochromatic and white light of equal energies incident on the samples. The period of time chosen allowed several generations to occur in the moderately growing samples yet without allowing either too heavy growth in the rapidly growing samples, or cell breakdown in the slow or non growing samples. All strains were grown at 25°C.

Growth was ascertained both by cell counts and packed cell volumes. Cell counts were made using a Coulter Electronics Model B Counter (Fig. 9). Packed cell volumes were found using 3 ml aliquots in graduated Bauer and Schenk cerebrospinal protein centrifuge tubes (Fig. 3). Centrifugation to constant volume was accomplished at 1900 x G for 60 min.

Pigment determination:

A. Spectroscopy:

Absorption spectra were obtained with a Cary 14 recording spectrophotometer equipped with scatter attachment (Fig. 10). Results using this unit

Figure 5: Overall View of Mercury and Tungsten Source Polychromator

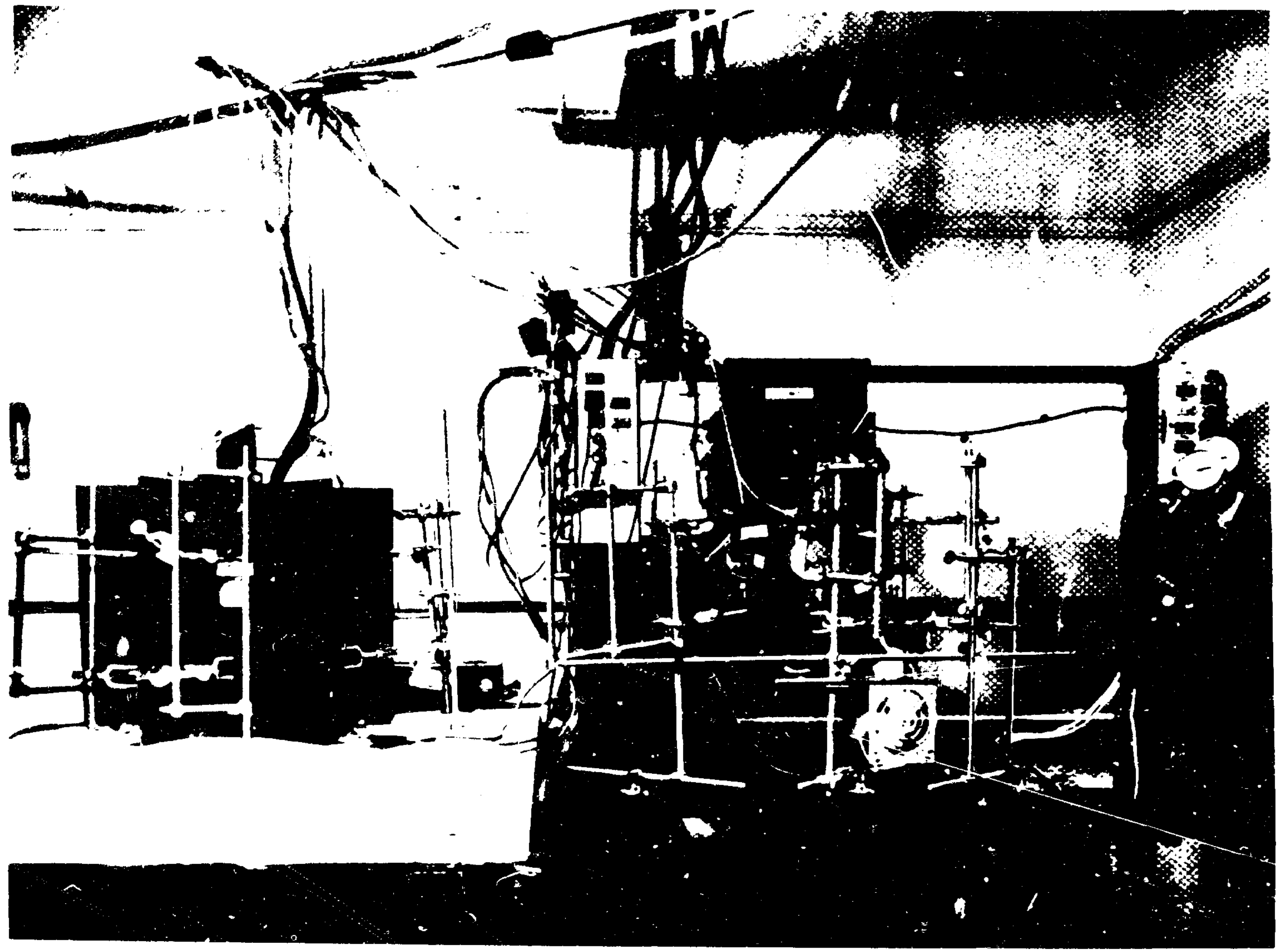


Figure 6: Closeup of Mercury Source, Water-Cooled Polychromator for Blue and Green Wavelengths

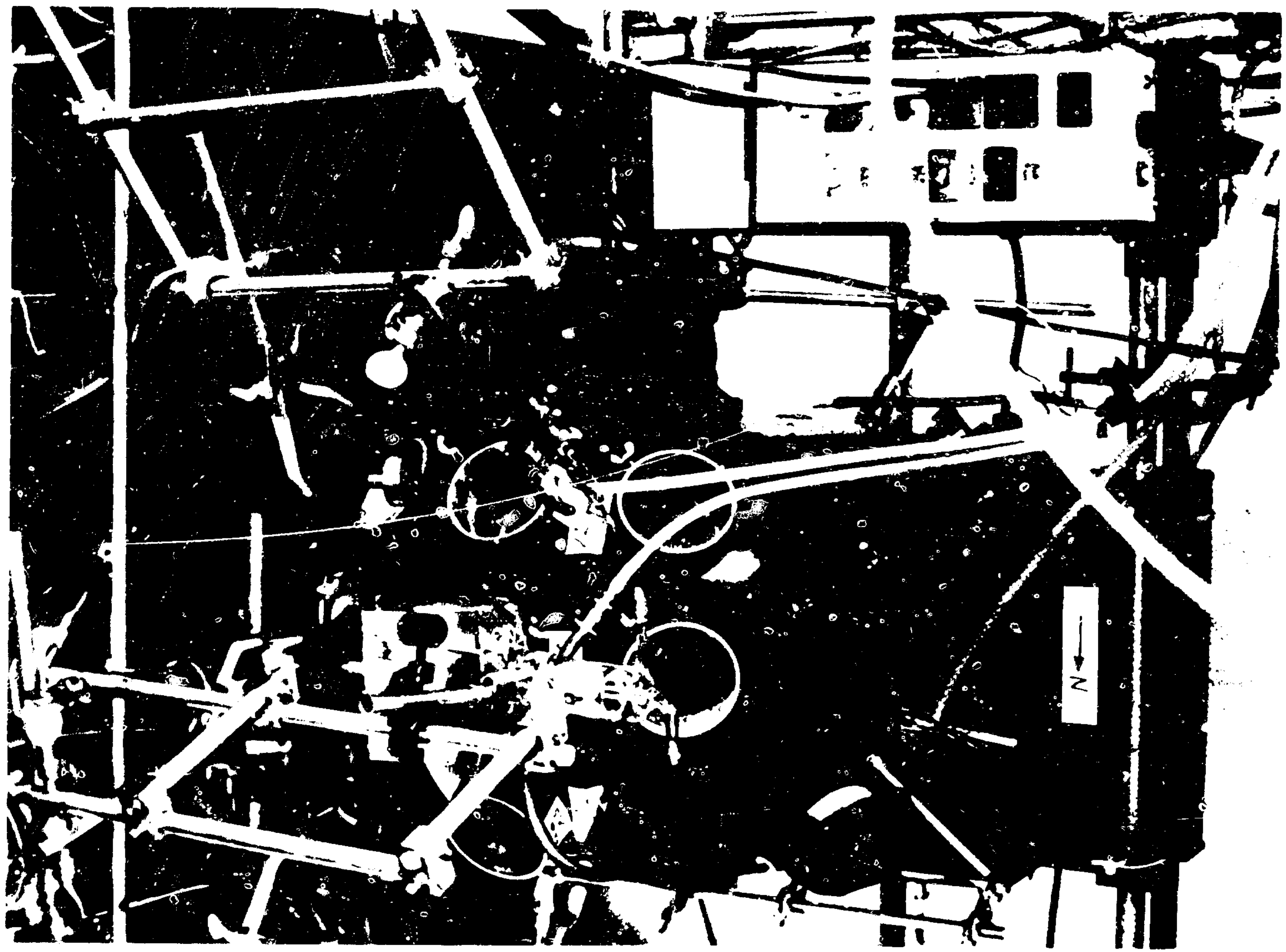


Figure 7: Closeup of Tungsten Source, Air-Cooled Polychromator for Red Wavelengths and White Illumination

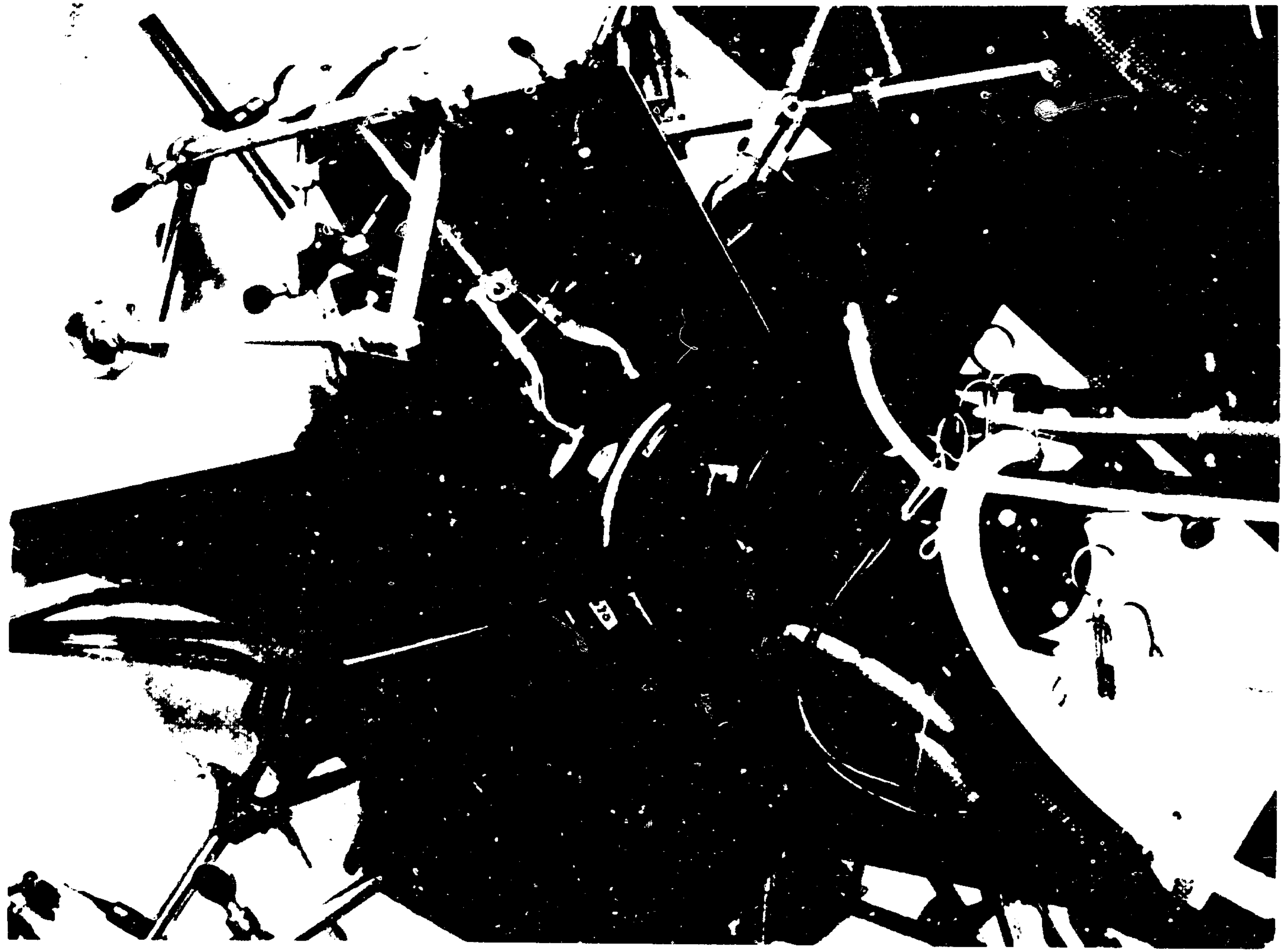


Figure 8: Flask Arrangement for Exposure of Algae to Monochromatic Light

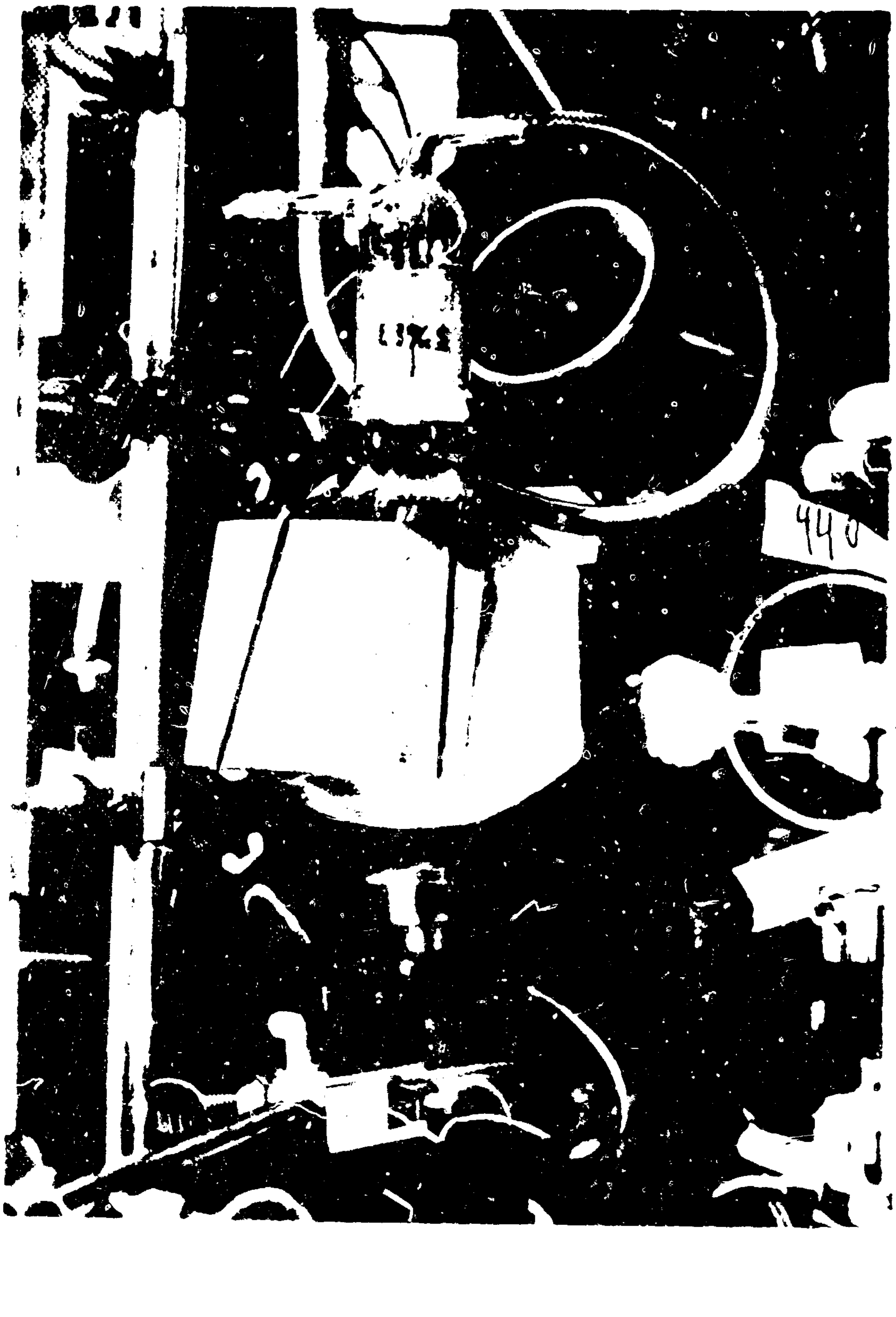


Figure 9: Coulter Electronics Model B Counter with Particle Size Distribution Plotter

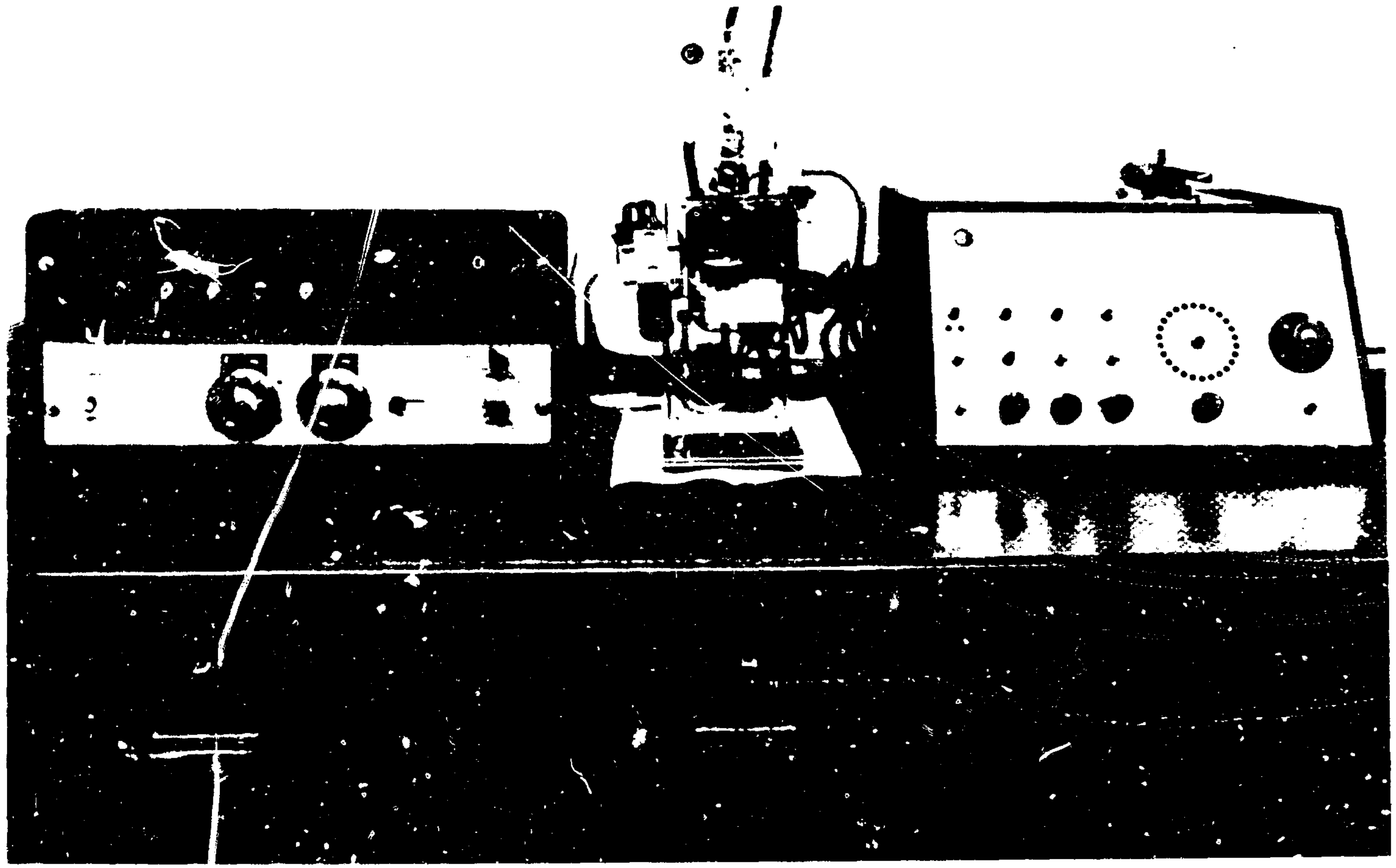
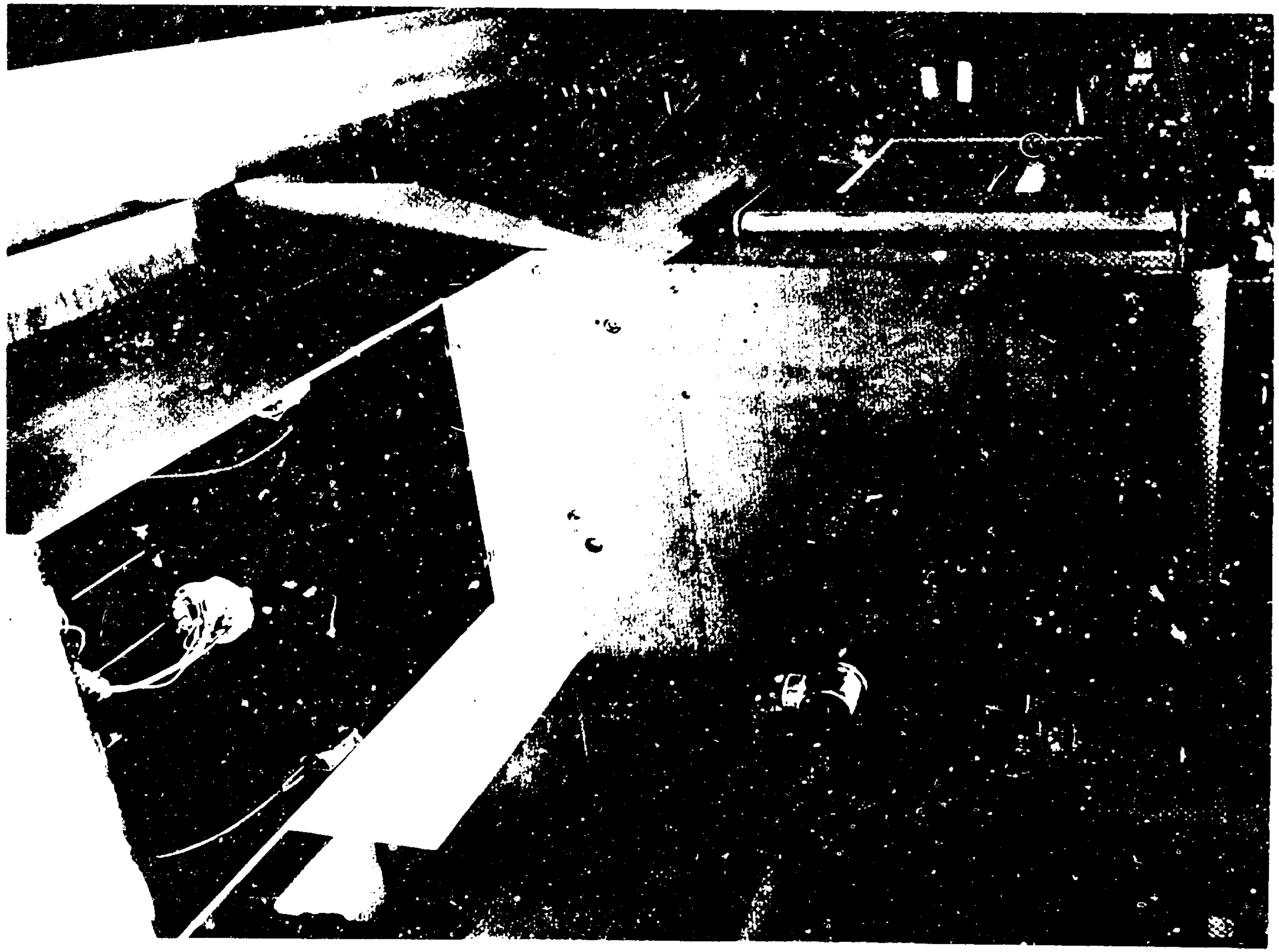


Figure 10: Cary 14 Spectrophotometer with Scatter Attachment (in Foreground)



compared with those of other methods are describ ... ier and Park (35) and the advantages discussed. Relative pigment content was determined by measuring absorption peak heights and subtracting background absorption at 740 nm. This technique is similar to that used by Brody (5). Samples for pigment determination consisted of whole cel... centrifuged from their media and resuspended in distilled water or in 1 M sucrose (for rapidly settling cells). Specific densities used are noted on the culture sheets for each alga. A ten mm light path was consistently used.

B. Chromatography:

As an assist to determine alterations in pig... t compositon, thin layer chromatographic analysis was routinely run for each sample. For qualitative separation, pre-layered Eastman Kodak Co. Type K301R2 sheets were used (silica gel G without fluorescent indicator). For preparative chromatography, glass plates were layered with 250 m/u silica gel G. In a few cases Brinkmann pre-activated chromatotubes were used.

Procedure:

1. Separate cells from media by least force and time necessary.
2. Rinse cells with distilled water and centrifuge as above.
3. Resuspend in boiling methanol (10 ml/25 μl cells).
4. Centrifuge and repeat methanol extraction if cells are not clear.
5. Evaporate total supernatent to 1-2 ml.
6. Dilute and rinse with a minimal volume of acetone.
7. Apply to substrate.
8. Develop with Petroleum ether: Benzene: Acetone: Ethyl acetate (3:2:1:1).

Photosynthesis and Respiration measurements:

The rates of photosynthesis and respiration were determined using the same regime of incident light which was used for growth of the particular alga. The light energy for these measurements was equalized at 12,000 ergs cm^{-2} sec^{-1} by neutral density filters and checked with a Radiometer as previously described. The measurements were made at 25°C, using a Gilson Medical Electronics Model K Oxygraph which was adapted for use with a Y.S.I. No. 5331 Clark polarographic type oxygen probe (Fig. 11). Use of this teflon membrane probe allowed measurements in growth media without the poisoning effect which occurs with the Gilson naked platinum electrode. A plastic cell and twin light units were designed to allow exposure of algal samples to light of single or two simultaneous wavelengths (Fig. 12). The illumination sources were 150 W GE type 1958 lamps. Generally each series required the use of new lamps. As with growth, wavelengths were segregated by interference filters listed in Table II.

Samples consisted of whole cells centrifuged from their growth medium and resuspended in 2-3 ml fresh medium at densities indicated on the individual culture sheets. A partial, typical, Oxygraph tracing is shown in Fig. 13. Measurements were made on two aliquots of each growth condition alternating respiration (dark) with photosynthesis (light) through the entire series of wavelengths and white light. The sequence of wavelengths was reversed for the second aliquot.

Cell Modifications:

A. Cell volume (size):

Alterations in cell volume were determined through use of the Coulter Counter with attached volume distribution plotter. A typical tracing is shown in Fig. 14. Microscopic examinations of each sample were made and observations recorded, to ascertain any gross structural changes. If changes were observed color photomicrographs were taken using a Zeiss Ultraphot II. Electron microscope examination of the lamellae of the photosynthetic apparatus has not yet been possible.

Figure 11: Equipment for Measuring Photosynthesis and Respiration as a Function of Wavelength of Simultaneous or Prior Illumination

Left: Gilson Oxygraph

Center: Illumination Chamber & Y.S.I. Probe

Right: Y.S.I.-Kettering Radiometer

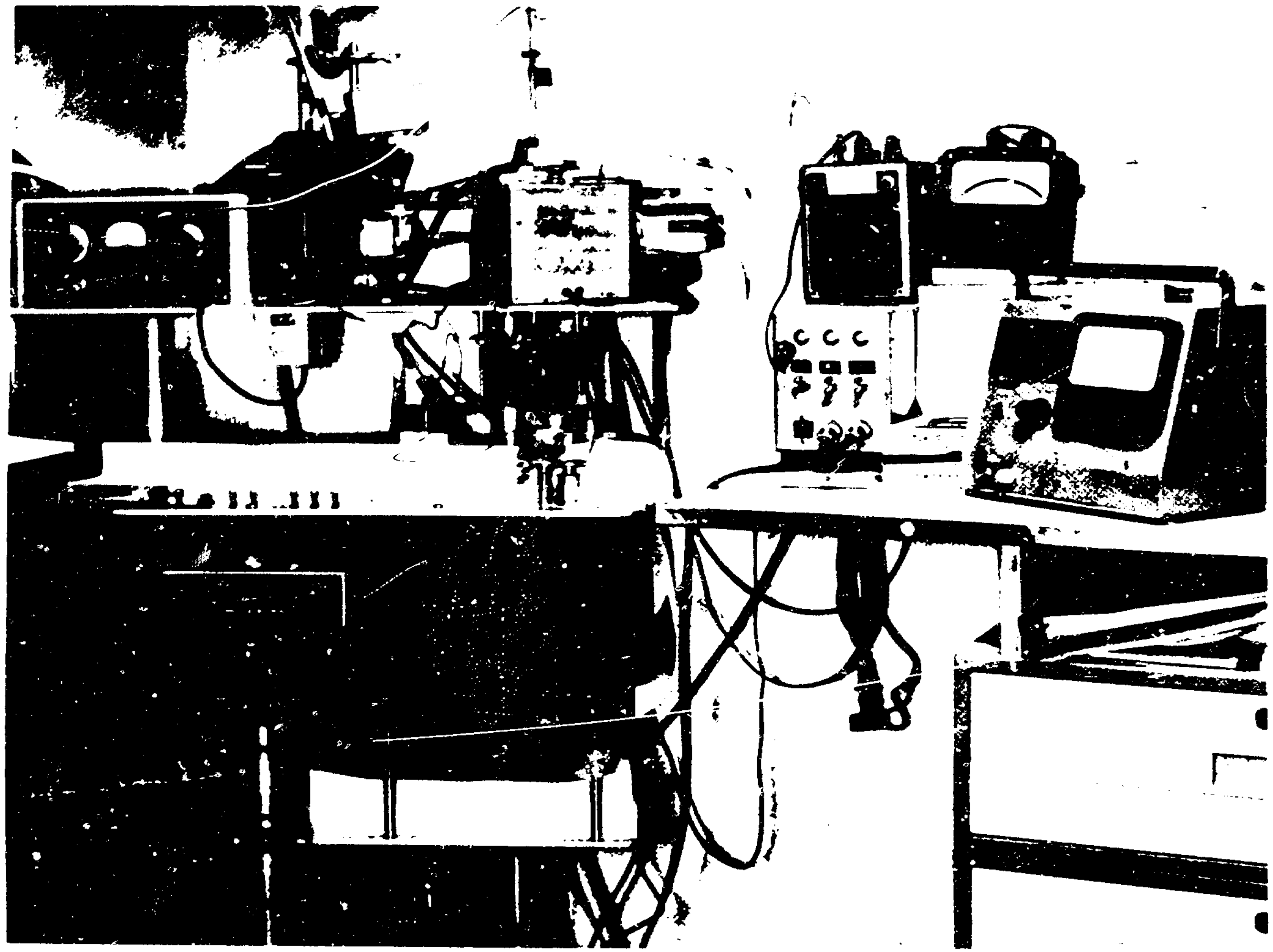


Figure 12: Closeup of Cell and Probe for Illumination of Algae with One or Two Wavelengths of Light During Photosynthesis and Respiration Measurements

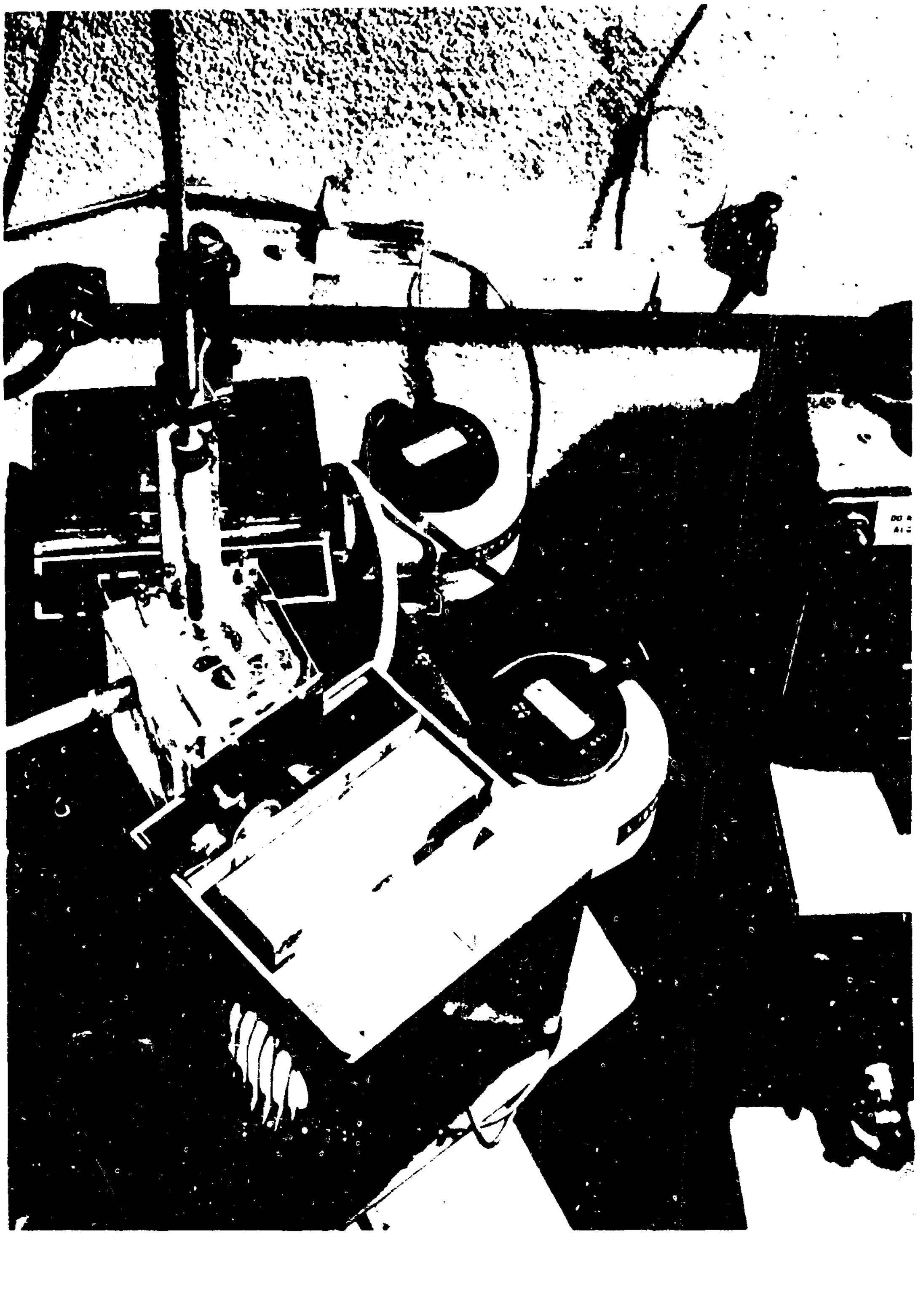


Figure 13: Typical Oxygraph Tracings of Oxygen Changes with *Phormidium persicinum* During a Series of Alternating Monochromatic Illumination and Dark Periods

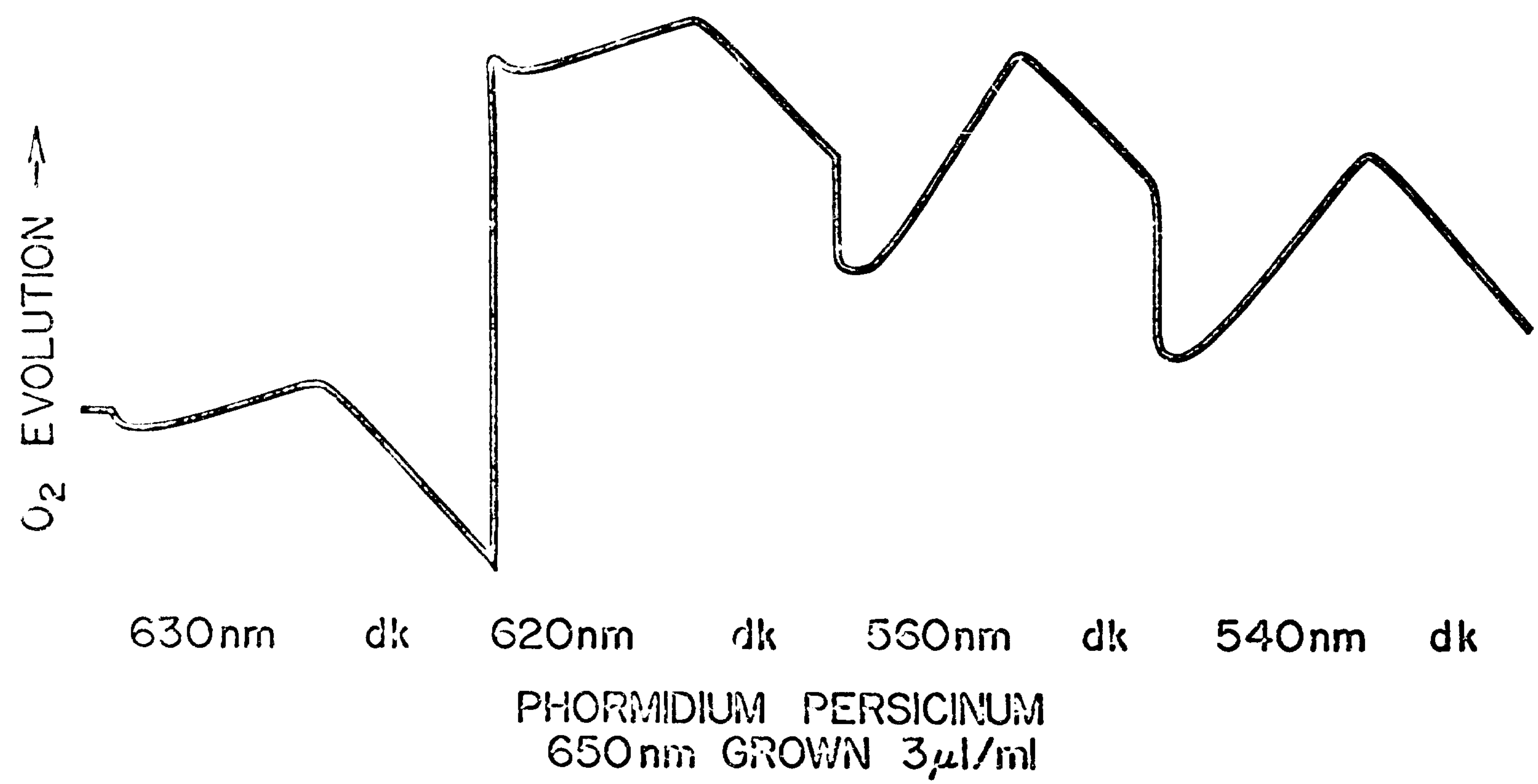
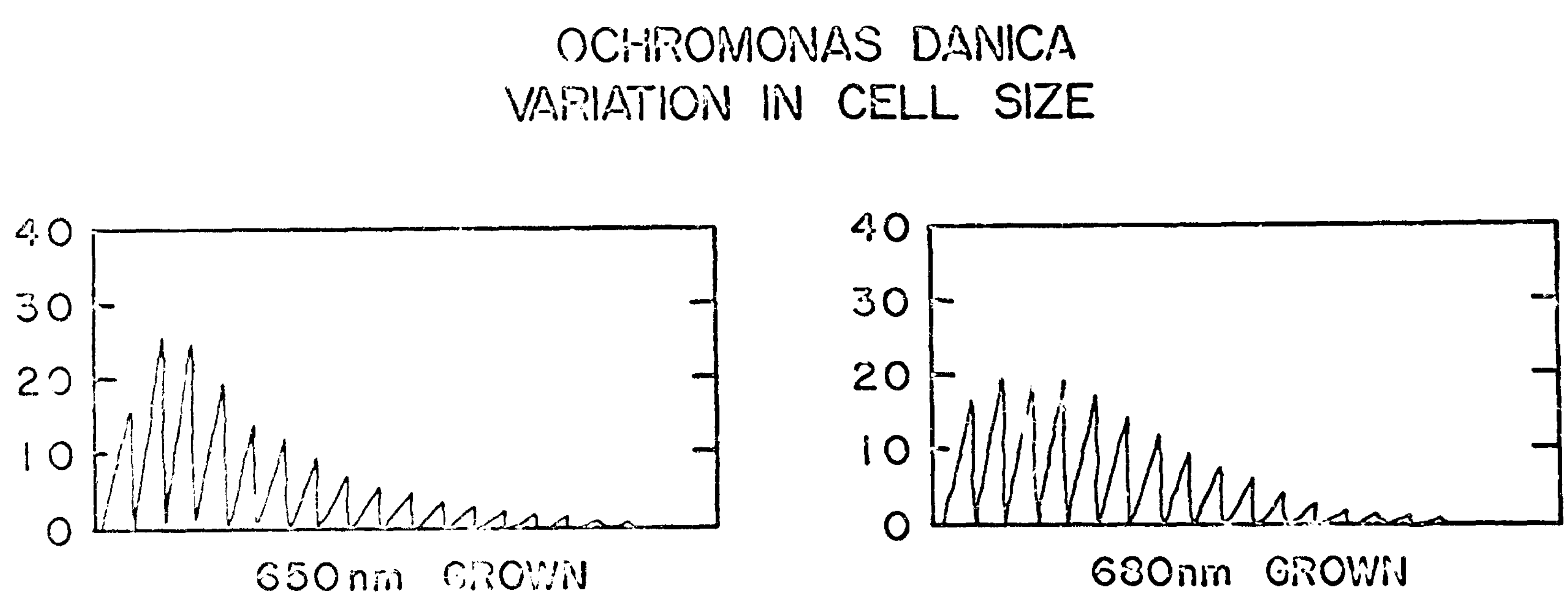


Figure 14: Typical Distribution Plots of Algal Cell Volumes Obtained with the Coulter Counter. (*Ochromonas danica* Cells Grown in Two Different Wavelengths of Light). Cell Volume Increases from Left to Right



Results and Discussion

Results for 15 individual algal species have been presented in progress reports 1,2 and 3. The current (final) report contains data for two additional algae plus summary tables of results in each measurement category for all species. Lastly, tangential studies which have grown out of this program are discussed.

Chlorella sorokiniana (7-11-( )).

This species of *Chlorella* is similar to *C. pyrenoidosa* except that it has a higher temperature tolerance. Both species exhibit physiological activity in light absorbed by chlorophyll *b* (650 nm) comparable to or exceeding that in light absorbed by chlorophyll *a* (680 nm). In line with the current measurements, the greatest difference found between the two species is the greater growth response of *C. sorokiniana* to 640 nm light (Fig. 15, Tab. III).

With *C. sorokiniana*, the synthesis of both chlorophylls *a* & *b* appears to be enhanced with illumination absorbed by chlorophyll *b* alone (Fig. 16, Tab. III). Absorption spectra (Fig. 17) show also that cells grown in illumination of 650 nm more nearly match (in terms of pigmentation) those grown in white light than do cells grown at 680 nm.

The pattern of photosynthesis rates is typical for green algae (Fig. 18, Tab. IV). Respiration rates clearly show a wavelength dependence and will be discussed later. While white light grown cells show little or no respiration response to wavelength of illumination, the wavelength adapted samples exhibit a wavelength dependency (Fig. 18, Tab.V.).

Nitzschia closterium:

The pigmentation of *N. closterium*, typical for diatoms, is dominated by fucoxanthin. However, in terms of growth, synthesis of pigments, photosynthesis and respiration, fucoxanthin apparently plays a direct role of

Figure 15: Growth of Chlorella sorokiniana as a Function of the Wavelength of Light Used for Growth Illumination

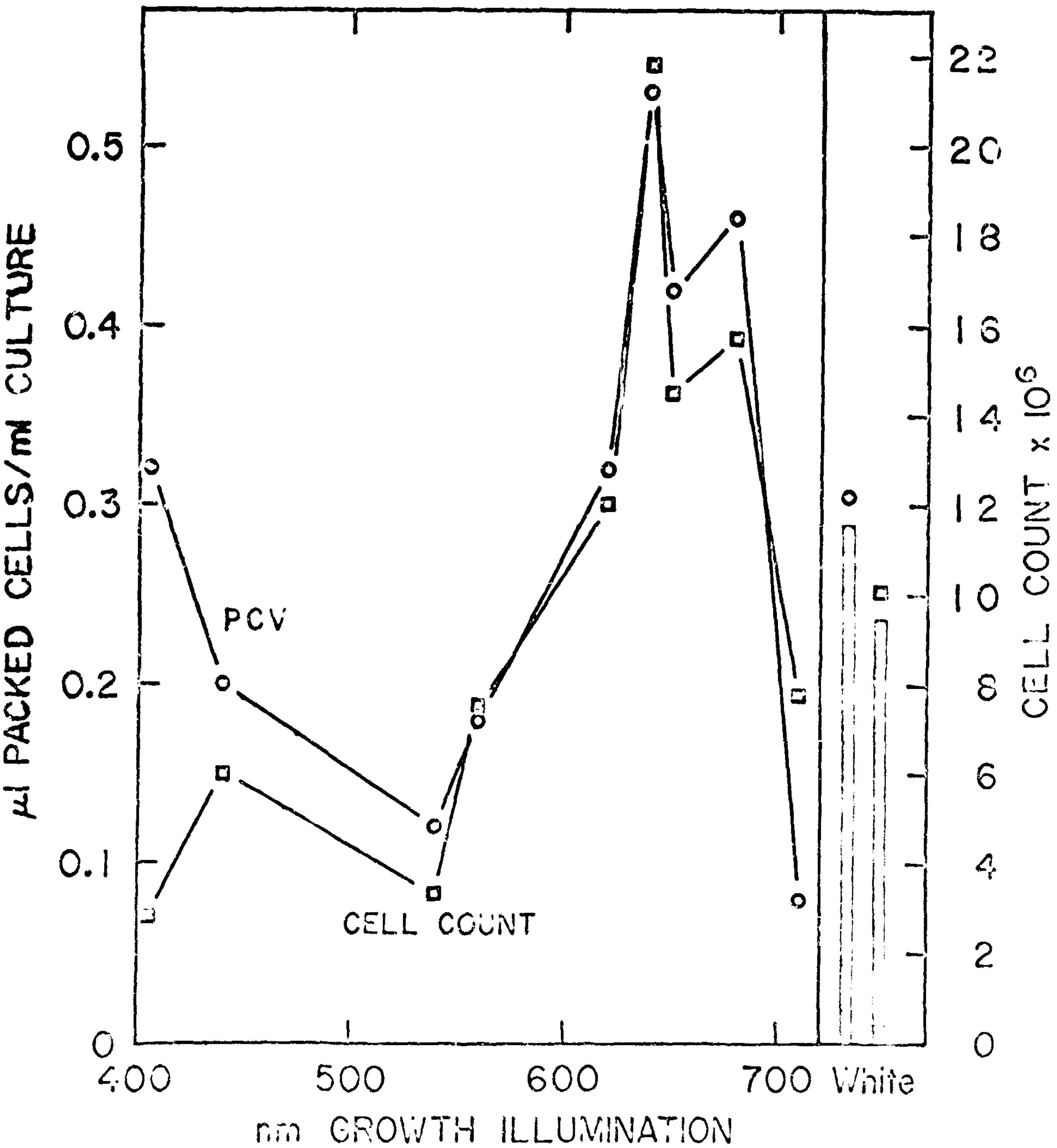


Table III

Growth and pigmentation data for <u>Chlorella sorokiniana</u> (7-11-05) as a function of the wavelength of light used for growth illumination.

Inoculum: 0.1 µl packed cells/ml

Incident energy: 11,500 ergs cm^{-2} sec^{-1}

Energy absorbed: ergs cm^{-2} sec^{-1} taken at 162 hours

Run time in hours: 228

Pigmentation: Optical densities of suspensions having a density of 1 µl packed cells/ml

Growth illum. (nm)	energy absorbed	Packed cell vol. µl/ml	Cell ct. per ml (x 10^4)	Pigmentation Chl. a 676 nm	Chl. b 654 nm	676/654
405	refrac-tion	0.32	280	0.50	0.35	1.43
440	of	0.20	603	0.79	0.58	1.36
540	media	0.12	329	0.61	0.41	1.49
560	and	0.18	746	0.62	0.41	1.51
620	density of	0.32	1198	0.60	0.40	1.50
640	cultures in	0.53	2176	0.62	0.42	1.48
650	validated	0.42	1453	0.70	0.45	1.56
680	results	0.46	1573	0.63	0.39	1.62
710		0.08	776	0.68	0.46	1.48
White		0.29	947	0.70	0.49	1.43

Figure 16: Content of the Major Photoactive Pigments of Chlorella sorokiniana as a Function of the Wavelength of Light Used for Growth Illumination

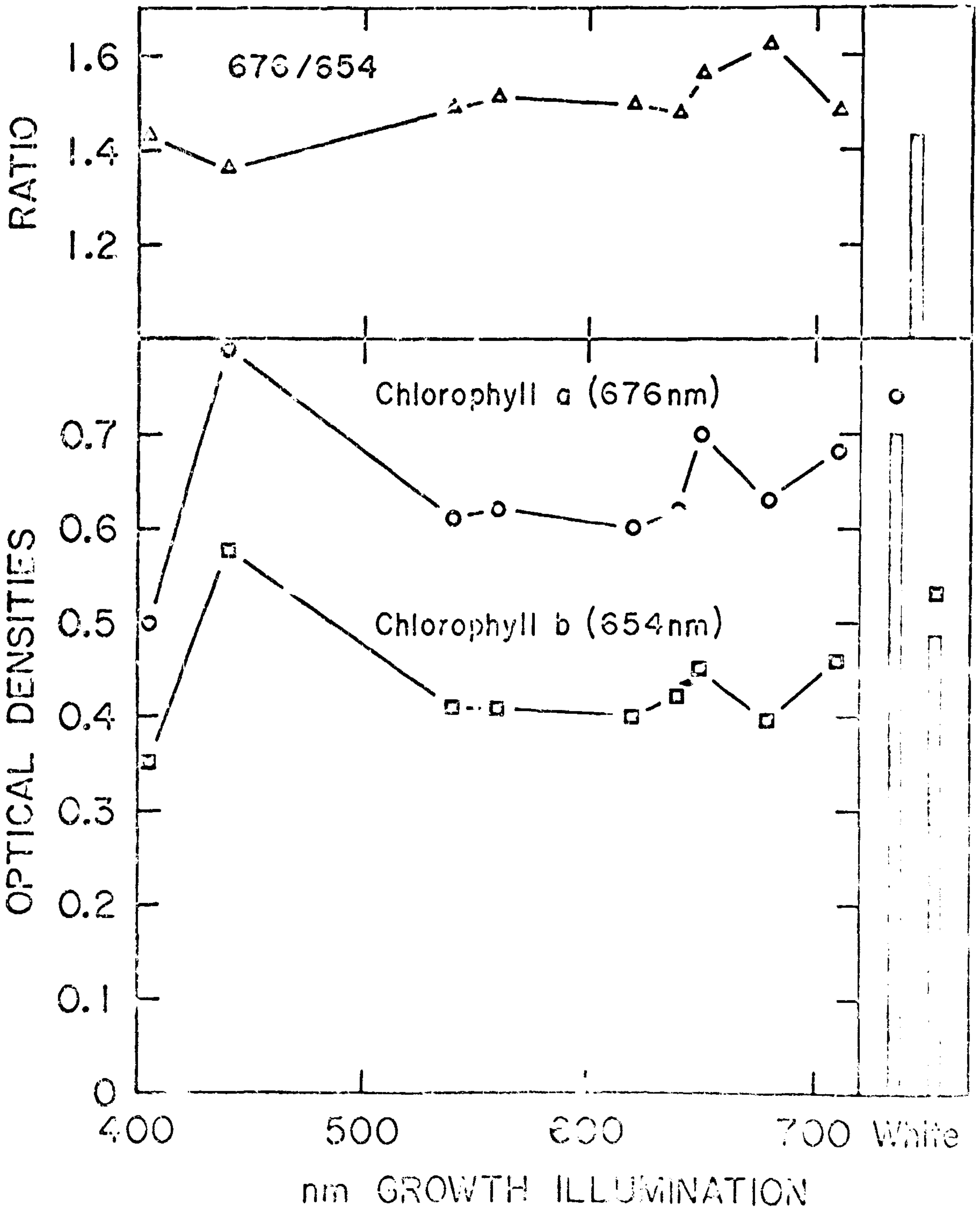


Figure 17: Selected Absorption Spectra of Equal Density Whole Cell Water Suspensions of *Chlorella sorokiniana* Grown in Monochromatic or White Illumination

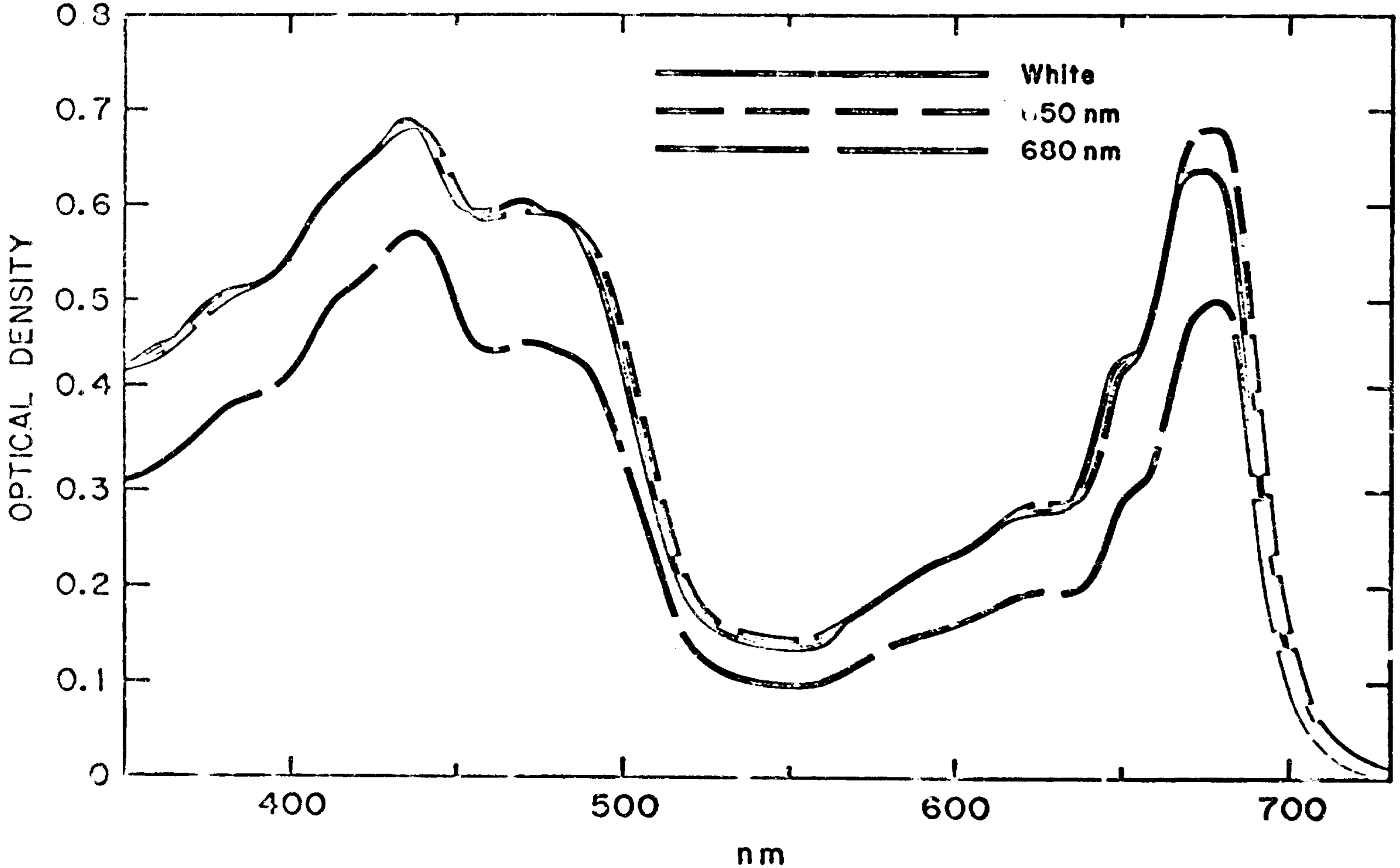


Figure 18: Comparison of Photosynthesis and Respiration Rates Between White-Light Grown and Wavelength-Adapted *Chlorella sorokiniana*. Wavelength Adaptation Refers to Growth and Process Measurement at the Same Wavelength of Simultaneous or Prior Illumination

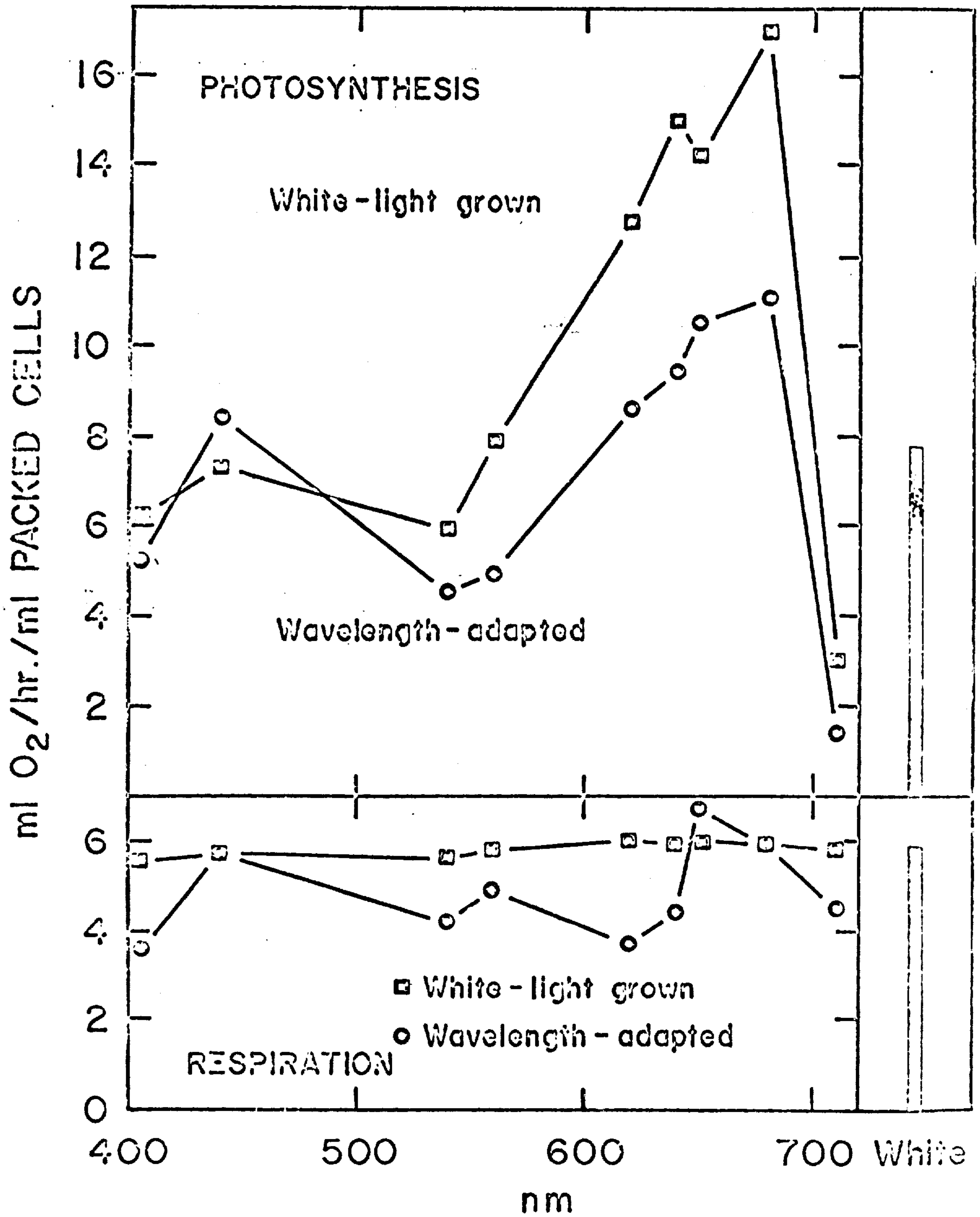


Table IV

Photosynthesis

Oxygen evolution with *Chlorella sorokiniana* as a function of the wavelength of light used for growth and subsequent measurement of photosynthesis rates.

Prior or actual measurement illum. (nm)	405	440	540	560	620	640	650	680	710	Wh.
Wh.	6.6	8.2	7.8	5.5	6.1	5.3	6.7	4.6	4.9	7.8
710	2.1	3.1	2.8	2.7	1.8	2.0	2.2	1.4	1.4	3.0
680	13.2	16.7	15.6	15.1	12.4	13.2	12.6	11.1	11.6	17.0
650	[illegible]	14.8	12.9	12.5	10.8	10.8	10.5	7.8	8.3	14.2
640	11.2	14.8	13.0	12.0	10.8	9.4	9.8	7.7	8.2	15.0
620	9.4	13.2	10.7	9.0	8.6	9.1	9.0	6.6	6.7	12.8
560	5.6	8.1	5.5	4.9	4.7	4.6	4.5	3.2	3.8	7.9
540	4.6	6.6	4.5	4.2	3.8	3.6	3.6	2.2	2.6	5.9
440	7.2	8.4	8.0	7.0	5.2	5.4	5.3	4.7	4.2	7.3
405	5.2	6.8	6.2	5.6	3.8	4.4	4.5	3.3	3.3	6.2

Growth illumination (nm)

Table V

Respiration

(*Chlorella sorokiniana*)

Oxygen uptake as a function of the wavelength of light used for growth and illumination immediately prior to respiration measurement.

µl Oxygen/hour/ml packed cells (x 10^3)

Prior or actual measurement illum. (nm)	405	440	540	560	620	640	650	680	710	Wh.
Wh.	3.6	6.3	4.8	4.6	4.2	4.0	6.6	5.6	4.8	5.9
710	3.5	6.0	4.6	5.0	3.5	3.6	6.3	5.0	4.5	5.8
680	4.1	5.6	4.6	5.5	3.8	5.1	6.7	5.9	5.7	5.9
650	4.0	5.7	4.5	5.5	3.9	4.5	6.7	5.7	5.1	6.0
640	4.0	5.8	4.8	5.5	3.9	4.4	6.4	5.4	4.8	5.9
620	4.0	5.6	4.4	5.3	3.7	4.2	6.5	5.3	4.6	6.0
560	3.8	6.7	4.1	4.9	3.4	3.8	6.1	5.1	4.5	5.8
540	3.7	5.6	4.2	5.0	3.4	3.5	6.2	4.8	4.5	5.6
440	4.0	5.7	4.5	5.5	3.7	4.0	6.5	5.6	4.2	5.7
405	3.6	5.7	4.6	5.3	3.5	3.8	6.3	5.4	4.8	5.5

Growth illumination (nm)

little or no significance. Chlorophyll c, although present in only small amounts, is of greater importance.

Growth response to monochromatic illumination is maximal in light absorbed by chlorophylls a and c with the former being of greatest significance (Fig. 19, Tab. VI). A comparison of packed cell volume and cell count curves show that cell division is more strongly influenced by wavelength than cell growth.

Although pigment synthesis is maximal in light absorbed by chlorophyll c (Figs. 20,21, Tab. VI), the response of both photosynthesis and respiration to monochromatic light is maximal in light absorbed by chlorophyll a. Response to white illumination is remarkably poor (Fig. 22, Tabs. VII and VIII).

Summation of results for 17 algal species:

Tables IX and X summarize growth response to monochromatic light as influencing packed cell volume and cell count. Maximum rates are noted in both cases. In general, blue illumination appears to be of significance only with the algae containing chlorophyll a alone or chlorophylls a & b as the major pigments. The dominance of phycocyanin, phyoerythrin and chlorophyll a is readily apparent. Chlorophyll c is important in growth response but the involvement of fucoxanthin is questionable.

With respect to pigmentation, Tables XI, XII and XIII summarize the effects of the growth regimes. Blue wavelengths are found to be of particular significance in seven instances while growth in white illumination is adequate for pigmentsynthesis in four cases.

In only two cases did 680 nm light (absorbed by chlorophyll a) significantly enhance pigmentation even chlorophyll a itself. This, again, points up the general lack of efficiency with which light absorbed by chlorophyll a is utilized.

Figure 19: Growth of *Nitzschia closterium* as a Function of the Wavelength of Light Used for Growth Illumination

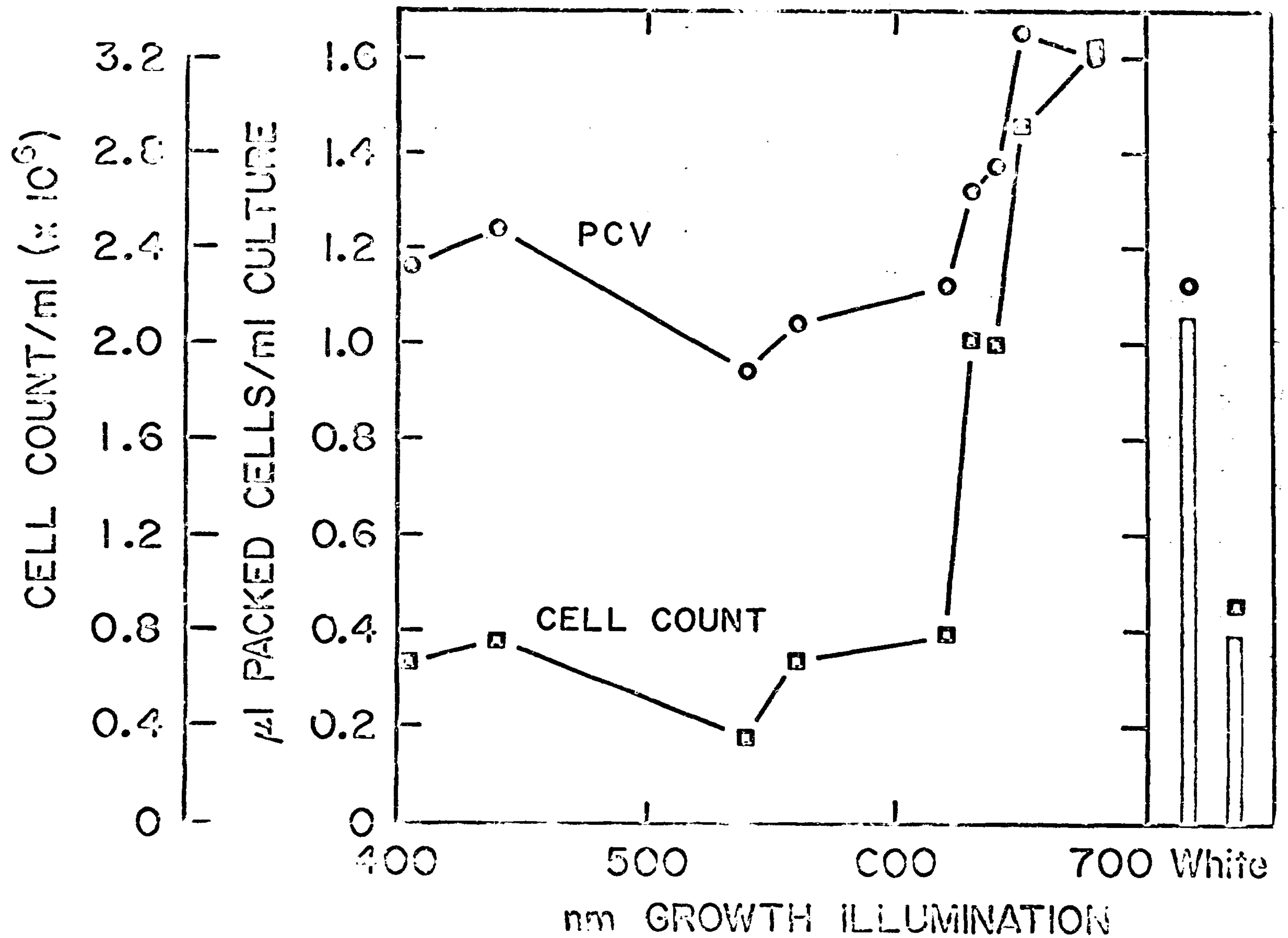


Table VI

Growth and pigmentation data for **Nitzschia closterium**

as a function of the wavelength of light

used for growth illumination.

Inoculum: 0.4 µl packed cells/ml

Incident energy: 12,750 ergs cm^{-2} sec^{-1}

Energy absorbed: ergs cm^{-2} sec^{-1} taken at 188 hours

Run time in hours: 240

Pigmentation: Optical densities of suspensions having a density of 5 µl packed cells/ml

Growth illum. (nm)	energy absorbed	Packed cell vol. µl/ml	Cell ct. per ml (x 10^3)	Pigmentation 674 Chl. a	632 Chl. c	532 focx.	674/632	674/532
405	1.30	1.16	656	.34	.14	.23	2.43	1.48
440	.95	1.24	748	.48	.18	.31	2.67	1.55
540	.85	.94	361	.26	.11	.16	2.36	1.62
560	1.05	1.04	676	.34	.14	.20	2.43	1.20
620	.55	1.12	775	.30	.11	.18	2.73	1.67
630	1.15	1.32	2016	.68	.24	.34	2.83	2.00
640	.85	1.37	1990	.44	.17	.27	2.59	1.63
650	1.23	1.65	2910	.64	.25	.38	2.56	1.68
680	.98	1.60	3232	.54	.20	.31	2.70	1.74
White	.20	1.06	794	.39	.16	.24	2.44	1.62

Figure 20: Content of the Major Photoactive Pigments of Nitzschia closterium as a Function of the Wavelength of Light Used for Growth Illumination

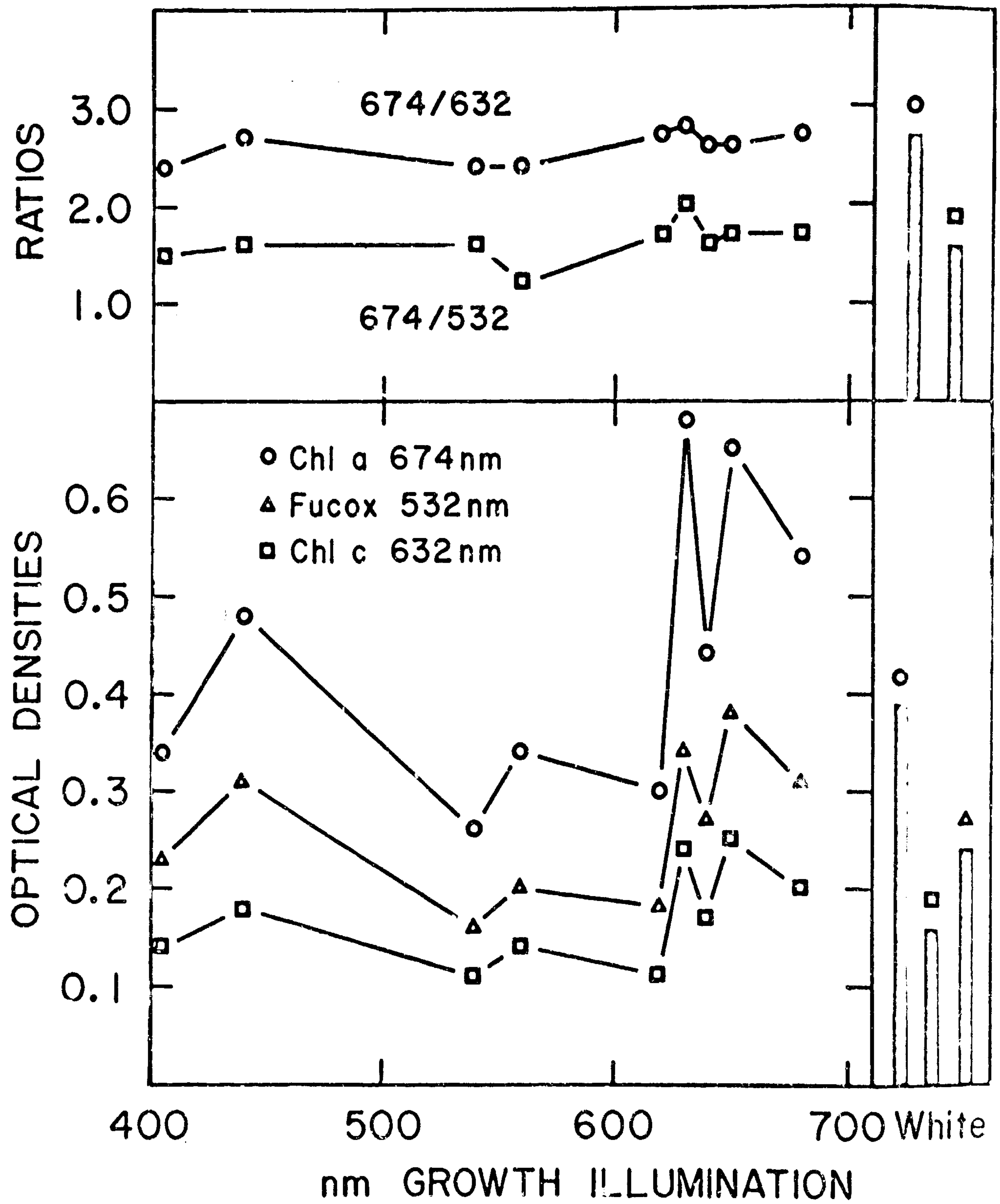


Figure 21: **Selected Absorption Spectra of Equal Density Whole Cell Water Suspensions of _Nitzschia closterium_, Grown in Monochromatic or White Illumination**

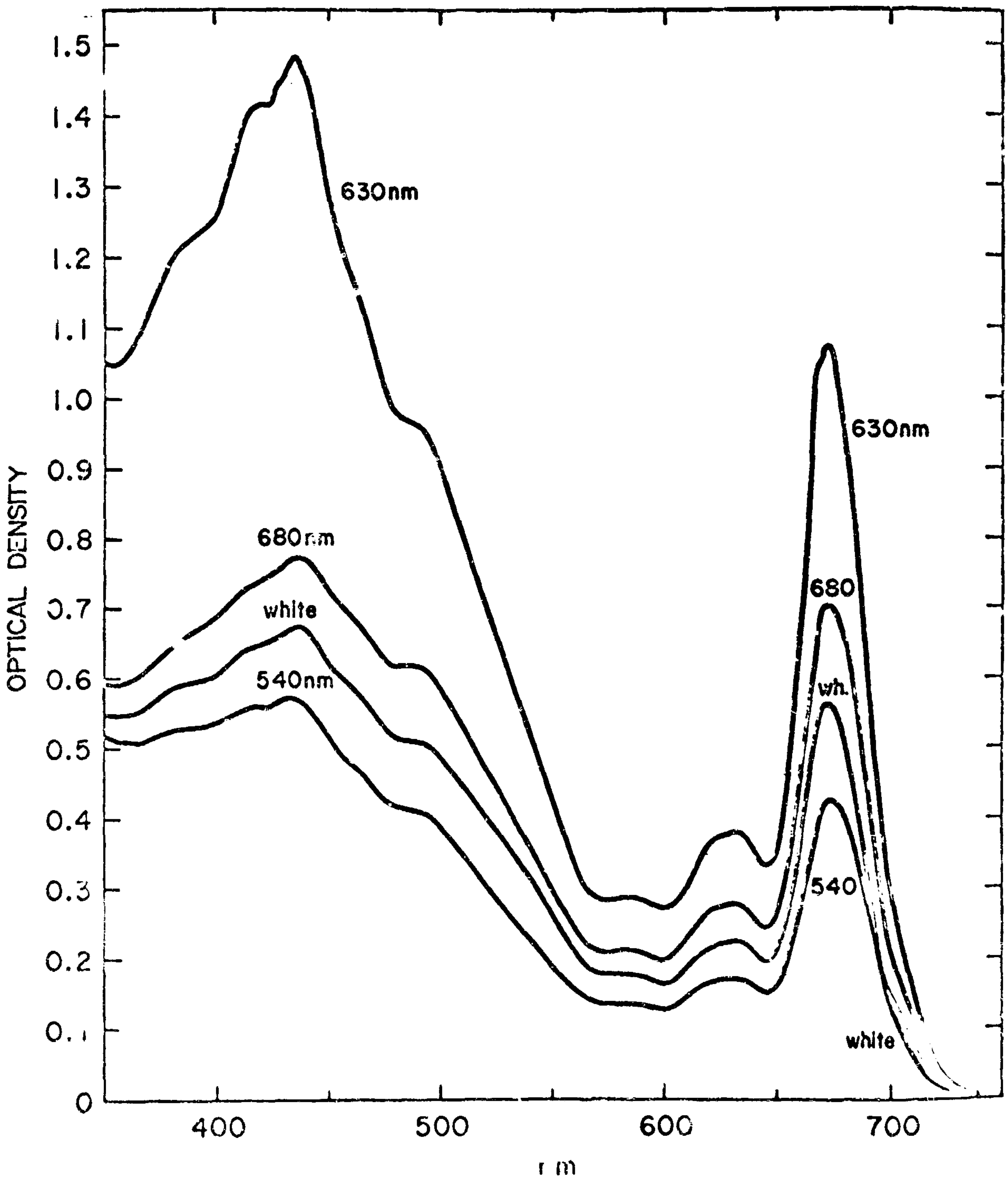


Figure 22: Comparison of Photosynthesis and Respiration Rates Between White-Light Grown and Wavelength-Adapted Nitzschia closterium. Wavelength Adaptation Refers to Growth and Process Measurement at the Same Wavelength of Simultaneous or Prior Illumination

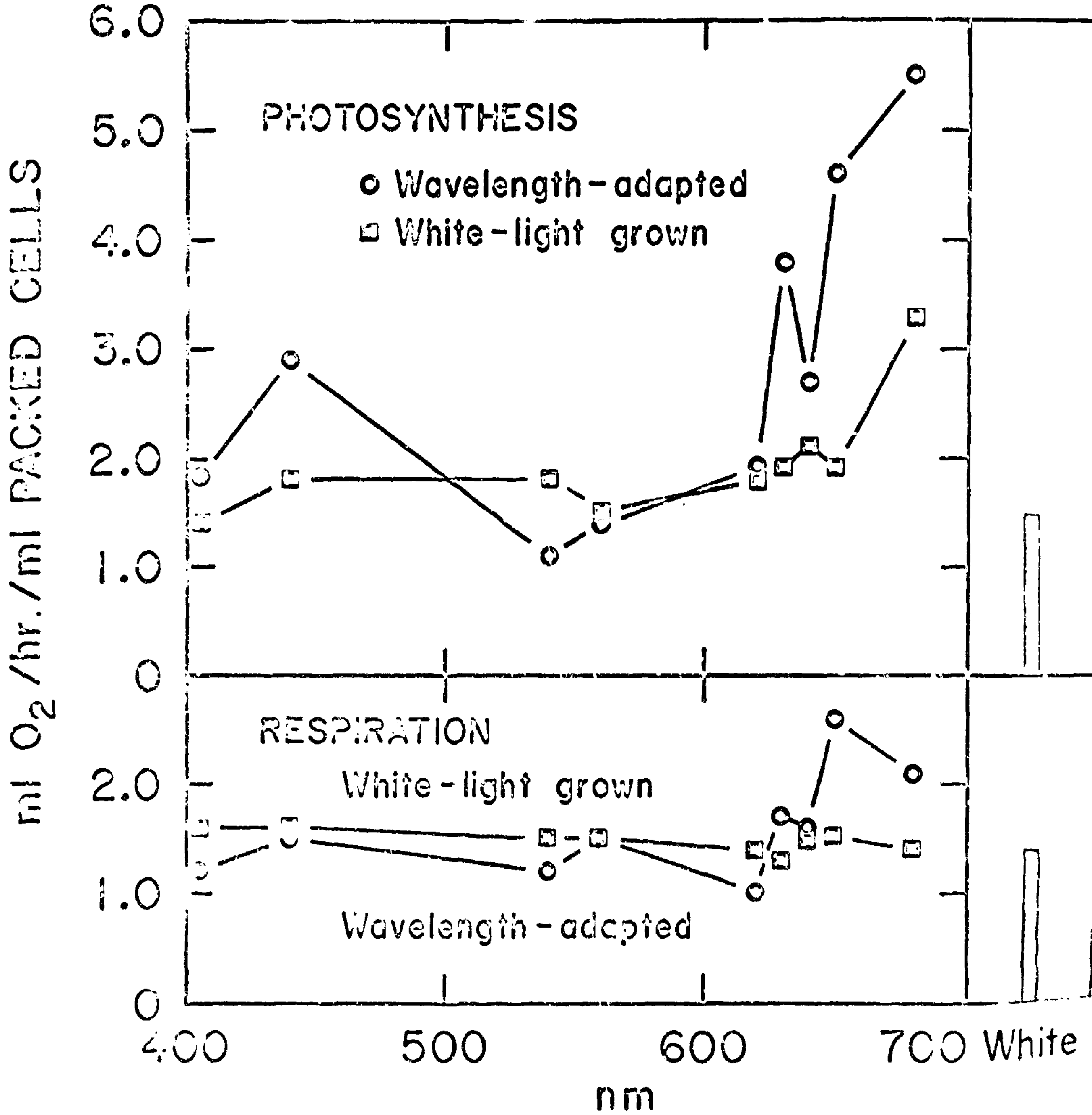


Table VII

Photosynthesis

Oxygen evolution with <u>Nitzschia closterium</u> as a function of the wave-length of light used for growth and subsequent measurement of photosynthesis rates.

μl Oxygen/hour/ml packed cells (x 10^3)

Prior or actual measurement illum. (nm)	405	440	540	560	620	630	640	650	680	White
White	2.0	2.3	1.2	1.1	1.5	2.6	2.1	3.2	2.6	1.5
680	3.7	4.6	2.3	3.0	3.4	5.6	4.5	7.2	5.5	3.3
650	2.4	3.2	1.4	1.8	1.9	3.9	2.6	4.6	3.4	1.9
640	2.4	3.4	1.4	1.9	2.1	3.9	2.7	4.7	3.6	2.1
630	2.3	3.5	1.4	1.9	2.1	3.8	2.8	4.4	3.5	1.9
620	2.3	3.1	1.2	1.8	1.9	3.3	2.6	4.5	3.1	1.8
560	1.8	2.4	1.0	1.4	1.3	2.7	2.1	3.5	2.2	1.5
540	2.2	2.9	1.1	1.6	1.8	2.9	2.3	3.8	2.8	1.8
440	2.5	2.9	1.4	1.8	2.1	2.8	2.8	[illegible].5	2.9	1.8
405	1.8	2.1	0.9	1.5	1.3	2.1	1.8	2.3	1.9	1.4

Growth illumination (nm)

Table VIII

Respiration

(*Nitzschia closterium*)

Oxygen uptake as a function of the wavelength of light used for growth and illumination immediately prior to respiration measurement.

µl Oxygen/hour/ml packed cells (x 10^3)

Prior or actual measurement illum. (nm)										
White	1.1	1.3	1.3	1.1	0.9	1.8	1.6	2.5	2.1	1.4
680	1.1	1.3	1.3	1.3	1.0	1.9	1.6	2.7	2.1	1.4
650	1.0	1.4	1.3	1.3	1.0	2.1	1.6	2.6	2.2	1.5
640	1.1	1.4	1.2	1.3	1.1	1.8	1.6	2.6	2.2	1.5
630	1.0	1.4	1.2	1.3	1.1	1.7	1.7	2.5	2.2	1.3
620	1.1	1.4	1.2	1.4	1.0	1.6	1.7	2.7	2.2	1.4
560	1.1	1.4	1.2	1.5	1.0	1.8	1.7	2.7	2.1	1.5
540	1.1	1.5	1.2	1.5	1.2	1.6	1.7	2.5	2.2	1.5
440	1.1	1.5	1.3	1.5	1.2	1.8	1.9	2.4	2.1	1.6
405	1.2	1.6	1.3	1.6	1.1	1.7	1.8	2.3	2.2	1.6
	405	440	540	560	620	630	640	650	680	White

Growth Illumination (nm)

Table IX

Growth of algae in white and monochromatic light of equal incident energy as percent over inoculum based on µl packed cells/ml culture

(Bracketed figures indicate maximum growth)

nm Growth illumination

Species	405	440	540	560	620	630	640	650	680	710	White
Amphidinium sp. 10 days	100	162	92	100	138	138	[177]	146	[177]	- -	77
Botrydiopsis alpina 8 days	236	[536]	28	6	60	- -	84	174	164	16	163
Chlamydomonas reinhardi 12 days	4,900	9,070	1,070	1,150	[illegible],730	- -	7,070	[15,400]	[14,670]	233	6,980
Chlorella pyrenoidosa 12 days	3,900	[5,000]	400	500	1,300	- -	1,[illegible]00	3,900	[4,700]	300	1,700
Chlorella 7-11-05 10 days	220	100	20	80	220	- -	[430]	320	360	0	190
Chlorococcum wimmeri 7 days	113	131	90	54	105	- -	[182]	169	[182]	90	120
Cryptomonas ovata 6 days	17	57	37	213	23	107	93	257	[292]	- -	20
Euglena gracilis 7 days	12[illegible]	250	150	200	210	- -	230	[310]	[315]	140	200
Gloeocapsa alpicola 7 days	29	88	135	182	[465]	418	400	382	206	- -	80
Nitzschia closterium 10 days	190	210	135	160	180	230	242	[312]	[300]	- -	165
Ochromonas danica [illegible] days	16,950	16,800	19,350	20,850	[26,400]	- -	24,950	25,100	[26,300]	19,450	18,850
Phormidium luridum 7 days	5	30	55	85	360	- -	[430]	230	150	10	135
Phormidium persicinum 7 days	125	150	458	[542]	169	308	288	238	262	- -	246
Porphyridium aerugineum 7 days	240	602	240	368	[1,370]	- -	900	836	836	155	474
Porphyridium cruentum 7 days	15	28	130	102	119	102	[198]	139	141	- -	43
Sphacelaria sp. 1[illegible] days	no measurable growth										
[illegible]bonema aequale 7 days	11[illegible]	282	21	0	221	- -	282	524	[548]	142	45

Table X

Growth of algae in white and monochromatic light of equal incident energy as percent over inoculum based on cell counts/ml culture

(Bracketed figures indicate maximum growth)

nm growth illumination

Species	405	440	540	560	620	630	640	650	680	710	White
Amphidinium sp.	183	337	251	228	336	[352]	321	302	309		199
Botrydiopsis alpina	no data due to clumping										
Chlamydomonas reinhardi	0	403	44	42	148		226	[698]	606	0	132
Chlorella pyrenoidosa	1620	2090	0	0	525		869	2100	[2380]	0	140
Chlorella 7-11-05	18	153	38	213	403		[814]	510	560	226	29[illegible]
Chlorococcum wimmeri	35	50	6	21	40		37	[61]	[69]	5	19
Cryptomonas ovata	17	111	88	241	100	162	149	359	[377]		107
Euglena gracilis	20	74	8	15	70		92	[152]	[160]	85	30
Gloeocapsa alpicola	127	106	198	172	[515]	450	411	363	140		185
Nitzschia closterium	169	206	48	177	218	726	715	1090	[1220]		225
Ochromonas danica	7540	7880	9040	9630	10160		7450	9900	[11740]	7900	7640
Phormidium luridum	Filamentous										
Phormidium persicinum	Filamentous										
Porphyridium aerugineum	348	647	176	276	[707]		281	401	317	0	346
Porphyridium cruentum	156	[203]	147	153	31	38	74	147	146		108
Sphacelaria sp.	Filamentous										
Tribonema aequale	Filamentous										

Table XI

Pigment synthesis of algae grown in white and monochromatic light of equal incident energy as optical densities of whole cell suspensions of 5 µl packed cells per ml H_2O through a 10 mm light path. Data are optical densities.

Species and Pigment	nm	405	440	540	560	620	630	640	650	680	710	White
		nm growth illumination										
Amphidinium sp.												
chlorophyll a	673	0.68	0.70	0.67	0.53	0.72	0.85	0.47	0.50	0.52	- -	0.85
chlorophyll c	633	0.20	0.22	0.20	0.17	0.23	0.23	0.13	0.13	0.13	- -	0.30
Peridinin	534	0.43	0.47	0.42	0.37	0.47	0.50	0.32	0.30	0.30	- -	0.57
Botrydiopsis alpina												
chlorophyll a	678	0.12	0.14	0.20	0.24	0.18	- -	0.12	0.06	0.05	0.26	0.20
Chlamydomonas reinhardi												
chlorophyll a	677	0.70	0.73	0.30	0.37	0.73	- -	0.67	0.60	0.53	0.25	0.60
chlorophyll b	654	0.53	0.53	0.23	0.28	0.48	- -	0.45	0.40	0.35	0.23	0.43
Chlorella pyrenoidosa												
chlorophyll a	678	1.90	3.00	1.15	1.20	2.35	- -	2.00	2.40	1.76	1.25	1.85
chlorophyll b	653	1.20	1.90	0.80	0.85	1.55	- -	1.30	1.50	1.40	0.85	1.25
Chlorella 7-11-05												
chlorophyll a	676	2.50	3.95	3.05	3.10	3.00	- -	3.10	3.50	3.15	3.40	3.50
chlorophyll b	654	1.75	2.90	2.05	2.05	2.00	- -	2.10	2.25	1.95	2.30	2.45
Chlorococcum wimmeri												
chlorophyll a	677	0.46	0.55	0.44	0.54	0.59	- -	0.50	0.44	0.46	0.40	0.45
chlorophyll b	654	0.35	0.41	0.32	0.38	0.42	- -	0.35	0.31	0.34	0.28	0.32
Cryptomonas ovata												
chlorophyll a	674	0.24	0.28	0.28	0.10	0.17	0.19	0.24	0.19	0.21	- -	0.48
chlorophyll c	628	0.13	0.13	0.12	0.05	0.08	0.08	0.10	0.09	0.10	- -	0.10
Phycoerythrin	564	0.30	0.27	0.31	0.11	0.21	0.25	0.25	0.19	0.19	- -	0.30
Euglena gracilis												
chlorophyll a	675	0.80	0.70	0.60	0.52	0.62	- -	0.82	0.95	0.92	0.88	0.70
chlorophyll b	652	0.58	0.50	0.42	0.40	0.46	- -	0.58	0.65	0.65	0.60	0.44

Gloeocapsa alpicola												
chlorophyll a	679	0.73	0.62	0.78	0.92	0.77	0.97	0.98	0.87	0.67	- -	0.83
Phycocyanin	624	1.07	0.80	0.97	1.12	0.75	0.93	0.97	0.93	0.88	- -	0.93
Nitzschia closterium												
chlorophyll a	674	0.34	0.48	0.26	0.34	0.30	0.68	0.44	0.64	0.54	- -	0.39
chlorophyll c	632	0.14	0.18	0.11	0.14	0.11	0.24	0.17	0.25	0.20	- -	0.16
Fucoxanthin	532	0.23	0.31	0.16	0.20	0.18	0.34	0.27	0.38	0.31	- -	0.24
Ochromonas danica												
chlorophyll a	675	0.73	0.77	0.27	0.35	0.53	- -	0.48	0.60	0.47	0.13	0.60
Fucoxanthin	540	0.35	0.37	0.22	0.23	0.28	- -	0.25	0.30	0.25	0.13	0.30
Phormidium luridum												
chlorophyll a	677	0.30	0.22	0.38	0.49	0.76	- -	0.85	0.74	0.46	0.16	0.42
Phycocyanin	628	0.40	0.26	0.42	0.49	0.79	- -	0.87	0.84	0.56	0.14	0.44
Phormidium persicinum												
chlorophyll a	676	0.32	0.30	0.34	0.30	0.50	0.34	0.38	0.31	0.25	- -	0.22
Phycoerythrin	565	0.81	0.78	0.78	0.68	1.14	0.75	0.89	0.78	0.64	- -	0.56
Porphyridium aerugineum												
chlorophyll a	672	0.22	0.14	0.28	0.31	0.19	- -	0.33	0.23	0.28	0.08	0.22
Phycocyanin	628	0.24	0.17	0.31	0.36	0.26	- -	0.38	0.29	0.38	0.09	0.26
Porphyridium cruentum												
chlorophyll a	679	0.24	0.21	0.10	0.08	0.13	0.18	0.11	0.08	0.12	- -	0.19
Phycocyanin	627	0.14	0.12	0.06	0.04	0.07	0.09	0.06	0.05	0.07	- -	0.10
Phycoerythrin	565	0.41	0.38	0.13	0.10	0.19	0.27	0.17	0.14	0.22	- -	0.30
Sphacelaria sp.												
chlorophyll a	670	0.02	0.02	0.06	0.06	0.07	- -	0.06	0.03	0.03	0.09	0.08
chlorophyll c	635	0.01	0.02	0.03	0.03	0.04	- -	0.03	0.02	0.02	0.04	0.04
Fucoxanthin	540	0.02	0.03	0.03	0.04	0.05	- -	0.04	0.03	0.03	0.05	0.05
Tribonema aequale												
chlorophyll a	676	0.10	0.12	0.11	0.08	0.12	- -	0.11	0.11	0.09	0.10	0.14

Table XII

Individual synthesis of pigments resulting from growth of algae in monochromatic and white light. Results are optical density averages taken from Table XI.

Pigment and abs. max.	No. of Samples	nm growth illumination										
		405	440	540	560	620	630	640	650	680	710	White
Chlorophyll a 676 nm	64	0.65	0.81	0.58	0.59	0.73	- -	0.72	0.76	0.66	0.69	0.73
Chlorophyll b 653 nm	20	0.88	1.25	0.76	0.79	0.98	- -	0.96	1.02	0.94	0.85	0.98
Chlorophyll c 632 nm	12	0.16	0.18	0.14	0.12	0.14	0.18	0.13	0.16	0.14	- -	0.19
Phycocyanin 627 nm	16	0.46	0.34	0.44	0.50	0.47	0.51	0.57	0.53	0.47	0.12	0.43
Phycoerythrin 565 nm	12	0.51	0.48	0.41	0.30	0.51	0.42	0.44	0.37	0.35	- -	0.39
Fucoxanthin 537 nm	8	0.29	0.34	0.19	0.22	0.23	0.34	0.26	0.34	0.28	0.13	0.27
Peridinin 534 nm	4	0.43	0.47	0.42	0.37	0.47	0.50	0.32	0.30	0.30	- -	0.57

Table XIII

Changes in accessory pigments in comparison with chlorophyll **a** as effected by growth of algae in monochromatic and white illumination. Data are ratios of optical densities of absorption maxima as indicated.

Pigments	No. of Samples	nm growth illumination										
		405	440	540	560	620	630	640	650	680	710	White
Chl b 654 / Chl a 677	20	0.72	0.71	0.73	0.72	0.69	- -	0.68	0.66	0.67	0.68	0.69
Chl c 632 / Chl a 673	16	0.46	0.45	0.40	0.42	0.42	0.35	0.38	0.43	0.43	- -	0.42
Phycocy. 627 / Chl a 677	12	1.28	1.22	1.16	1.13	1.12	- -	1.04	1.16	1.29	1.02	1.10
Phycoeryth. 565 / Chl a 676	12	1.80	1.7[illegible]	1.57	1.53	1.65	1.68	1.66	1.75	1.76	- -	1.82
Fucoxanthin 537 / Chl a 676	12	0.75	0.82	0.65	0.65	0.60	- -	0.61	0.70	0.70	0.80	0.56
Peridinin 534 / Chl a 673	4	0.63	0.67	0.62	0.69	0.65	0.59	0.68	0.60	0.58	- -	0.67

Pigment ratios are not stable even with respect to that of Chlorophylls a & b. Pigment amounts vary independently of each other each with its own dependence upon wavelength.

Since photosynthesis and respiration were measured using several wavelengths of light with each sample of several growth regimes, summation of data becomes cumbersome. Tables XIV and XVI provide the data for species grown and measured in the same wavelength; tables XV and XVII show the three highest values for photosynthesis regardless of wavelength combinations and the highest and lowest values for respiration. Maximum rates of respiration and photosynthesis do not coincide. Comparing tables XIV and XV, it can be seen that considerable enhancement of photosynthesis occurs through growth at one wavelength and measurement at another as opposed to growth and measurement at the same wavelength-regardless of that wavelength. The pattern of this enhancement appears to be that of measurement at wavelengths longer than the growth illumination rather than the reverse. The major exception to this is Phormidium persicinum whose pigmentation is dominated by phycoerythrin and contains only a trace of phycocyanin. In general the reverse pattern is true for maximum rates of respiration, being provided by measurement at wavelengths shorter than those of growth.

Maximum rates for the various algal types were found as follows:

Chlorophyll a & b containing - growth at 440 measurement 680

Chlorophyll c containing - growth at 630 measurement 680

Phycocyanin containing - growth at 620 measurement 620-650

Fucoxanthin containing - no correlation to pig. absorp.

The effect of continued exposure to monochromatic light.

Due to the significant variation of pigmentation in Gloeocapsa alpicola, a series of sub-cultures was set up in 680 nm illumination. Fig. 23 shows that such additional sub-culturing and continued exposure to chlorophyll a

Table XIV

Photosynthesis* of wavelength-adapted algae (growth and measured oxygen production at same wavelength).
Photosynthesis measurements made at 12,000 ergs cm^{-2} sec^{-1}
Data are ml oxygen/hour/ml packed cells

*Corrected for Res.

nm growth and measurement illumination

Algae	405	440	540	560	620	630	640	650	680	710	White
Amphidinium sp.	1.6	2.8	3.0	2.7	2.7	3.5	2.5	2.3	2.6	---	2.5
Botrydiopsis alpina	0.9	1.6	0.3	0.5	0.9	---	0.8	0.4	1.1	0.5	1.3
Chlamydomonas reinhardi	3.9	6.6	0.4	1.1	5.5	---	6.0	6.1	8.3	0.5	2.8
Chlorella pyrenoidosa	7.8	9.7	3.2	3.7	12.0	---	14.6	18.3	15.6	1.2	11.3
Chlorella 7-11-05	5.2	8.4	4.5	4.9	8.6	---	9.4	10.5	11.1	1.4	7.8
Chlorococcum wimmeri	1.0	1.0	0.7	0.9	2.0	---	3.0	3.3	4.4	0.7	1.2
Cryptomonas ovata	1.6	1.3	1.3	0.6	1.7	1.6	0.9	0.7	0.9	---	0.9
Euglena gracilis	4.6	6.1	2.8	2.8	7.6	---	8.6	9.0	10.2	5.8	3.5
Gloeocapsa alpicola	0.4	0.2	0.2	0.7	1.0	1.2	1.1	1.1	0.9	---	0.5
Nitzschia closterium	1.8	2.9	1.1	1.4	1.9	3.8	2.7	4.6	5.5	---	1.5
Ochromonas danica	1.6	1.6	0.3	0.2	1.5	---	1.6	1.7	3.2	0.1	1.6
Phormidium luridum	0.5	0.3	0.8	2.0	6.5	---	7.6	5.5	2.3	0.3	2.1
Phormidium persicinum	1.1	1.5	4.6	4.8	5.7	4.7	5.6	4.3	3.3	---	2.3
Porphyridium aerugineum	1.0	0.7	1.5	2.6	8.9	---	7.1	8.2	7.2	0.2	1.9
Porphyridium cruentum	0.9	1.3	1.8	1.3	1.4	2.0	1.3	1.1	2.0	---	1.6
Sphacelaria sp.	0.05	0.09	0.08	0.09	0.26	---	0.24	0.26	0.08	0.04	0.14
Tri[illegible]	0.25	0.18	0.06	0.05	0.20	---	0.13	0.07	0.21	0.07	0.12

Table XV

Maximum rates of photosynthesis* of algae as effected by monochromatic and white illumination for growth and measurement. Data are the three highest rates expressed as ml oxygen/hour/ml packed cells

*Corrected for Res.

	nm growth illumination										
Algae and wavelength (nm) for Ps measurements.	405	440	540	560	620	630	640	650	680	710	White
Amphidinium sp.											
1. 680						4.5					
2. 680											4.4
3. 680			4.1								
Botrydiopsis alpina											
1. 680											3.7
2. 680										3.4	
3. 680		2.9									
Chlamydomonas reinhardi											
1. 680		11.2									
2. 680					9.8						
3. 680							9.0				
Chlorella pyrenoidosa											
1. 680		20.5									
2. 650		19.0									
3. 680	18.9										
Chlorella 7-11-05											
1. 680											17.0
2. 680		16.7									
3. 680			15.6								
Chlorococcum wimmeri											
1. 680								5.2			
2. 680							5.0				
3. 680		4.9									
Cryptomonas ovata											
1. 680	2.8										
2. 680						2.5					
3. 680					2.4						

Euglena gracilis										
1. 680									11.4	
2. 680		11.2								
3. 680							10.7			
Gloeocapsa alpicola										
1. 620			1.4							
2. 620 and 640			1.35							
3. 620 and 630	1.3							1.3		
Nitzschia closterium										
1. 680							7.2			
2. 680					5.6					
3. 680								5.5		
Ochromonas danica										
1. [illegible]		5.2								
2. [illegible]50										4.5
3. 640 and 680	3.4			3.4			3.4			
Phormidium luridum										
1. 640						7.6				
2. 620						7.4				
3. 650						6.8				
Phormidium persicinum										
1. 560						7.6				
2. 560				7.4						
3. 560					7.3					
Porphyridium aerugineum										
1. 640							10.1			
2. 640									9.8	
3. 620				9.7						
Porphyridium cruentum										
1. 560		3.4								
2. 540 and 560		3.3			3.3					
3. 560	3.1									

Sphacelaria sp.							
1. 650 and 680				0.28			
2. 540							0.27
3. 620 and 650				0.26	0.26		0.26
Tribonema aequale							
1. 6[illegible]0	0.5	0.5				0.5	
2. [illegible]80				0.4			
3. 630			0.3				0.3

Table XVI

Respiration of wavelength-adapted algae (growth and prior illumination for assimilation at same wavelength). Prior illumination was 12,000 ergs cm^{-2} sec^{-1}. Data are ml oxygen assimilated per hour per ml packed cells.

Algae	nm for growth and prior measurement illumination										
	405	440	540	560	620	630	640	650	680	710	White
Amphidinium sp.	2.6	2.8	3.0	2.7	2.7	3.4	3.2	4.0	3.6	- -	2.6
Botrydiopsis alpina	0.6	0.8	1.1	0.9	1.0	- -	1.4	1.1	1.1	1.6	0.7
Chlamydomonas reinhardi	2.5	3.3	3.5	3.6	3.3	- -	3.1	2.8	3.0	5.8	2.1
Chlorella pyrenoidosa	4.5	5.2	2.9	3.0	4.5	- -	5.5	5.2	4.5	2.6	4.6
Chlorella 7-11-05	3.6	5.7	4.2	4.9	3.7	- -	4.4	6.7	5.9	4.5	5.9
Chlorococcum wimmeri	1.0	1.0	1.0	1.1	1.7	- -	2.6	3.0	3.0	1.1	1.0
Cryptomonas ovata	1.0	1.9	1.8	1.0	1.0	1.1	1.3	1.4	1.4	- -	1.1
Euglena gracilis	2.5	2.9	2.3	2.6	2.8	- -	3.2	3.6	3.6	3.4	2.3
Gloeocapsa alpicola	0.2	0.3	0.2	0.3	0.3	0.3	0.2	0.3	0.2	- -	0.2
Nitzschia closterium	1.2	1.5	1.2	1.5	1.0	1.7	1.6	2.6	2.1	- -	1.4
Ochromonas danica	6.3	4.2	3.7	5.5	2.8	- -	5.0	2.5	6.0	3.3	4.9
Phormidium luridum	1.0	0.9	0.9	1.5	1.8	- -	2.4	2.1	1.3	1.3	1.0
Phormidium persicinum	1.5	2.5	1.7	1.6	2.8	2.1	2.9	4.0	2.6	- -	1.3
Porphyridium aerugineum	1.5	0.9	1.3	1.5	3.6	2.2	2.2	3.5	3.0	3.9	0.9
Porphyridium cruentum	0.7	0.8	0.6	0.6	0.4	0.8	0.8	0.6	0.8	- -	0.8
Sphacelaria sp.	0.20	0.29	0.34	0.44	1.05	- -	0.99	0.82	0.28	0.18	0.46
Tribonema aequale	0.4	0.5	0.3	0.4	0.6	- -	0.7	0.6	0.8	0.7	0.4

Table XVII

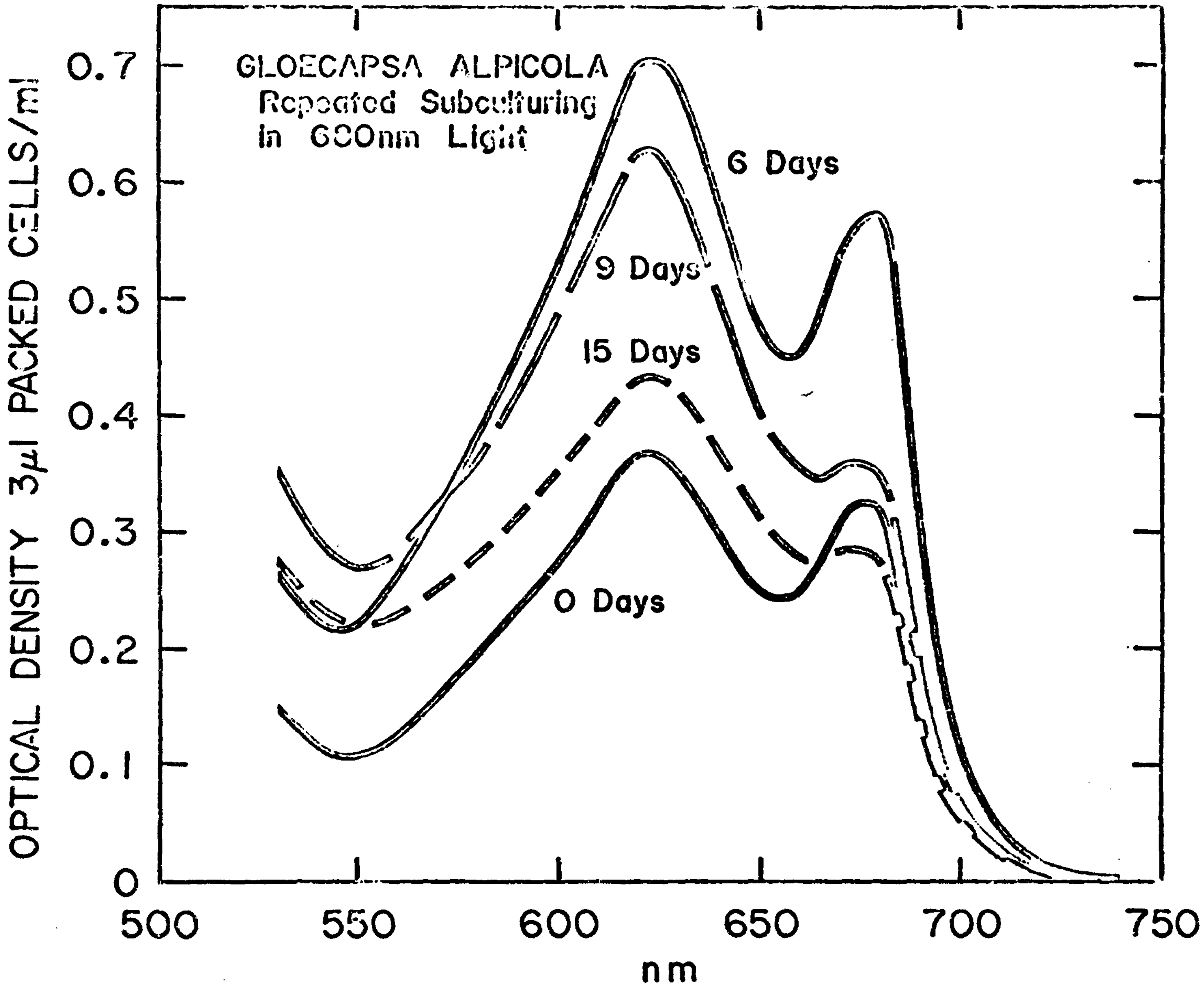
Maximum and minimum rates of respiration of algae effected by monochromatic and white illumination for growth and measurement. Data are ml oxygen assimilated per hour per ml packed cells.

nm growth illumination

Algae and nm prior measurement illum.	405	440	540	560	620	630	640	650	680	710	White
Amphidinium sp.											
max. 680											4.1
min. white						2.3	2.3				
Botrydiopsis alpina											
max. 710								1.7			
min. white								0.5	0.5		
Chlamydomonas reinhardi											
max. 405										6.1	
min. white											2.1
Chlorella pyrenoidosa											
max. 405									6.4		
min. 540 and 560										2.2	
Chlorella 7-11-05											
max. 680 and 650								6.7			
min. 540 and 560					3.4						
Chlorococcum wimmeri											
max. 680 and 710							3.2	3.2			
min. white										0.8	
Cryptomonas ovata											
max. 440,540,620, 630 and 640		1.9									
min. 405,680 and white				0.9	0.9	0.9					

Euglena gracilis										
max. 620 and 650									4.1	
min. 710			1.6							
Gloeocapsa alpicola										
max. 560		0.6								
min. 680										0.1
Nitzschia closterium										
max. 560,620 and 680							2.7			
min. white				0.9						
Ochromonas danica										
max. 650			7.4							
min. white							1.5	1.5		
Phormidium luridum										
max. 560						2.5				
min. 440,540 and white									0.4	
Phormidium persicinum										
max. 650							4.0			
min. white										1.3
Porphyridium aerugineum										
max. 710				4.0			4.0	4.0		
min. 405, 440 and white		0.9								
Porphyridium cruentum										
max. 440 and 540					1.0					
min. 640	0.4									
Sphacelaria sp.										
max. 620				1.05						
min. white	0.16									
Tribonema aequale										
max. 710		1.0								
min. white			0.2							

Figure 23: Absorption Spectra of Subcultures of Gloeocapsa alpicola from Continued Exposure to 680 nm Illumination



absorbing light, enhances production of phycocyanin while reducing chlorophyll a. Subsequent sub-cultures lead to an apparent "fatigue effect" and reduction of both of these pigments. It is judged that there is a critical time of exposure to single wavelengths which is specific for each organism, beyond which only harmful effects will come about. Coupled pairs of wavelengths are therefore recommended (see Tab. XVIII) for long time growth of algae in qualitatively limited illuminations.

Production of far-red absorbing pigmentation.

We found that the absorption spectra of Nitzschia closterium grown in green light exhibited a shoulder absorbing at 708 nm. Allen, et al (3) found 710 nm absorption in several UV-induced mutants of Chlorella pyrenoidosa and in illuminated Ochromonas danica (2). They found that the absorption disappeared upon extraction and determined that any pheophytin formation could not account for the degree of this absorption. We confirm both of these observations, in the case of N. closterium. Later, J.S. Brown (9) found similar absorption in dark-stored O. danica and dark-aged Euglena gracilis and observed that it does reside in the chloroplasts.

Figure 24 shows the formation of 708 nm absorption with respect to wavelength of green growth illumination in comparison to the lack of such absorption in white-light grown material. This far-red absorption can be enhanced, to the detriment of other pigments, by repeated sub-culturing in green light (Fig. 25). A difference spectra indicating the specific absorption of this pigmentation is also shown. If cells of N. closterium, showing this pigmentation, are placed in any wavelength of the visible region other than green, the 708 nm absorption totally disappears within a few hours. This alga was unique, in this respect, among the algae studied under conditions of the project.

Tab. XVIII Recommended Light Sources for Algal Studies
(wavelengths are inclusive, not either/or, unless so stated)

Algae	General	Growth	Pigment Formation	Photosynthesis
	Wavelengths in nm			
Amphidinium sp.	440, 630	440, 630-680	630 or white	440-540, 630
Botrydiopsis alpina	440, 680	440	710	440, 680
Chlamydomonas reinhardi	440, 650-680	440, 650	405-440	440, 680
Chlorella pyrenoidosa	440, 650-680	440, 650-680	440	640-680
Chlorella sorokiniana (7-11-05)	405-440, 650-680	405, 640-680	440	650-680
Chlorococcum wimmeri	440, 680	440, 640-680	440	680
Cryptomonas ovata	405-440, 630-680	560, 680	440 or white	405, 620-630
Euglena gracilis	440, 650-680	440, 650-680	650-680	640-680
Gloeocapsa alpicola	620-640	620-640	630-640	620-650
Nitzschia closterium	440, 630-680	440, 630-680	630-650	650-680
Ochromonas danica	440, 630-680	620-680	440	680
Phormidium luridum	620-640	620-640	620-650	620-640
Phormidium persicinum	540-630	540-560	620	620-640
Porphyridium aerugineum	620-640	620-640	640-680	620
Porphyridium cruentum	440-540, 630-640	440-540, 640	405	540, 630-680
Sphacelaria sp.	440, 620-650	----	710 or white	620-650
Tribonema aequale	440, 680	440, 650-680	White	440, 680

Figure 24: Far Red Absorption in Nitzschia closterium as a Function of the Wavelength of Growth Illumination. White Light is Equivalent to Blue and Red Wavelengths.

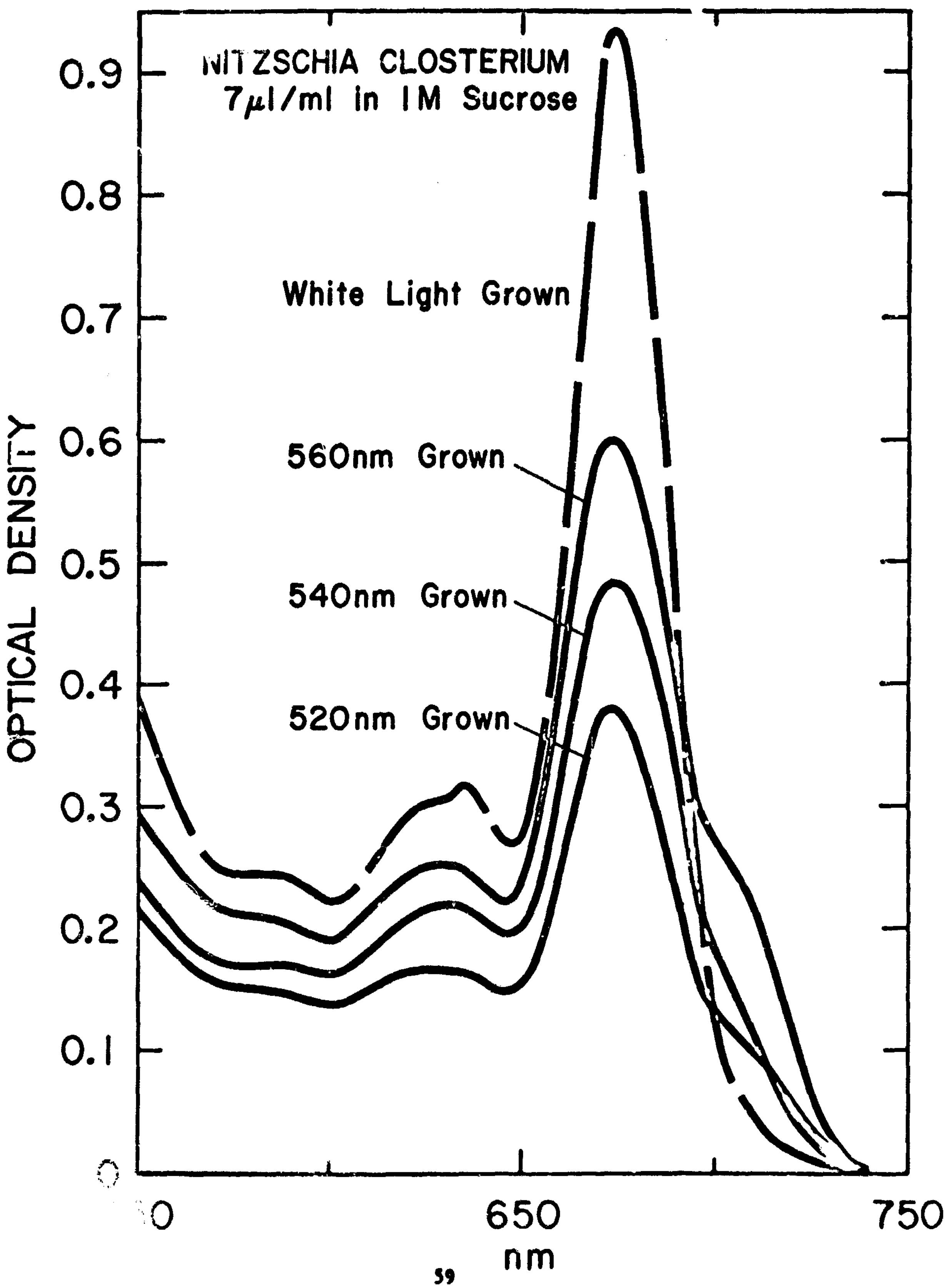
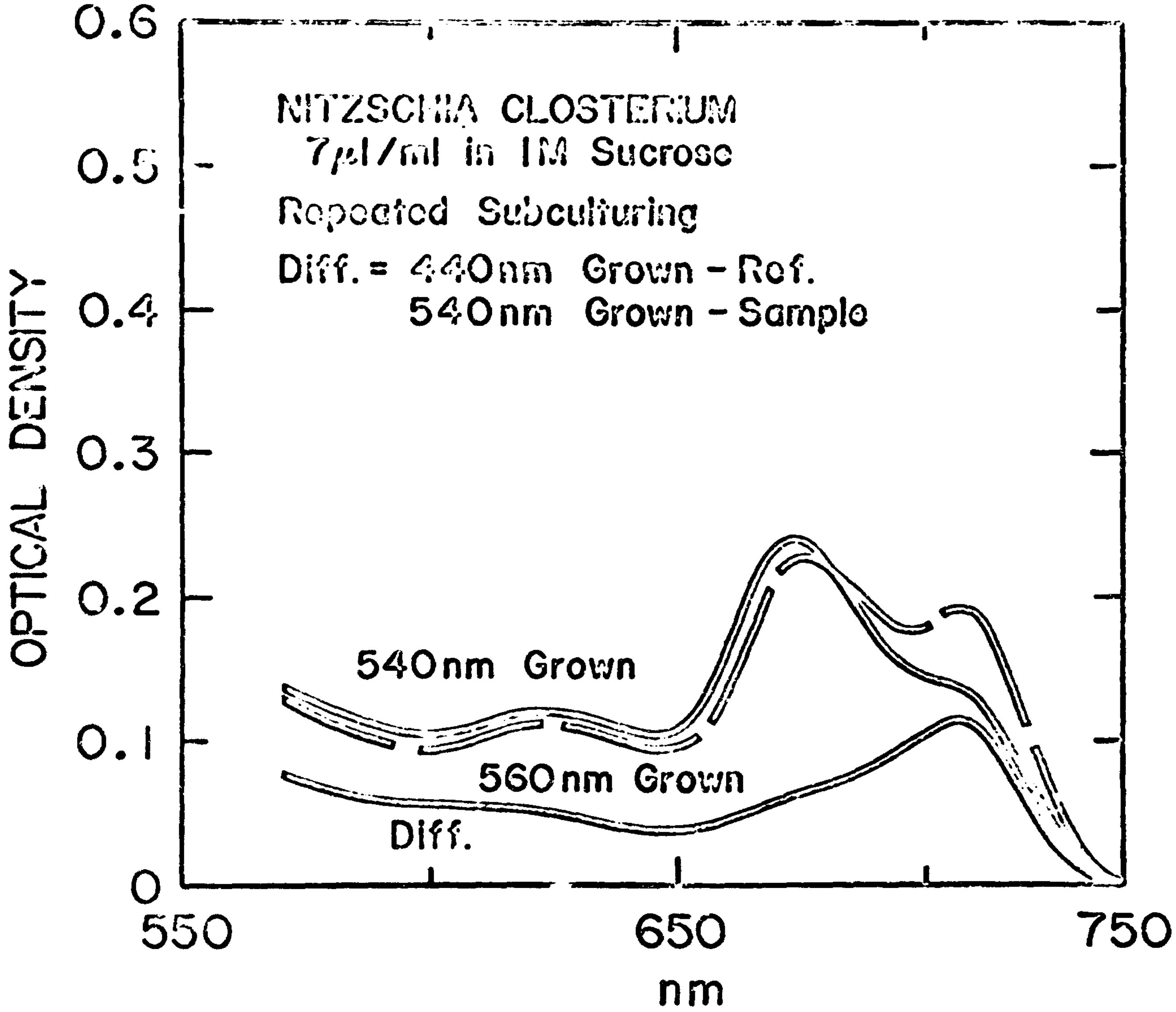


Figure 25 Absorption Spectra of Subcultures of *Nitzschia closterium* in Green Light and a Difference Spectra Between Samples With and Without Far Red Absorption



Wavelength dependence of respiration.

That there is a wavelength dependence of respiration has been discussed in the introduction. The present work allows a direct comparison of the degree of this dependence between algae (Tables XVI and XVII). We have investigated this effect further with the colorless alga Astauia longa, by running a light quality growth series with this alga and comparing respiration rates with those of the other algae studied. In no case did we find an influence of illumination wavelength. Earlier, however, we did show a dependence of respiration rate with intensity of illumination (10). It would appear that the presence of photoactive pigments or a photosynthetic apparatus, or both, are necessary for the observed wavelength response of respiration.

Conclusions:

From this work we can recommend specific compositions of illumination sources for continued growth and metabolism of seventeen diverse algae; such recommendations are offered in Table XVIII.

At least one general observation can be made which opposes previous views. This is, that Cyanophyta and apparently Rhodophyta instead of exhibiting a broad physiological response to wavelengths of illumination (relativelyindependent from wavelength) show greater limitation and hence dependence than any other group. Chlorophyll c containing algae, on the other hand, exhibit the greatest freedom from wavelength dependence. Could this be a reason for the success and universal occurrence of diatoms?

Since it has been shown that significant enhancement of oxygen production can be brought about by application of two different wavelengths of light, the next logical step in boosting the biological output of algae and arriving closer to realization of the full photosynthetic potential of these organisms is to grow selected pigment varying species in light of wavelengths maximally absorbed by each photoactive pigment present. Such work should be combined with that of light duration (continuous vs. intermittent illumination). In continuous light, cells are forced into organic materials production over and above their normal needs for cell synthesis. This stockpiling may well inhibit or alter the normal cyclic processes and lead to reduced efficiency of energy utilization. Therefore, more efficient use of applied light energy should come about through reducing this stockpiling by intermittent illumination.

It is obvious that in some cases, due to the severe conditions of illumination imposed on the algae in the current study, endogenous respiration is greater than photosynthesis or net oxygen evolution. If this were directly extrapolated to nature, such organisms could not survive. However, it is equally obvious that they do survive in nature. The conclusion can be drawn that the native metabolism of most, if not all, of the wild species is a combination of both autotrophic and heterotrophic forms. Many differences between characteristics of wild and laboratory forms of any given species may be due to forced autotrophism of the organism in the laboratory.

Literature Cited

1. Allen, M.B., 1959, Arch. Mikrobiol. 32:270-277, Studies with *Cyanidium caldarium*, an anomalously pigmented chlorophyte.

2. Allen, Mary Belle, 1961, In Light and Life, McElroy and Glass, Eds., Johns Hopkins:479-482, Evidence for pigments absorbing at 705-710 nm in photosynthetic organisms.

3. Allen, M.B., Bendix, S.A., Murchio, J.C., 1962, Arch. Mikrobiol. 42: 36-39, Concerning "Long wavelength" pigments in algae.

4. Björn, L.O., Suzuki, Y., Nilsson, J., 1963, Physiol. Plant. 16:132-141, Influence of wavelength on the light response of excised wheat roots.

5. Brody, M., 1958. The participation of chlorophyll and phycobilins in the photosynthesis of red algae. II. Observations on cellular structure of *Porphyridium cruentum*. Ph.D. Dissertation, Univ. of Ill. Microfilms L.C. Card. No. Mic. 58-1686

6. Brody, Marcia, Emerson, Robert, 1959, Amer. Jour. Bot. 46:433-440, The effect of wavelength and intensity of light on the proportion of pigments in *Porphyridium cruentum*.

7. Brody, Marcia, Brody, Seymour Steren, 1962, Arch. Biochem. Biophys. 96:354-359, Induced changes in the photosynthetic efficiency of *Porphyridium cruentum*. II.

8. Brown, A.H., 1953, Amer. J. Bot. 40:719-729, The effects of light on respiration using isotopically enriched oxygen.

9. Brown, Jeanette S., 1963, B.B.A. 75:299-305, The separation of the forms of chlorophyll *a* and the absorption changes in *Euglena* during aging.

10. Brown, Thomas E., Richardson, Frances L., 1967, Jour. Phycol. (in press), The effect of growth environment on the physiology of algae: Light intensity.

11. Cayle, T., Emerson, R., 1957, Nature 179:89-90, Effect of wavelength on the distribution of carbon-14 in the early products of photosynthesis.

12. Emerson, Robert, Chalmers, Ruth V., 1958, Phycol. News Bull. 35:51-56, Speculations concerning the function and phylogenetic significance of the accessory pigments of algae.

13. Fork, D.C., 1963, In: Photosynthesis Mechanisms in Green Plants, Nat. Acad. Sci. Pub. #1145, Nat. Res. Counc. 352-361, Observations on the function of chlorophyll a and accessory pigments in photosynthesis.

14. Franck, James, 1958, Proc. N.A.S.:941-948, Remarks on the long-wave-length limits of photosynthesis and chlorophyll fluorescence.

15. Fujita, Yoshihiko, Hattori, Akihiko, 1960, Pl. and Cell Physiol. 1:293-303, Effect of chromatic lights on phycobilin formation in a blue-green alga, Tolypothrix tenuis.

16. Govindjee, Rabinowitch, E., 1960, Science 132:355, Two forms of chlorophyll a in vivo with distinct photochemical functions.

17. Halldal, Per, 1964, Physiol. Plant. 17:414-421, Ultraviolet action spectra of photosynthesis and photosynthetic inhibition in a green and a red alga.

18. Hattori, Akihiko, Fujita, Yoshihiko, 1959, J. Biochem. 46:521-524, Formation of phycobilin pigments in a blue-green alga, Tolypothrix tenuis, as induced by illumination with colored lights.

19. Hauschild, A.H.W., Nelson, C.D., Krotkov, G., 1962, Can. J. Bot. 4:179-189 and 1619-1630, The effect of light quality on the products of photosynthesis in Chlorella vulgaris and, the effect of light quality on the products of photosynthesis in green and blue-green algae, and in photosynthetic bacteria.

20. ———, ———, ———, 1965, Naturwiss, 52:435, On the mode of action of blue light on the products of photosynthesis in Chlorella vulgaris.

21. Haxo, F.T., Blinks, L.R., 1950, J. Gen. Physiol. 33:389-422, Photosynthetic action spectra of marine algae.

22. Hoch, George, Owens, Olga V.H., Kok, Bessel, 1963, Arch. Biochem. & Biophys. 101:171-180, Photosynthesis and respiration.

23. Jones, Larry W., Myers, Jack, 1965, J. Phycol. 1:6-13, Pigment variations in Anacystis nidulans induced by light of selected wavelengths.

24. Karlander, Edward P., Krauss, Robert W., 1962, Pl. Physiol. 37, Suppl: lxiv, Effects of monochromatic light on the growth of Chlorella vulgaris.

25. Karlander, Edward P., Krauss, Robert W., 1966, Pl. Physiol. 41:1-6, Responses of heterotrophic cultures of Chlorella vulgaris Beyerinck to darkness and light. I. Pigment and pH changes.

26. Karlander, Edward P., Krauss, Robert W., 1966, Pl. Physiol. 41:7-14, Responses of heterotrophic cultures of Chlorella vulgaris Beyerinck to darkness and light. II. Action spectrum for and mechanism of the light requirement for heterotrophic growth.

27. Kowallik, Wolfgang, 1963, Planta 60:100-108, DieZellteilung von Chlorella im verlaufe einer Farblichtkultur.

28. Kowallik, Wolfgang, 1965, Planta 64:191-200, Die Proteinproduktion von Chlorella im Licht verschiedener Wellenlängen.

29. Kowallik, Wolfgang, 1967, In: Energy conversion by the photosynthetic apparatus, Brookhaven Symp. 19:467-477, Chlorophyll-independent photochemistry in algae.

30. Kowallik, Wolfgang, 1967, Pl. Physiol. 42:672-676, Action spectrum for an Enhancement of endogenous respiration by light in Chlorella.

31. Meier, Florence E., 1936, Smithson. Misc. Coll. 94 (17):1-12, Growth of a green alga in isolated wavelength regions.

32. Myers, Jack, Graham, Jo-Ruth, 1963, Pl. Physiol. 38:105-116, Enhancement in Chlorella.

33. McLeod, G.C., 1961, Pl. Physiol. 36:114-117, Action spectra of light-saturated photosynthesis.

34. McLeod, G.C., 1961, Science 133:192-193, Variation of enhancement of photosynthesis with conditions of algal growth.

35. Sauer, K., R.B. Park, 1964, Molecular orientation in quantasomes. II. Absorption spectra, Hill activity and fluorescence yields. Biochem. Biophys. Acta. 79:476-489.

36. Shihira, Ikuko, Krauss, Robert W., 1963, Univ. Md. Press, College Pk., Md., Chlorella, Physiology and Taxonomy of Forty-one Isolates.

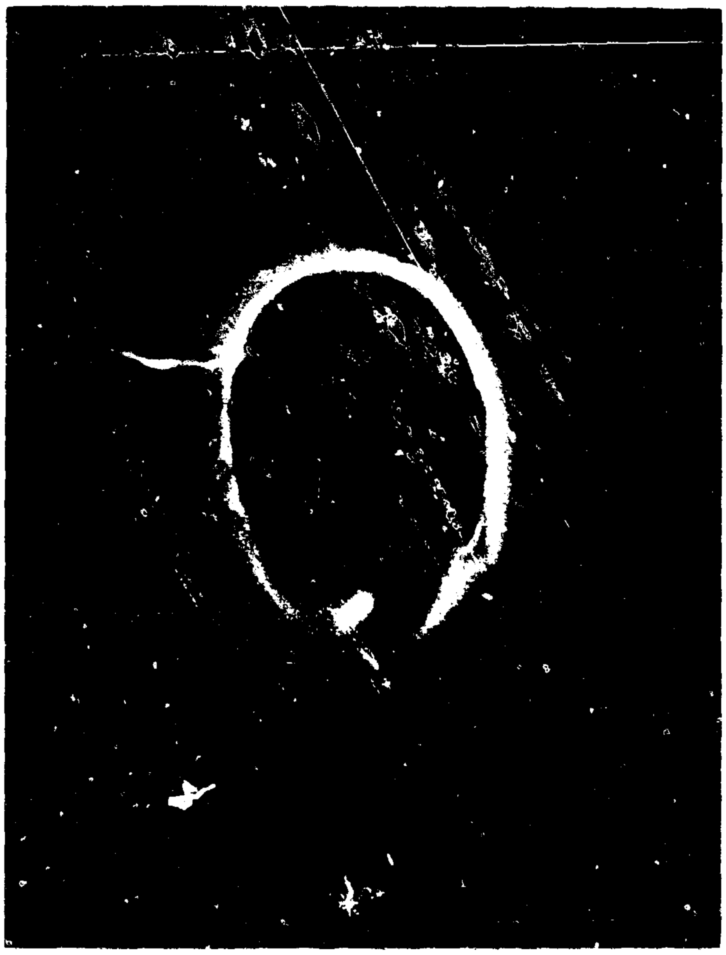
37. Vidaver, Wm., 1965, Pl. Physiol. 41:87-89, Separate action spectra for the two photochemical systems of photosynthesis.

Appendix A

Photomicrographs of typical forms of the algae studied accompanied by descriptions of normal material and cells grown in monochromatic light.

The photomicrographs were taken on a Zeiss Ultraphot II microscope using the automatic exposure control and tungsten illumination. The 4 x 5 in. Tri-X sheet film used was processed with D-76 developer.

Figure 26: *Amphidinium sp.*, X 2600



Amphidinium sp.

Brown oblong naked unicells with two unequal length flagella emerging from a girdle or deep furrow. A single chromatophore contains chlorophyll *a* and peridinin as major photoactive pigments.

Growth illum: 13,500 ergs cm^{-2} sec^{-1}
Time: 240 hours

Illumination	Description
405 nm	normal golden brown appearance with good motility.
440	same as with 405 nm
540	Brass-colored cells with excellent motility but smaller than 405 or 440 nm cells.
560	Same as with 540 nm except cells slightly smaller
620	Cells of normal color and size with excellent motility
630	Same as with 620 nm
640	Pale but active cells smaller than normal
650	Pale, sluggish, small cells
680	Same as 650 nm but with excellent motility
White	Average size cells with good color and motility

Figure 27: Botrydiopsis alpina, X 2600

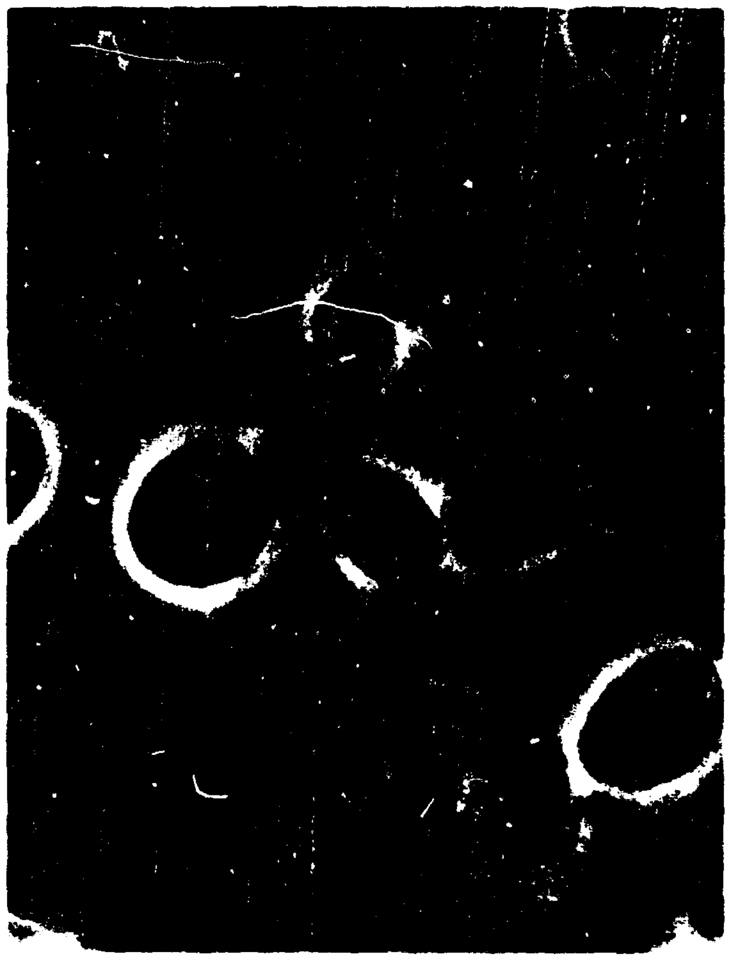
Botrydiopsis alpina

Bright yellow-green, spherical free-living unicells of variable size. Cell wall thin in proportion to size. The one or more chloroplasts contain only chlorophyll a as a major photoactive pigment. Some "cells" may be zoospores or aplanospores.

Growth illum: 11,000 ergs cm^{-2} sec^{-1}
Time: 184 hours

Illumination	Description
405 nm	Clumps of 3-12 average sized cells of good color with ~5 percent ghost cells and a few large green cells with heavy walls.
440	Clumps of 6-12 cells of average to large size of good color with ~10 percent ghost cells. Some cells appear "pregnant" without signs of division internally.
540	Clumps of 2-10 cells of average size of sl. pale color with ~20 percent ghosts. "Pregnant" cells noticed as in 440 nm.
560	Clumps of 4-8 cells of better color than 540 nm with ~5 percent ghosts--otherwise as 540 nm.
620	Clumps of 1-6 cells of pale to good color and of variable size with ~15 percent ghosts. Some large green cells with heavy walls or containing daughter cells.
640	More frequent clumps and larger than 620 nm of better color, otherwise as 620 nm.
650	Clumps of cells as 640 nm of good color and of small to medium size with 10 percent ghosts and a few "pregnant" shaped.
680	As 650 nm except ~25 percent ghosts and some very large, heavy-walled green cells.
710	Clumps of up to 12 cells of best color and overall appearance and of variable size with very few ghosts but with a few heavy-walled as in 680 nm.
White	Clumps of 6-12 cells of good color and of smaller size than normal with some ghosts. A few misshapen cells noticed.

Figure 28: Chlamydomonas reinhardi, X 2600



Chlamydomonas reinhardi

Green, flattened spherical motile unicells with two equal length flagella arising from the anterior region of the cell. The single cup-shaped chloroplast contains chlorophylls a and b as the major photoactive pigments.

Growth Illum: 12,750 ergs cm^{-2} sec^{-1}
Time: 287 hours

Illumination	Description
405	Small cells of pale to good coloring and with fair to good motility. Some small clumping noticeable.
440	Large cells of good coloring and motility grouped largely in tetrads.
540	Small to medium size granular cells colorless or pale in color with minimal motility. Clumps common.
560	As 540 nm with somewhat better pigmentation.
620	Medium size granular cells of pale to good color with poor motility.
640	Medium size cells with clear to granular appearance of pale to good color with fair motility.
650	Medium size cells less granular than 640 nm with good color and fair motility. Some clumping present.
680	Same as 650 nm with many cell doublets.
710	Few living very small granular cells of good color but negligible motility.
White	Small to medium size somewhat granular cells with good color and poor motility. Many tetrads of cells present.

Figure 29: Chlorella pyrenoidosa, X 2600

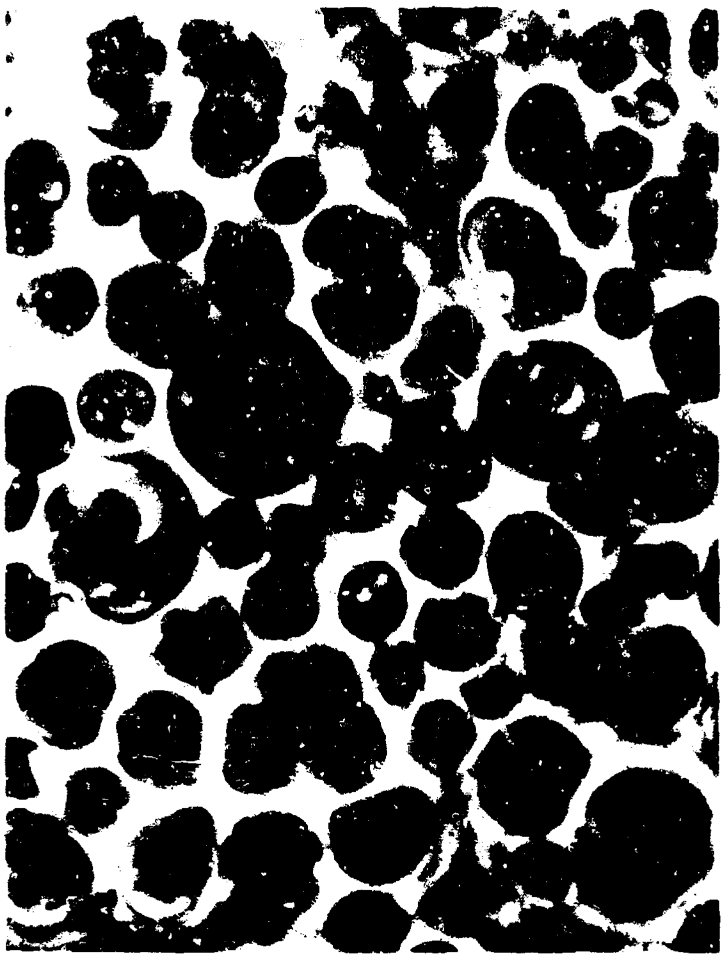
Chlorella pyrenoidosa

Small, spherical, unicells. The single cup-shaped chloroplast contains chlorophylls a and b as the major photoactive pigments.

Growth Illum: 15,000 ergs cm^{-2} sec^{-1}
Time: 256 hours

Illumination	Description
405 nm	Some clumping of small to average sized cells with good color.
440	As 405 nm except cell size ranges from small to large.
540	Increased clumping of variable sized irregular shaped cells from colorless to good coloration.
560	Clumping with medium to large size cells of irregular outline and good color.
620	Clumping with very small cells of irregular outline and good color.
640	Some clumping of small cells with single cells being of variable size and a few of irregular shape. Color is good.
650	Minimal clumping with cells of small to medium size of good color but irregular shape.
680	As 650 nm but with somewhat increased clumping.
710	Normal appearing cells but of a yellow-green color.
White	Considerable clumping of somewhat irregular shaped cells of good color.

Figure 30: Chlorella sorokiniana, (7-11-05), X 2600



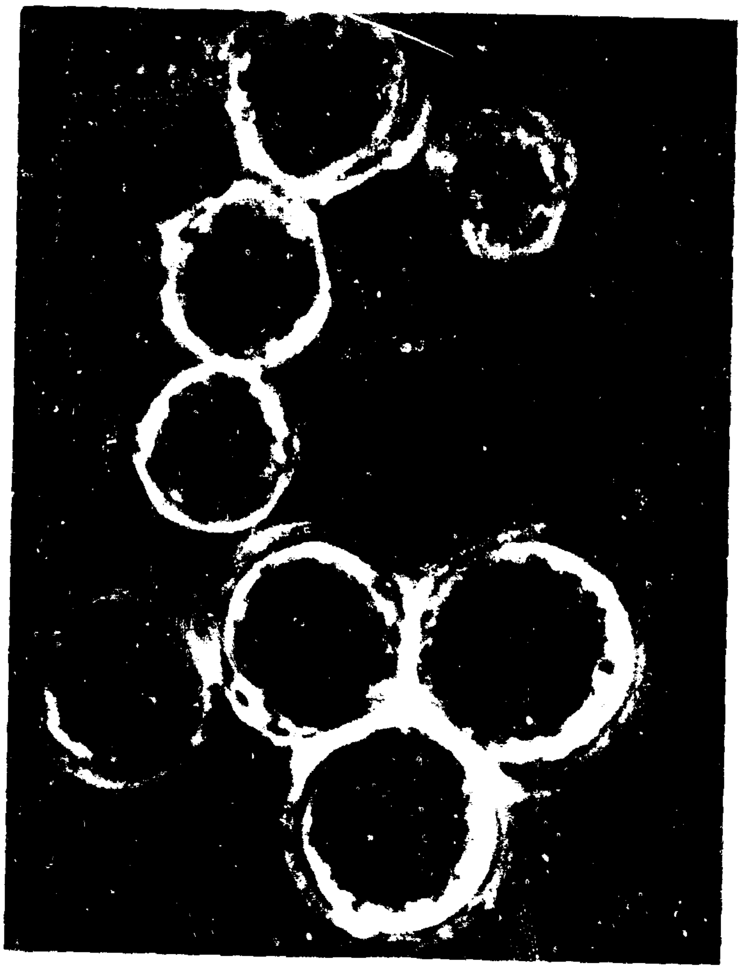
Chlorella sorokiniana (7-11-05)

General description as of *Chlorella pyrenoidosa*.

Growth Illum: 11,500 ergs cm^{-2} sec^{-1}
Time: 228 hours

Illumination	Description
405 nm	Small to oversized cells of good color with clumping of 6-30 cells.
440	As 405 nm with more clumping.
540	Small, uniform sized cells paler than 405 or 440 nm with occassional small clumps of 3-4 cells.
560	Cells of variable size from small to large of a color between 440 and 540 with clumps of 2-10 cells.
620	Small to medium sized cells of light green color with occasional clumps of 3-4 cells.
640	Cells of larger size than 620 nm of same color with increased clumping and many > 10 cells.
650	As 640 nm with some irregular-shaped cells.
680	Medium to oversized cells of good color with clumping of large numbers of cells.
710	Mostly large cells of good color with singles and small clumps of 2-10 cells.
White	Small to average sized cells of good color with occasional small and large clumps.

Figure 31: Chlorococcum wimmeri, X 2600



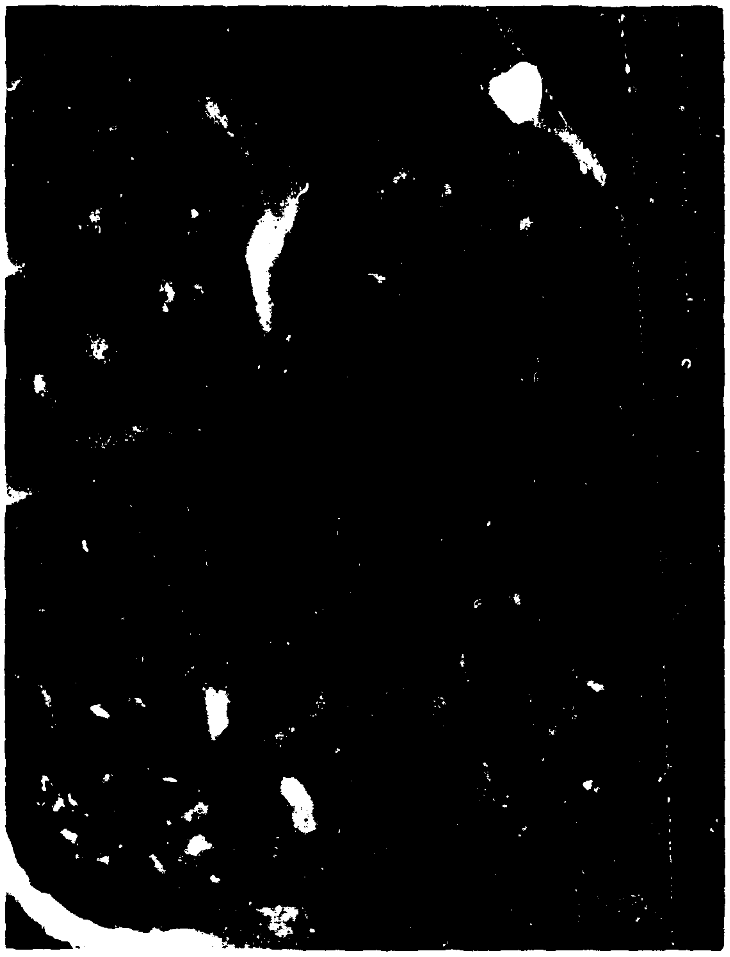
Chlorococcum wimmeri

Large green ovoidal unicells reaching 45 μ. Both aplanospores and zoospores can be seen in cultures. Large cup-shaped chloroplast contains chlorophylls a and b as the major photoactive pigments.

Growth Illum: 14,250 ergs cm^{-2} sec^{-1}
Time: 193 hours

Illumination	Description
405 nm	Bright green cells of small to medium size with many in strings and clumps of 5-15 cells.
440	Green cells of all sizes, some with red centers and occasional zoospores. Cells contain 1-2 large vacuoles. Clumping more pronounced than 405 nm but smaller clumps.
540	Very granular bright green cells of all sizes, some containing red centers and usually with a large vacuole. Small irregular clumps common.
560	Egg-shaped, granular, green cells of all sizes. Some small clumps.
620	Granular, bright-green cells of all sizes. Virtually no clumping, but some in pairs or triplets.
640	Ghost cells and pale green to red cells of all sizes with moderate clumping.
650	Green and bright green cells of all sizes with occasional red centers. Mostly single but a few small clumps and zoospores are prevalent.
680	Mostly small green to red cells with 10 percent ghost cells, largely single but a few small clumps. Some zoospores noticed.
710	Green to bright green mostly small but variable sized with a few ghost cells. Pronounced and irregular clumping.
White	Bright green variable size cells with occasional red centers and 5 percent ghost cells. Some strings and irregular clumping.

Figure 32: Cryptomonas ovata, X 2600



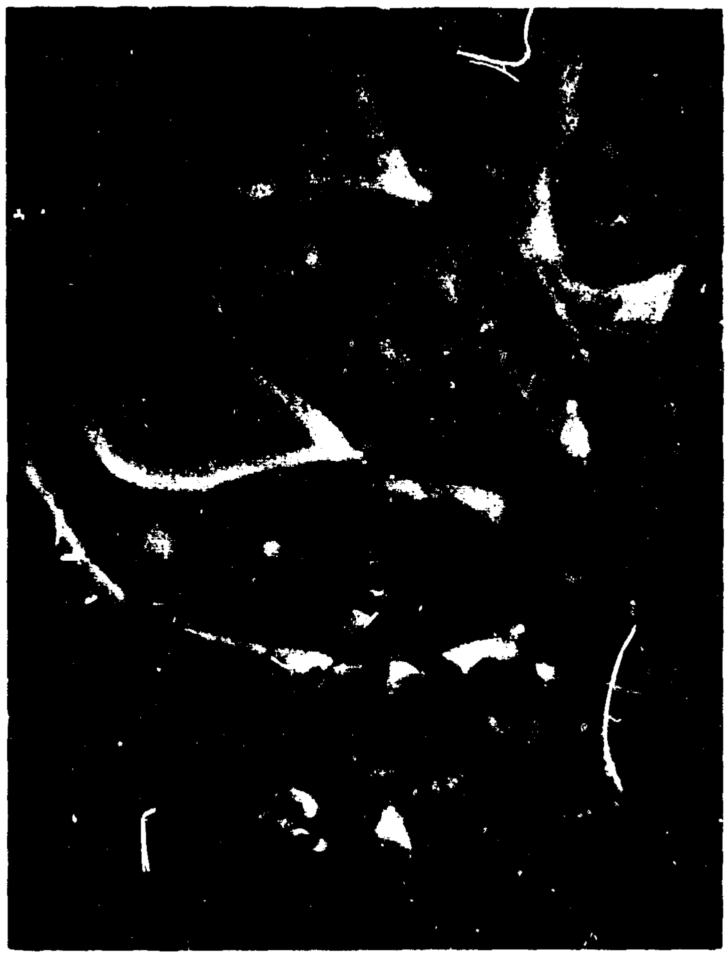
Cryptomonas ovata

Brown to olive to red-green oblong motile unicells with two equal flagella inserted in a gullet. Chloroplasts contain chlorophylls a and c and phycoerythrin as the major photoactive pigments.

Growth Illum: 13,000 ergs cm^{-2} sec^{-1}
Time: 205 hours

Illumination	Description
405 nm	Good cell appearance and color with an occasional green cell $<$ 1/3 motile and clumping.
440	Pale to good coloring with small chloroplasts and approx. 50 percent motility together with clumping.
540	Paler than 405 or 440 nm with poor motility and small clumps.
560	Fair color and motility with some small clumping.
620	Pale, small, non-motile cells with good structure and majority in small clumps.
630	As 620 nm with a few single motile cells.
640	Pale cells with poor motility in large clumps of 20-30 cells.
650	Very pale cells with fair motility in large but loose clumps.
680	Cells of good color with best motility in occasional small loose clumps.
White	Small cells of good color with negligible motility and some clumping.

Figure 33: <u>Euglena gacilis</u>, X 2600



Euglena gracilis

Flexible, elongated free-swimming (motile) green unicell with one flagellum and an eye spot at the anterior end. Numerous (~10) discoid chloroplasts contain chlorophylls a and b as the major photoactive pigments.

Growth Illum: 11,000 ergs cm^{-2} sec^{-1}
Time: 166 hours

Illumination	Description
405 nm	Small cells of very good color and fair to good motility with distinct chloroplasts.
440	Cells 50 percent larger than 405 nm but paler with very good motility and distinct chloroplasts.
540	Cells mostly rounded and other than fully extended, of good color and with distinct chloroplasts but poor motility.
560	Small cells but more fully extended than 540 nm, of good color and with distinct chloroplasts and fair to good motility.
620	Large elongated cells of pale to good color with good motility and distinct chloroplasts.
640	Sl. smaller cells than 620 nm with most somewhat bulbous and of good color but with motility less than 620 nm and less distinct chloroplasts.
650	Large cells with many rounded and pale to good color having very good motility and distinct chloroplasts.
680	Largest elongated cells of good color with good motility and clear and distinct chloroplasts.
710	Variable sized cells, pale, with fair to good motility, granular contents and rather indistinct chloroplasts.
White	Cell size between 405 and 440 nm with many rounded, of good but sl. pale color having distinct chloroplasts but sluggish to good motility.

Figure 34: Gloeocapsa alpicola, X 2600

Gloeocapsa alpicola

Small, spherical, blue-green unicells containing chlorophyll a and phycocyanin as the major photoactive pigments. Prominent sheath usually found.

Growth Illum: 15,000 ergs cm^{-2} sec^{-1}
Time: 170 hours

Illumination	Description
405 nm	Large cells with sheaths and of good color occurring as singles, doublets, tetrads, and clumps.
440	Small cells with large sheaths, of good color occurring as doublets, tetrads, and small clumps.
540	Small to medium cells with sheaths and of good color occurring largely as doublets with few tetrads or clumps.
560	Cells and sheaths as 540 nm cells of good color and mostly in tetrads with some doublets and clumps.
620	Small cells of good color occurring as singles, doublets and small clumps.
630	Small to average sized cells of good color in doublets, tetrads and small clumps.
640	Average to large cells of good color as singles, doublets, tetrads and small clumps.
650	As 640 nm with considerably more clumping.
680	As 650 nm with fewer single cells.
White	Large cells of good color occurring as singles, doublets, and tetrads with very few clumps.

Figure 35: Nitzschia closterium, X 2600

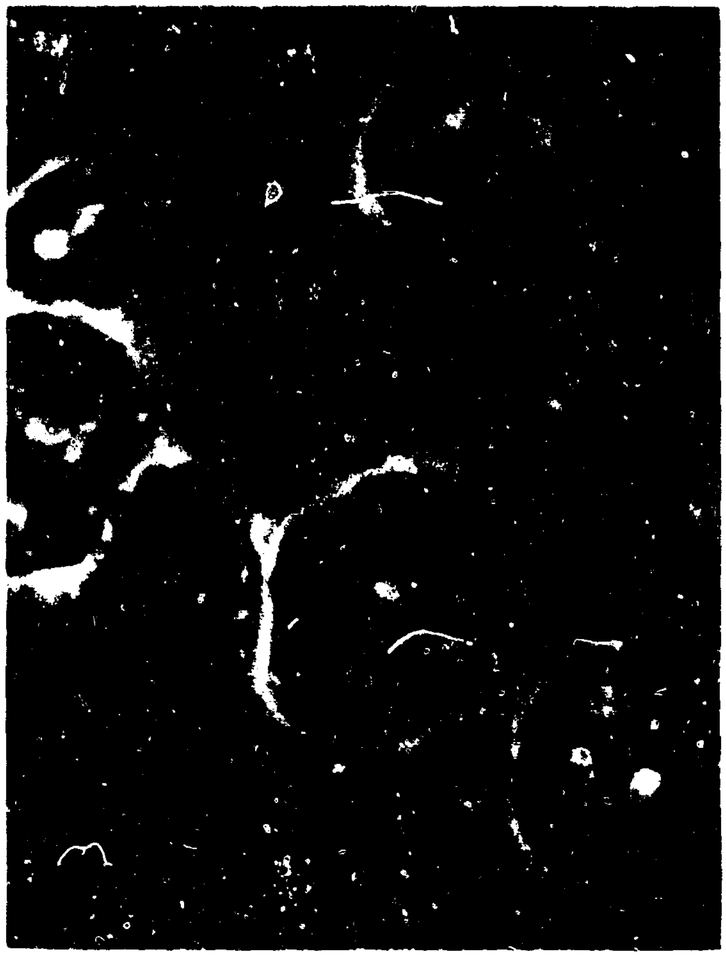
Nitzschia closterium

Elongate unicell with central chromatophores and containing two valves. Chloroplast, yellow to olive green stretched diagonally across cell and into each valve face, contains chlorophylls a and c, and fucoxanthin as major photo-active pigments.

Growth Illum: 12,750 ergs cm^{-2} sec^{-1}
Time: 240 hours

Illumination	Description
405 nm	Large typical to irregular shaped cells yellow to olive green in color with asymmetrical chloroplast. Large clumps noticeable.
440	Cells as 405 nm, golden to green in color with large, distinct chloroplast, generally in large, loose clumps (more so than 405 nm).
540	Cells as 405 nm, yellow green to green with large, distinct chloroplasts and generally in tight clumps.
560	As 540 nm with some baseball-bat-shaped cells in smaller clumps.
620	Some bat-shaped green cells with relatively large chloroplasts and clumping.
630	Majority, typical shaped yellow green cells with larger chloroplasts than 620 nm and in very large clumps.
640	Some asymmetrical cells, green with large chloroplasts and clumping.
650	Green cells, some large and round, in large clumps.
680	As 640 nm, quite green with smaller chloroplasts and in smaller clumps with more single cells.
White	Smaller, green cells with variable-sized chloroplasts in small clumps with many single cells.

Figure 36: Ochromonas danica, X 2600



Ochromonas danica

Golden yellow-green motile unicells with two unequal length flagella. Chloroplasts of irregular shape containing chlorophyll a and fucoxanthin as major photoactive pigments.

Growth Illum: 15,000 ergs cm^{-2} sec^{-1}
Time: 164 hours

Illumination	Description
405 nm	Variable sized cells with many atypically shaped having bulbous tails, large vacuoles but distinct chloroplasts. Some dead and some small and rounded. Good color with 40-60 percent motile.
440	Cells as 405 nm with 50 percent having bulbous tails and rare dead cells. Good color with distinct chloroplasts and 75 percent motility.
540	Cells of uniform size without bulbous tails but with large vacuole. Colorless to pale with a few small green chloroplasts and 80 to 90 percent motility.
560	Cells with bulbous tails and large vacuoles. Majority colorless but with more greens than 540 nm and small chloroplasts, 80 percent motile. Colorless cells often with side bulges.
620	Variable sized cells with some very large, mostly of typical shape with occ. misshapen and bulbous tails, large vacuole. Color pale-green to green with 25-75 percent motile.
640	Variable size cells from very small to large with some in division, many granular with large vacuole. Color golden with some green cells, non-motile or sluggish with occ. clumps of granular cells.
650	Variable size cells with some very large of generally typical shape with occ. bulbous tails, many with large vacuoles. Color fair with 25-75 percent motile.
680	Bulbous-tailed cells of variable but smaller size than 650 nm having less well discernable vacuoles with some granular. Color paler but more active than 650 nm.
710	Variable size and shaped cells from very unusual to typical with bulbous and otherwise shaped "tails", large vacuole. Poor color with poorly discerned chloroplasts, sluggish (~25 percent motile).
White	Cells of typical shape with occ. bulbous tails and variable sized chloroplasts and vacuoles. Approx. 25 percent colorless cells with rest of good color and mostly motile.

Figure 37: Phormidium luridum, X 2600

Phormidium luridum

Blue-green filaments of cylindrical shape with gelantinous sheaths. Cells granular in appearance (no chloroplasts) and contain chlorophyll a and phycocyanin as major photoactive pigments.

Growth Illum: 14,250 ergs cm^{-2} sec^{-1}
Time: 170 hours

Illumination	Description
405 nm	Short to medium length filaments of good color with majority of approx. 10 cells in length.
440	As 405 nm.
540	Long to medium length filaments (a few short) of good color.
560	As 540 nm with filaments somewhat longer.
620	Short to long length filaments of good color with majority medium to long.
640	Filaments overall somewhat better than 620 nm; of good color and short to long with majority long.
650	Short to long filaments of good color with majority short to medium and some very short.
680	Short to long filaments of paler color than others; majority short to medium.
710	Short filaments of good color with a generally "fat" appearance.
White	Short to long filaments of good color.

Figure 38: Phormidium persicinum, X 2600

Phormidium persicinum

Red cylindrical filaments of granular appearance. Cells contain chlorophyll a, phycoerythrin and trace amounts of phycocyanin as major photoactive pigments.

Growth Illum: 14,250 ergs cm^{-2} sec^{-1}
Time: 170 hours

Illumination	Description
405 nm	Majority of filaments single and medium to long in length, of pale to good color and in some small, loose clumps.
440	As 405 nm with somewhat more clumping.
540	Filaments very long with very good color and many in loose clumps.
560	As 540 nm except more single filaments.
620	Filaments short to long of good color and occ. in small to large loose clumps.
630	As 620 nm except filaments longer.
640	Filaments medium to long with majority long, of good color and better appearance than 620 and 630 nm samples; some in small tight clumps.
650	Filaments loose and medium to long in length, of good color and only occ. clumps.
680	Filaments short to long, of good color with some loose clumping.
White	Filaments short to long, generally shorter than other samples but of good color.

Figure 39: *Porphyridium aerugineum*, X 2600

Porphyridium aerugineum

Blue-green spherical unicells with gelatinous sheath. Massive single chloroplast contains chlorophyll a, phycocyanin and a possible trace of phycoerythrin as major photoactive pigments.

Growth Illum: 15,550 ergs cm^{-2} sec^{-1}
Time: 169 hours

Illumination	Description
405 nm	Many large single cells with small cells in clumps having large chloroplasts of blue-green to green-blue color. Cells with average sheath.
440	Mostly single cells of average to larger size than 405 nm with smaller chloroplast of blue-green to quite green in color and thicker sheath than 405 nm.
540	Singles and clumps of cells larger than 440 nm of good color (quite blue-green) with large chloroplast. Excellent appearing cells.
560	Single and small clumps (2,4,6) of cells of variable size with chloroplasts as 440 nm of green-blue to green color. Cells in clumps having heavy sheath.
620	Cells of irregular shape relatively uniform in size with occasional large cell. All having large chloroplast of good blue-green color. Considerable clumping from small to large groups.
640	Cells of variable size, from small to large with large chloroplast of pale to good color generally in small to large clumps.
650	Mainly small to large single cells with large chloroplast of good to green-blue color and clumping.
680	Cells of irregular shape and variable in size as singles or in clumps with large chloroplast of good color and heavy sheaths.
710	Primarily single cells of large size with large chloroplast of good blue-green color.
White	Mainly solitary cells of average to large size with large chloroplast of blue-green to green-blue in color.

Figure 40: Porphyridium cruentum, X 2600

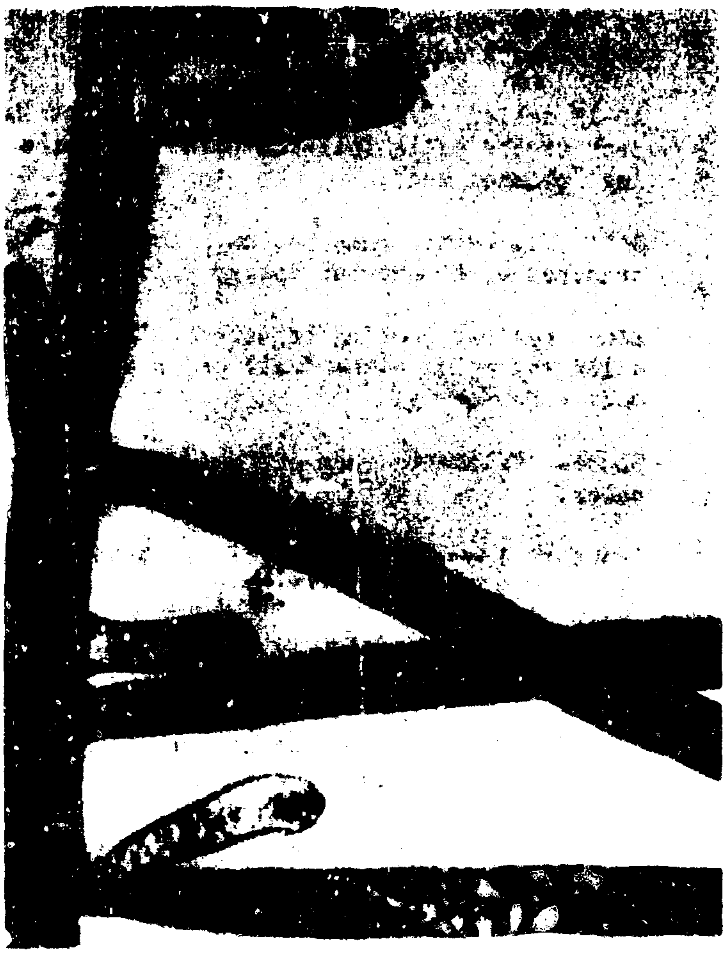
Porphyridium cruentum

Red spherical unicell with cells surrounded by a gelatinous sheath. Single massive, stellate chloroplast contains phycocyanin, phycoerythrin and chlorophyll a as major photoactive pigments.

Growth Illum: 14,500 ergs cm^{-2} sec^{-1}
Time: 174 hours

Illumination	Description
405 nm	Average size cells solitary or in clumps of 2-4, somewhat granular with a few ghost cells. Cells of good color with heavy sheaths.
440	Average size largely paired cells of good color.
540	Average size cells with crenated edges, solitary or in pairs, of pale green to green color.
560	Average size cells with occasional large (2 x normal) and tending to pair, of pale color with many colorless cells present, heavy sheath.
620	Average to sl. small in size, some as singles with majority in clumps of 2-5, of green color or green with red centers.
630	Average size cells with increased clumping (>620) in 2-4's of fair color with both green and red areas, heavy sheaths.
640	Average to large size with increased clumping in groups of 2-8, having color as 630 nm; some with heavy sheaths.
650	Size as 640 nm, somewhat granular with less clumping but a few large clumps (~10 cells) and color as 640 nm, a few with heavy sheaths.
680	Cells variable in size from small to large, solitary or in groups of 2-4 of good color with heavy sheaths.
White	Cells of average size, paired or in small clumps, rarely solitary, of good color.

Figure 41: Sphacelaria sp., X 500



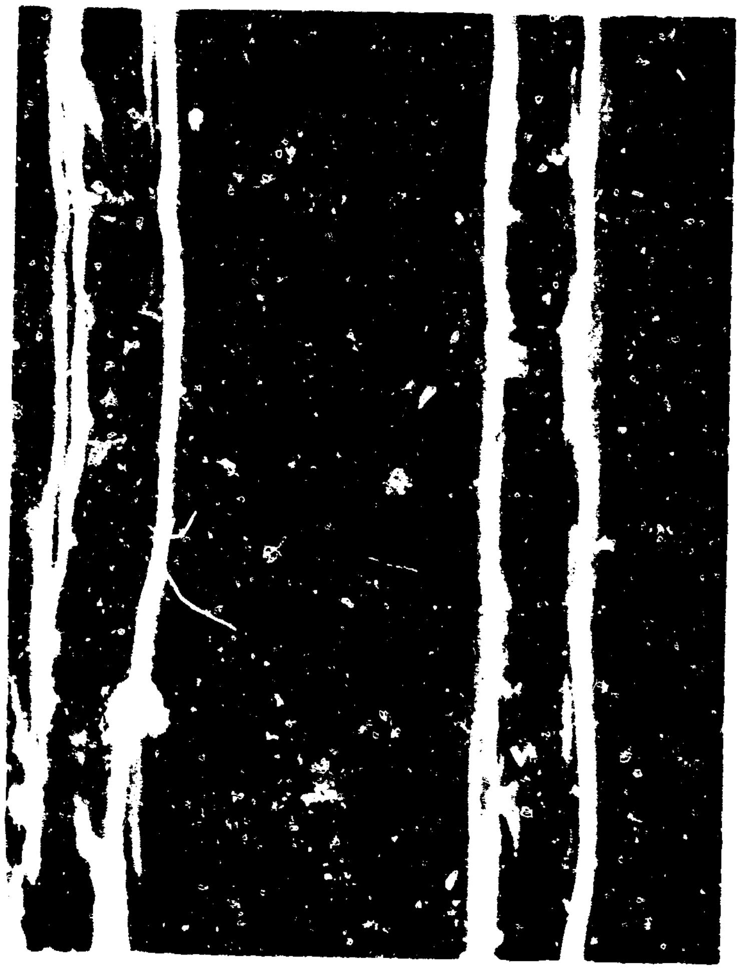
Sphacelaria sp.

Deep brown branched filaments normally growing in colonial tufts. Filaments usually more than one cell in width. Numerous small chloroplasts contain chlorophylls a and c and fucoxanthin as major photoactive pigments.

Growth Illum: 13,500 ergs cm^{-2} sec^{-1}
Time: 278 hours

Illumination	Description
405 nm	15-20 celled branched filaments with 50 percent of the cells full and of good color, the rest being empty.
440	As 405 nm except only 25 percent of the cells are full and of good color.
540	10-15 celled largely unbranched filaments with 75 percent of the cells full but pale in color and many appearing "plasmolyzed".
560	Generally better than 540 nm. Longer filaments with small branches with somewhat darker color and fewer "plasmolyzed".
620	Long, typical looking branched filaments of pale to good color and cells either full or "plasmolyzed" appearing with only a few empty.
640	Shorter filaments than 620 nm with bulbous branching and paler.
650	Short to long filaments with few branches having granular cells of pale color with 50 percent full and some empty.
680	Short, branched filaments of granular appearing cells of pale to good color.
710	Short filaments with bulbous branching of very pale, largely empty cells.
White	Typical filaments having 75 percent filled cells of fair color.

Figure 42: Tribonema aequale, X 2600



Tribonema aequale

Unbranched yellow green filaments with uniform barrel-shaped cells whose wall consists of two equal, slightly overlapping halves. Evidence of broken cells are the appearance of cell wall "H" pieces. Variable number of elipsoid chloroplasts contain chlorophyll a and a trace of e as major photoactive pigments.

Growth Illum: 12,250 ergs cm^{-2} sec^{-1}
Time: 170 hours

Illumination	Description
405 nm	Medium to long (10-20 cells) filaments of good color with 3-8 large chloroplasts per cell. ~20 percent broken, short (2-4 cells), hollow filaments present.
440	Medium to long filaments of good color with 3-5 large chloroplasts per cell. ~50 percent H-pieces and hollow cells present.
540	Short to medium (~10 cells) filaments of pale green color with 2-4 large amorphous appearing chloroplasts per cell. ~ 80 percent broken, hollow cells and H-pieces present.
560	Short to medium (mostly short) filaments of pale green color with 3-5 large chloroplasts per cell. 80-90 percent hollow cells and H-pieces present.
620	Long to very long filaments (~ 30 cells) of sl. pale color with ~4 large, distinct chloroplasts per cell. 25 percent broken filaments and H-pieces, few hollow cells, some filaments with "balled" contents.
640	Medium to very long filaments of good color with 4-6 large chloroplasts per cell. 30-40 percent broken, hollow cells and H-pieces present.
650	Long to very long filaments of good color with 4-6 large chloroplasts per cell. 20 percent hollow cells and H-pieces present with some cells having "balled" contents.
680	Long to very long filaments of good color with 2-4 very large chloroplasts per cell. 10 percent hollow cells and H-pieces, some cells with "balled" contents.
710	Short to medium (10-15 cells) filaments of good color with ~ 4 large and distinct chloroplasts per cell. 30-40 percent hollow cells and H-pieces present.
White	Long filaments of good color with 3-5 large chloroplasts per cell. 10 percent hollow cells and H-pieces present.

Appendix B

Absorption spectra of whole algal cells grown in white light. Pertinent data are indicated on each spectrum.

Fig. 43: *Amphidinium sp.*

Fig. 44: *Botrydiopsis alpina*

Fig. 45: *Chlamydomonas reinhardi*

Fig. 46: *Chlorella pyrenoidosa*

Fig. 47: *Chlorella sorokiniana* (7-11-05)

Fig. 48: *Chlorococcum wimmeri*

Fig. 49: *Cryptomonas ovata*

Fig. 50: *Euglena gracilis*

Fig. 51: *Gloeocapsa alpicola*

Fig. 52: *Nitzschia closterium*

Fig. 53: *Ochromonas danica*

Fig. 54: *Phormidium luridum*

Fig. 55: *Phormidium persicinum*

Fig. 56: *Porphyridium aerugineum*

Fig. 57: *Porphyridium cruentum*

Fig. 58: *Sphacelaria sp.*

Fig. 59: *Tribonema aequale*

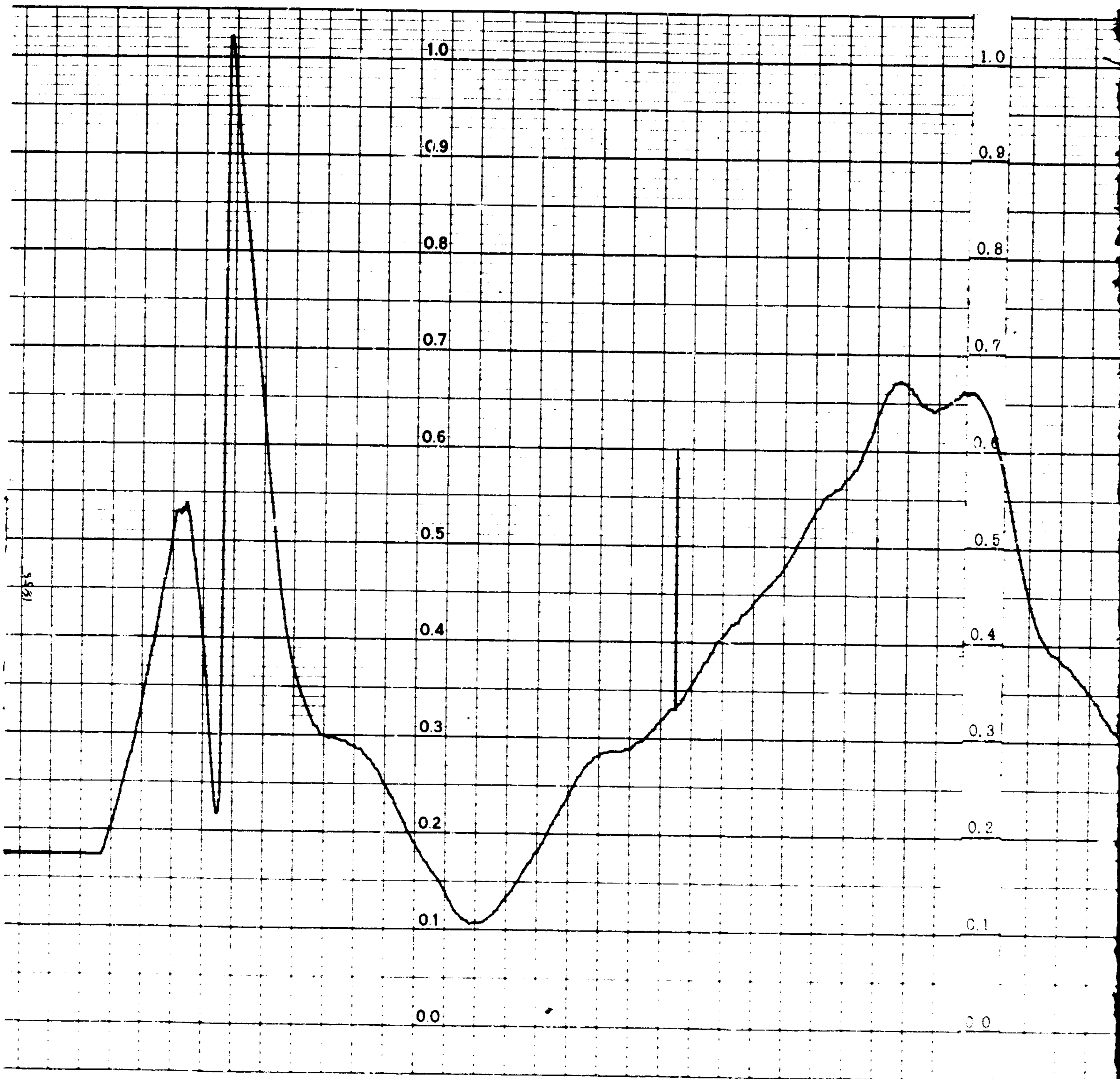
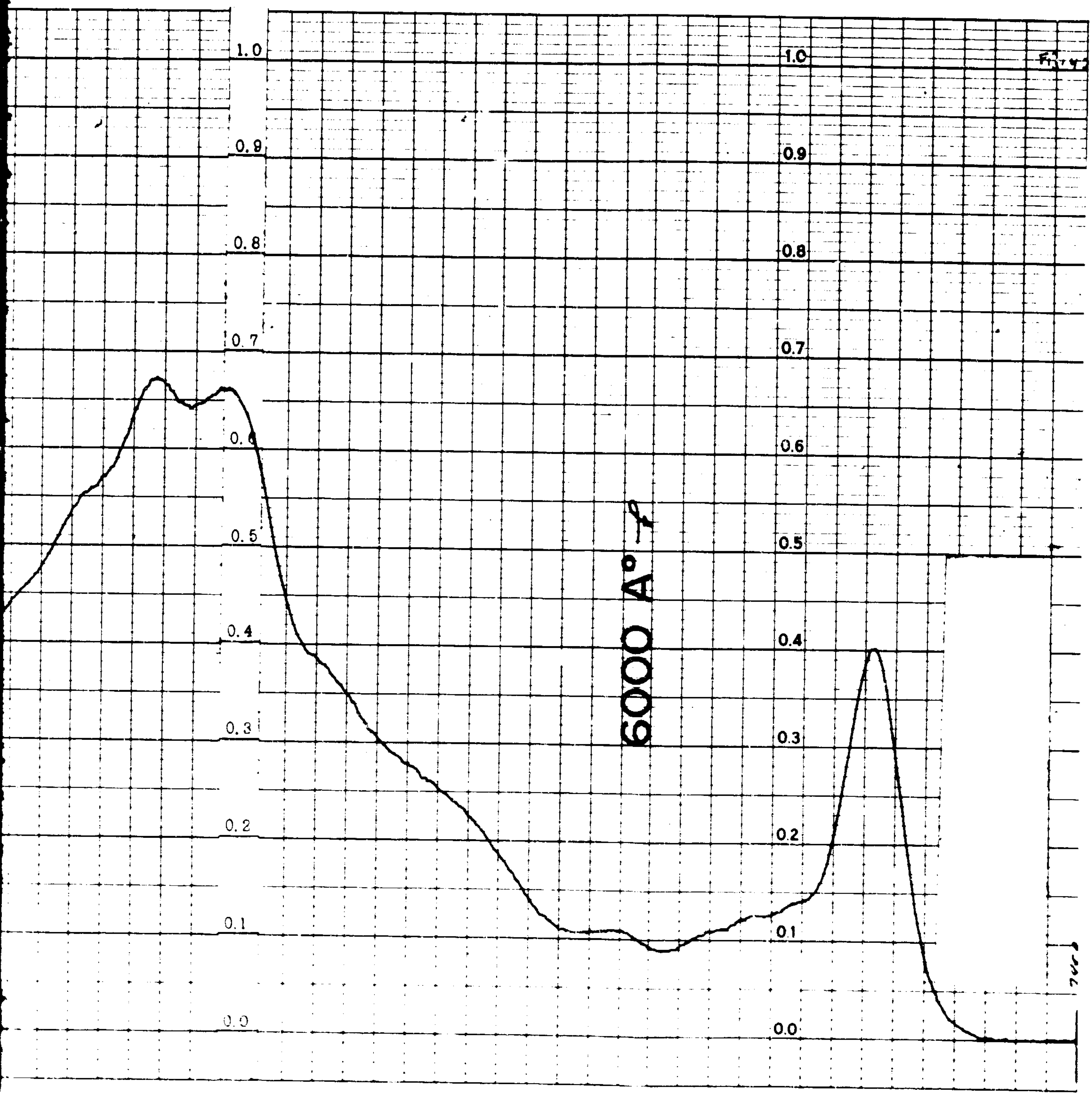


Figure 43: Sample: Amphidinium sp. Sample Conc: 1
25 A°/sec. Scan: 100 A°/div. Solvent:
5 in/min.

A



Amphidinium sp. Sample Conc: 1 ul/ml Scan Speed:
c. Scan: 100 A°/div. Solvent: H_2O Chart Speed:

B

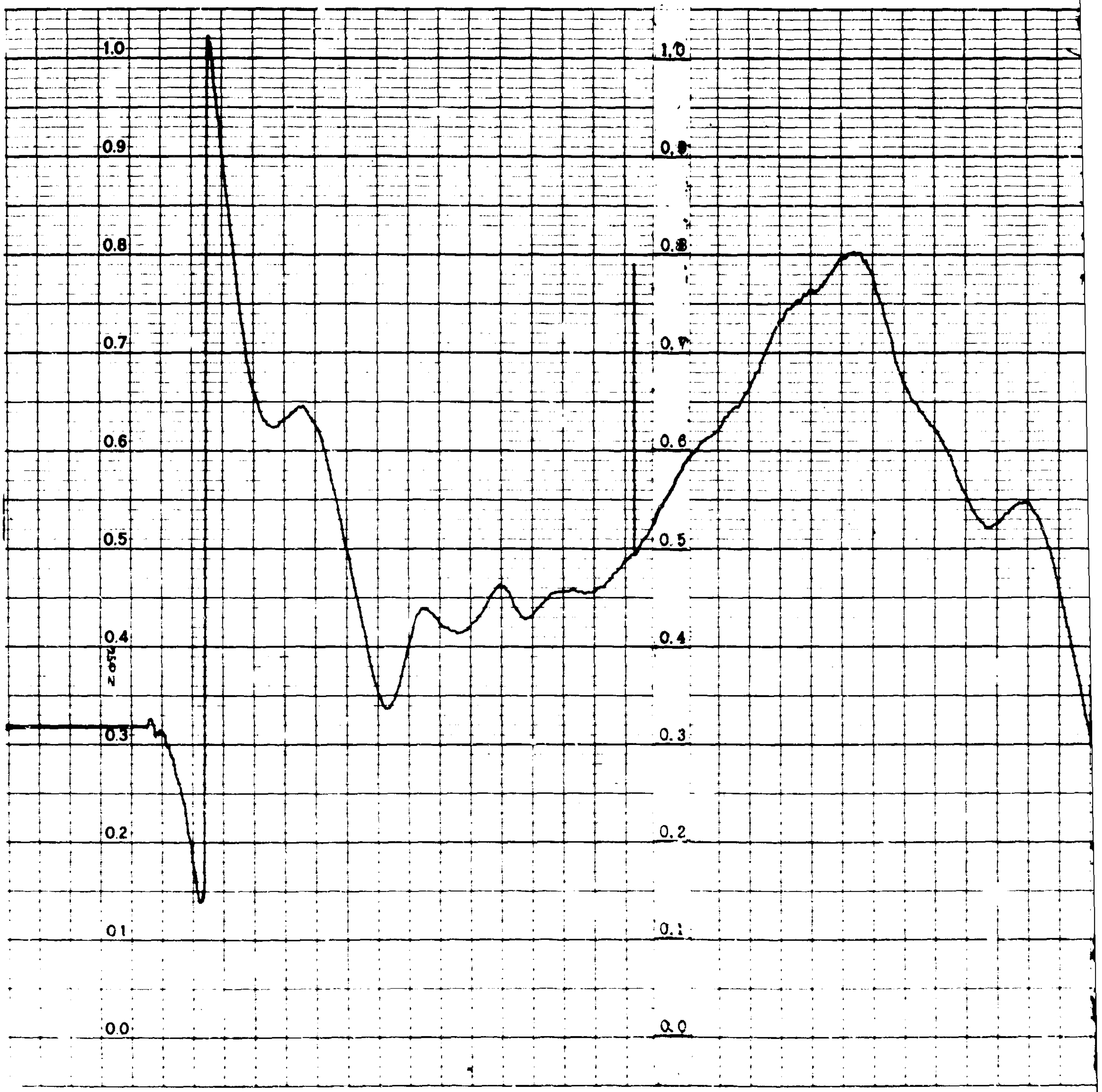
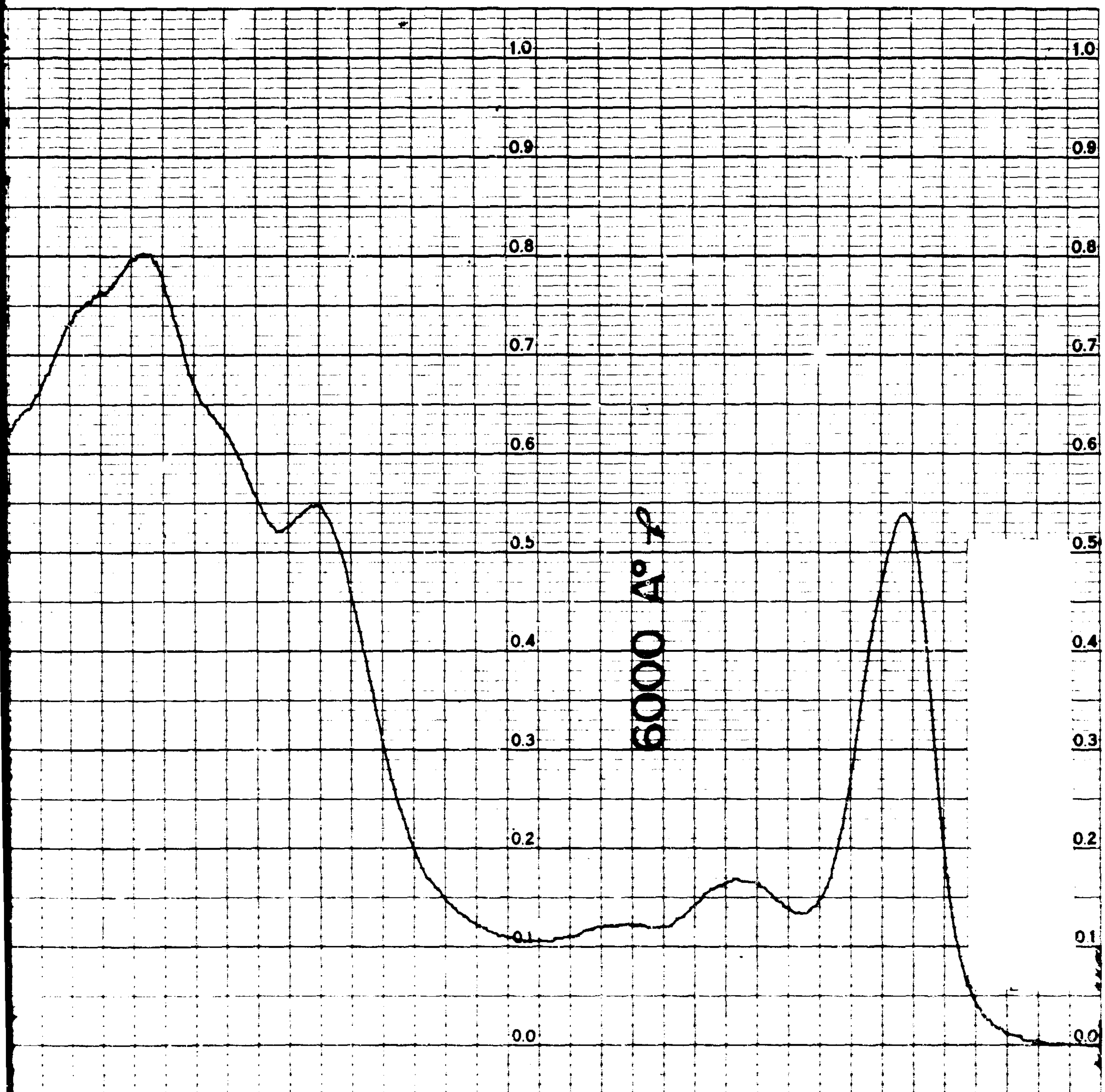


Figure 44: Sample: Botrydiopsis alpina Sample Conc
Speed: 2? A°/sec. Scan: 100 A°/div. S
Speed: 5 in/min.

f)



Botrydiopsis alpina Sample Conc: 20 ul/ml Scan 25 A°/sec. Scan: 100 A°/div. Solvent: H_2O Chart 5 in/min.

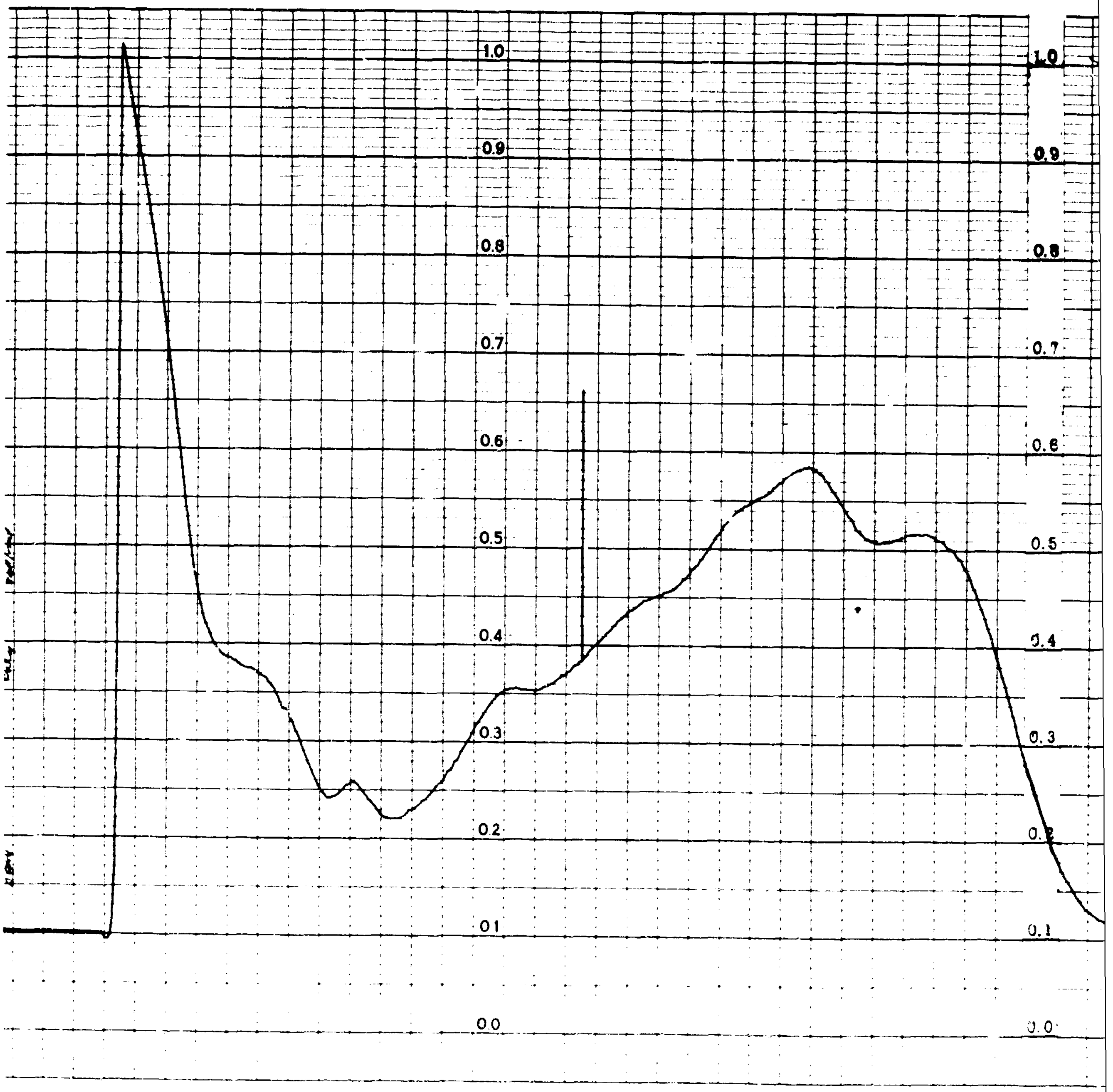
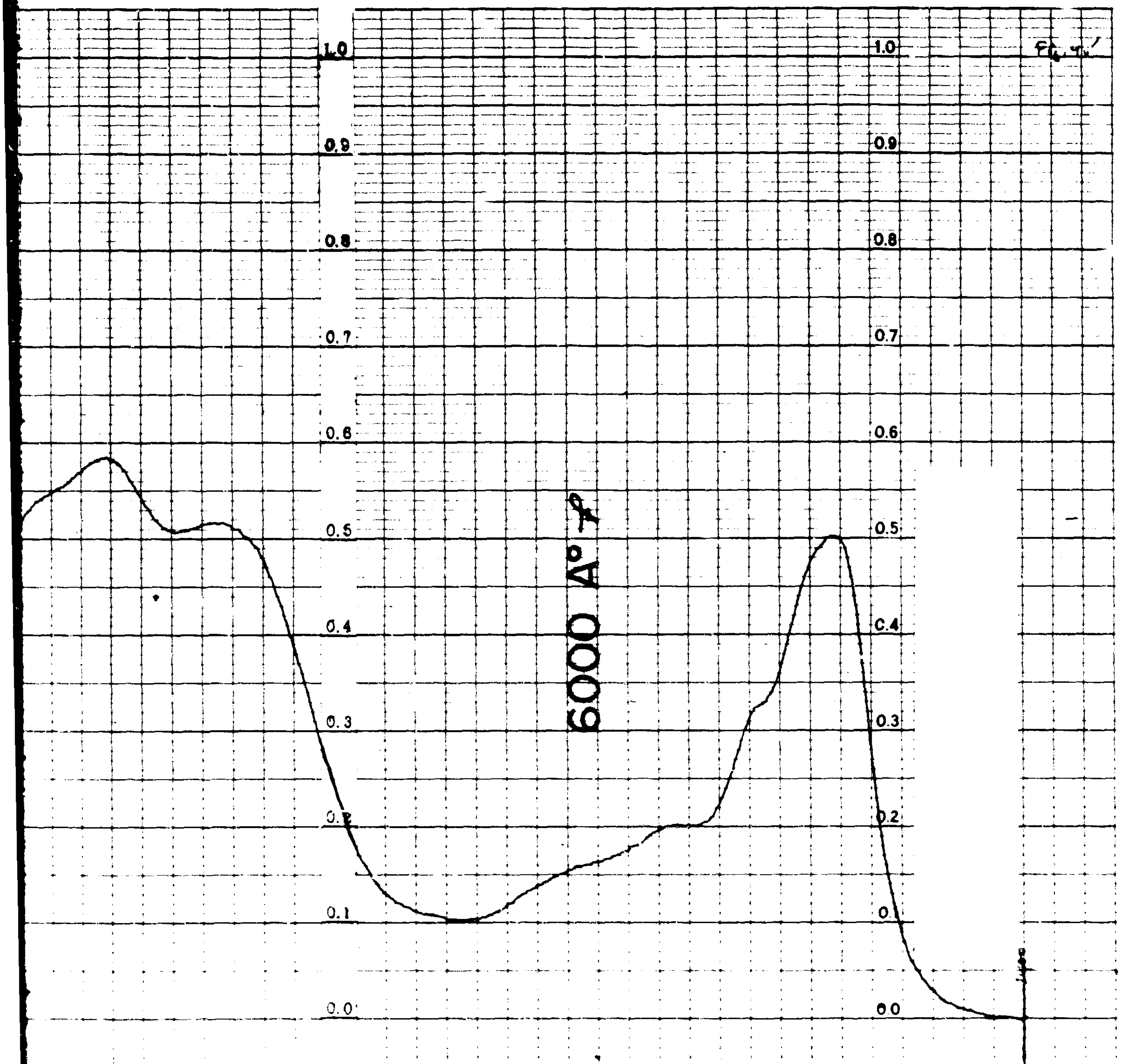


Figure 45: Sample: Chlamydomonas reinhardi Sample Co
Speed: 25^{o}A /sec. Scan: 100^{o}A /div. Sol
Speed: 5 in/min.

A



<u>Chlamydomonas</u> <u>reinhardi</u> Sample Conc: 3 ul/ml Scan 25°A /sec. Scan: 100°A /div. Solvent: H_2O Chart 5 in/min.

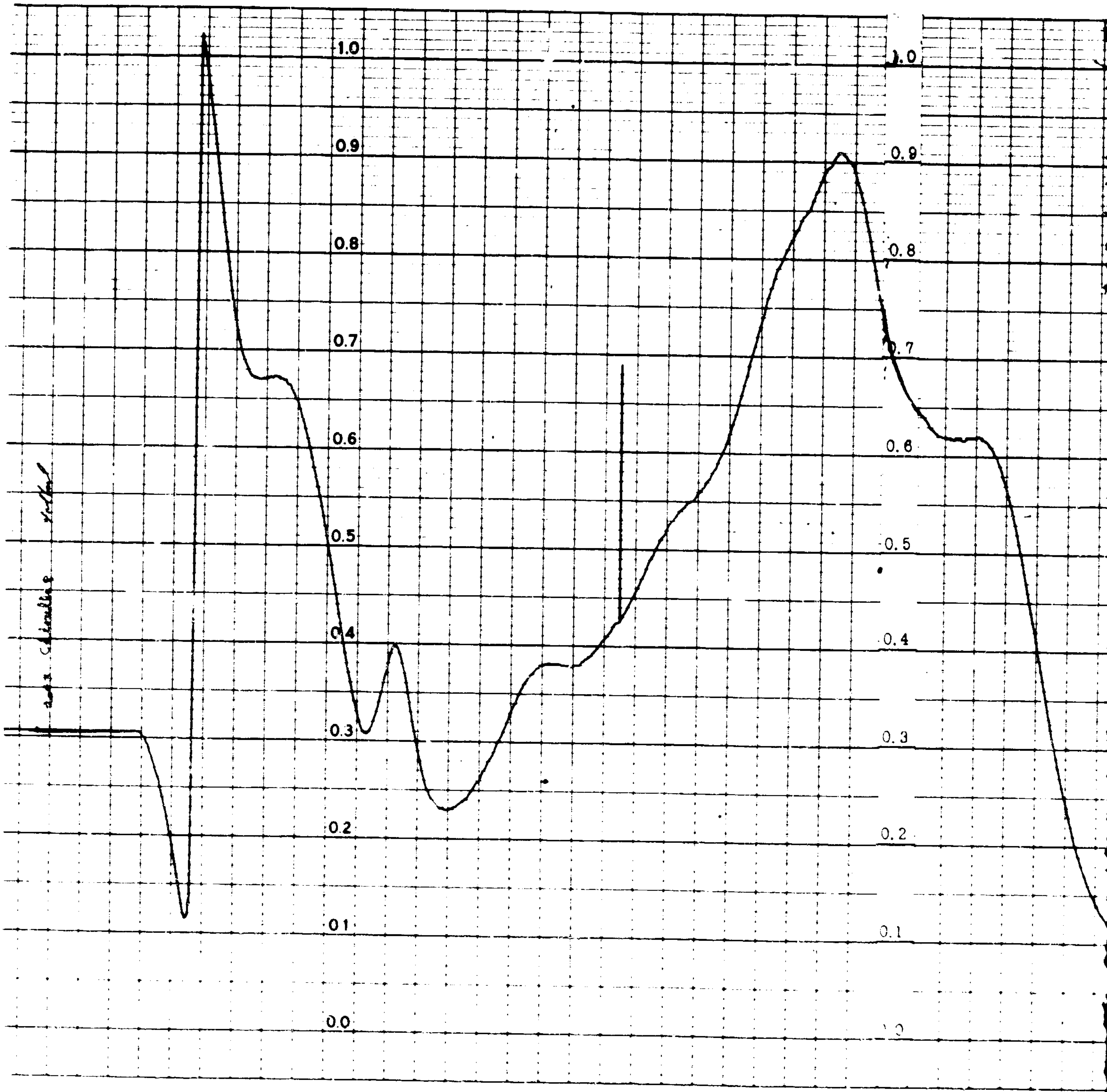
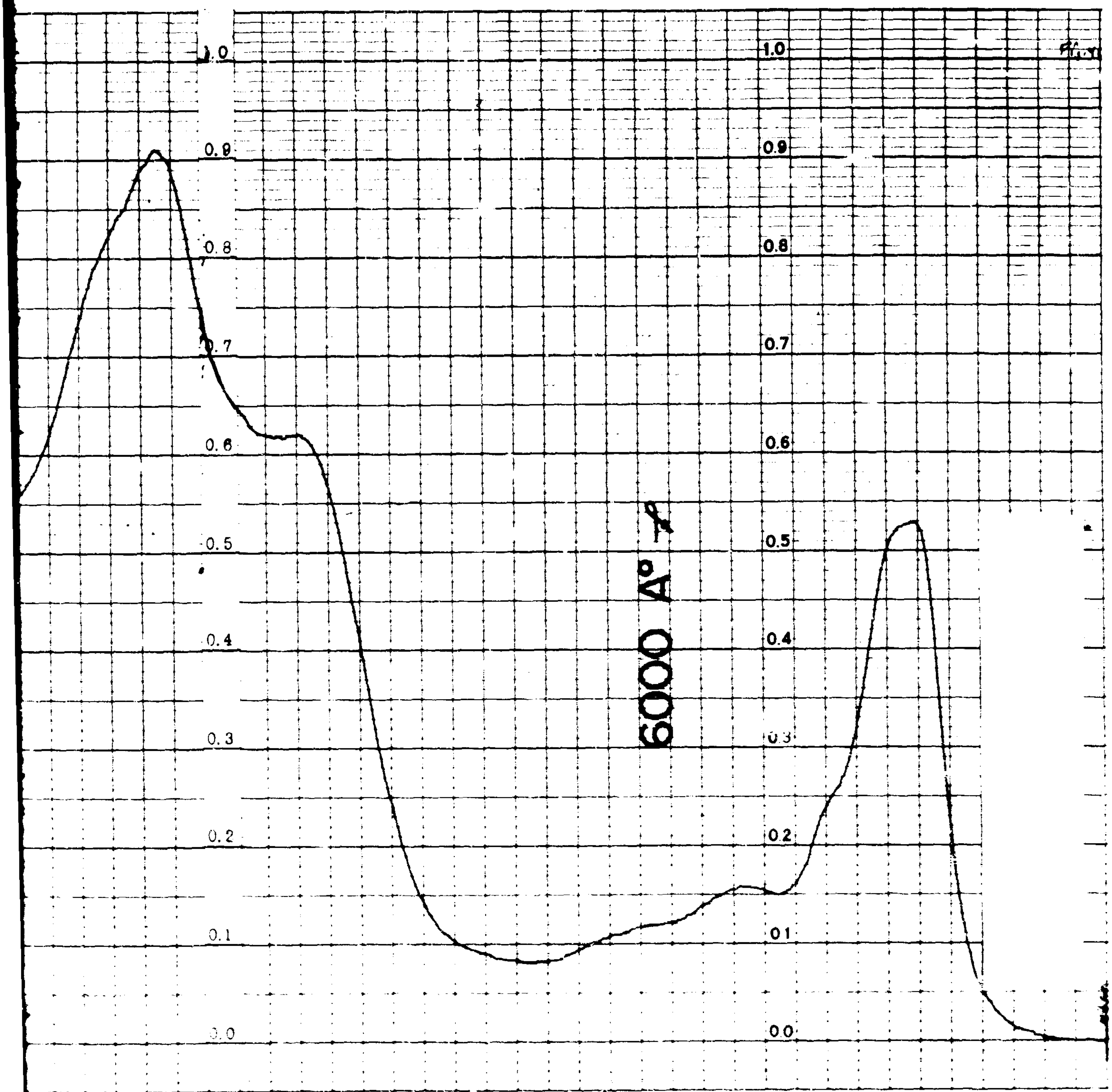


Figure 46: Sample: Chlorella pyrenoidosa Sample Con
Speed: 25 A°/sec. Scan: 100 A°/div. So
Speed: 5 in/min.

A



■: Chlorella pyrenoidosa Sample Conc: 4 ul/ml Scan
: 25 A°/sec. Scan: 100 A°/div. Solvent: H_2O Chart
: 5 in/min.

B

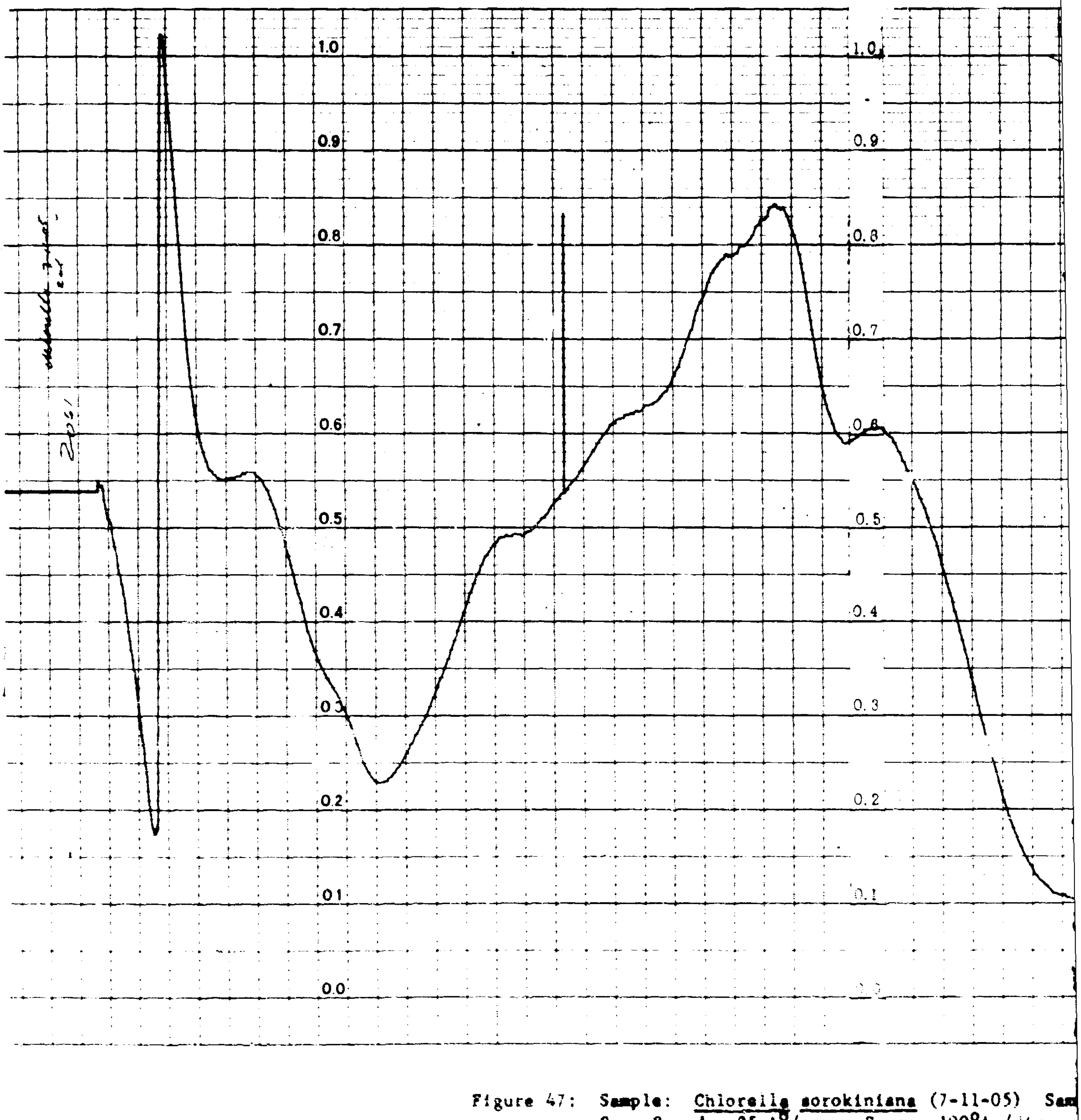
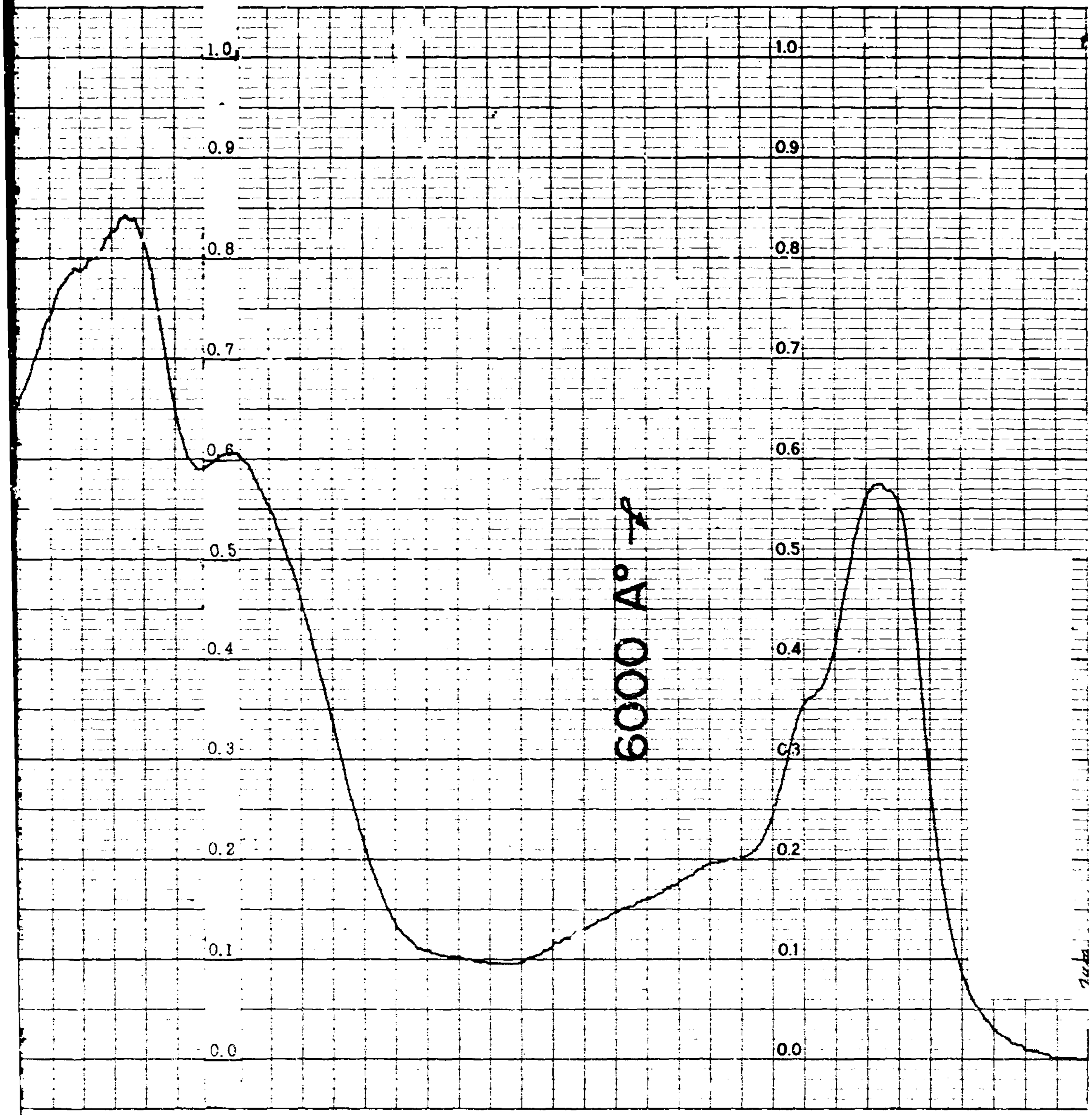


Figure 47: Sample: Chlorella sorokiniana (7-11-05) Sam
Scan Speed: 25 A°/sec. Scan: 100°A /div.
Chart Speed: 5 in/min.



Chlorella sorokiniana (7-11-05) Sample Conc: 2 ul/ml
eed: 25 A°/sec. Scan: 100°A /div. Solvent: H_2O
peed: 5 in/min.

B

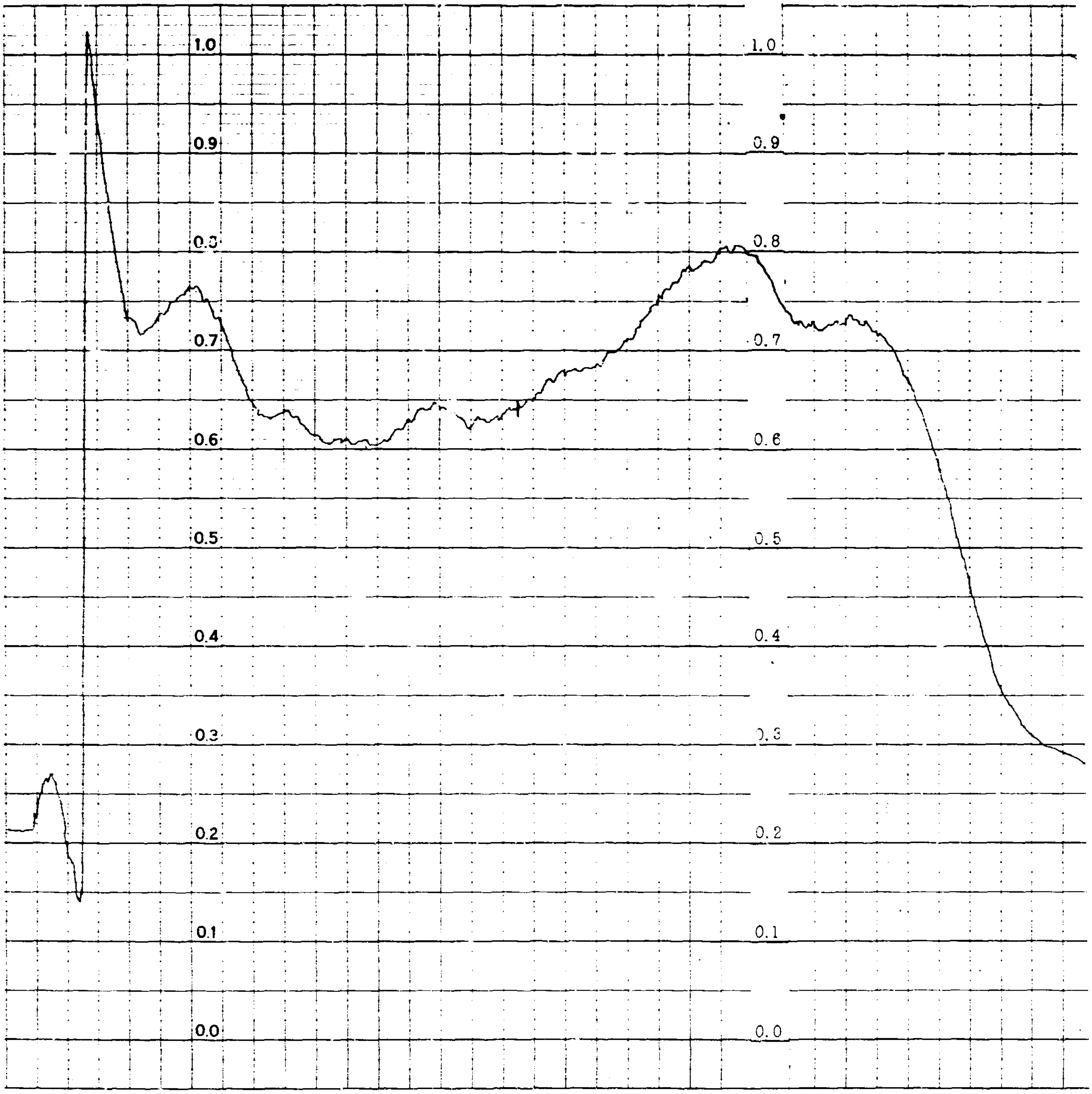
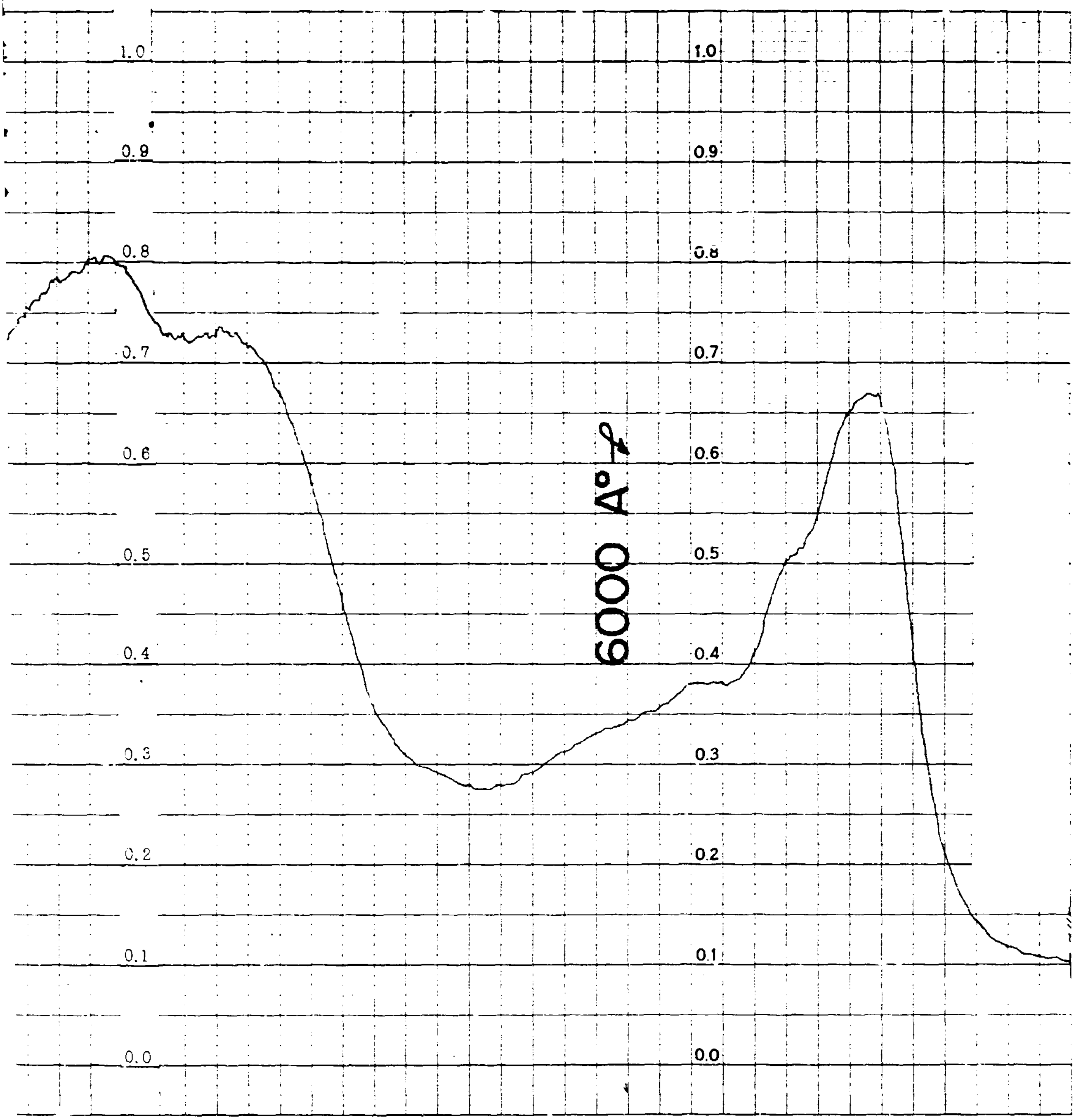


Figure 48: Sample: Chlorococcum wimmeri Sample Conc: 4
Speed: 25 A°/sec. Scan: 100 A°/div. Solven
Speed: 5 in/min.

A

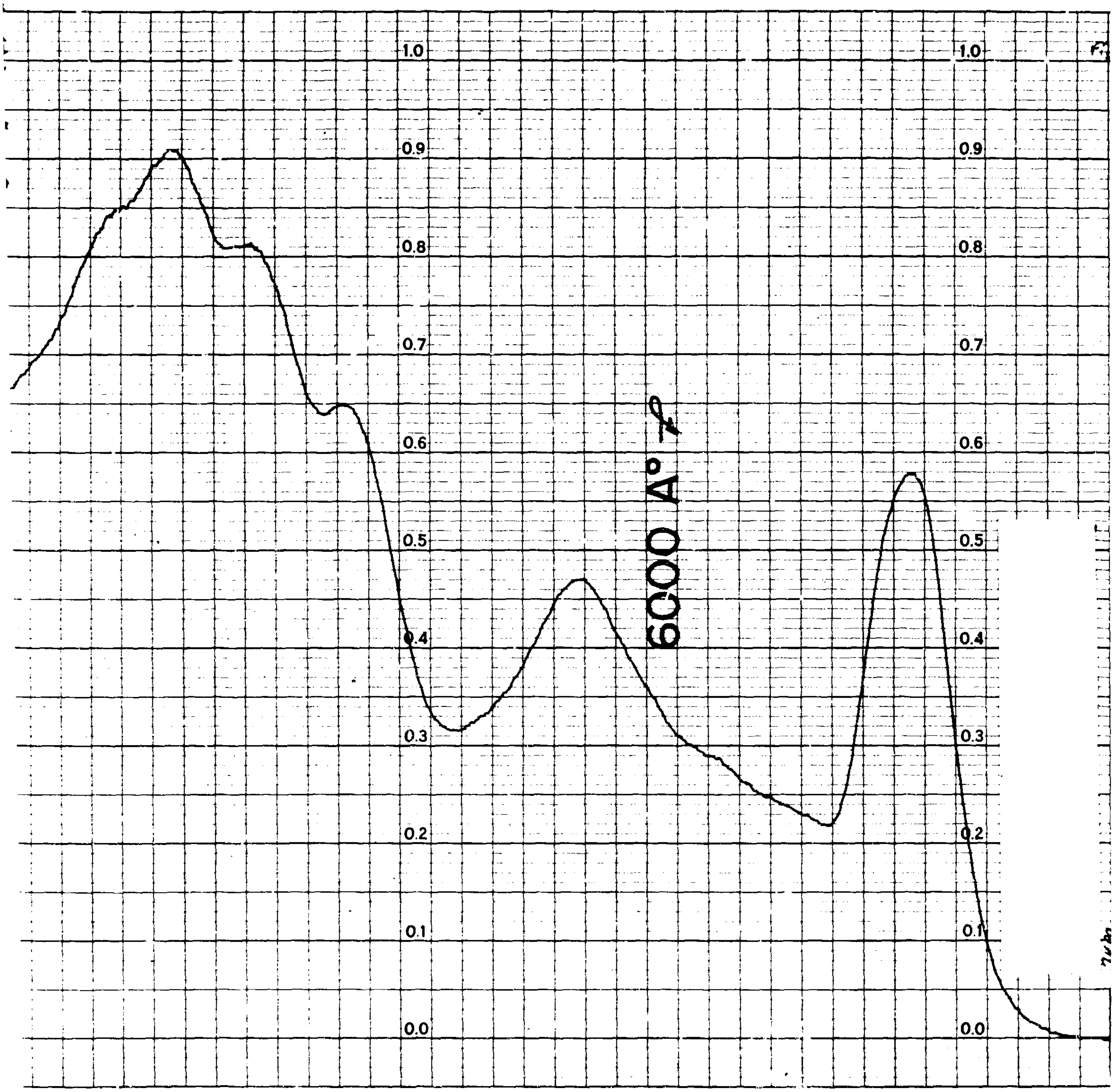


: Chlorococcum wimmeri Sample Conc: 4 ul/ml Scan 25 A°/sec. Scan: 100 A°/div. Solvent: H_2O Chart 5 in/min.

B

Figure 49: Sample: Cryptomonas ovata Sample Conc: 25 A°/sec. Scan: 100 A°/div. Solvent: Speed: 5 in/min.

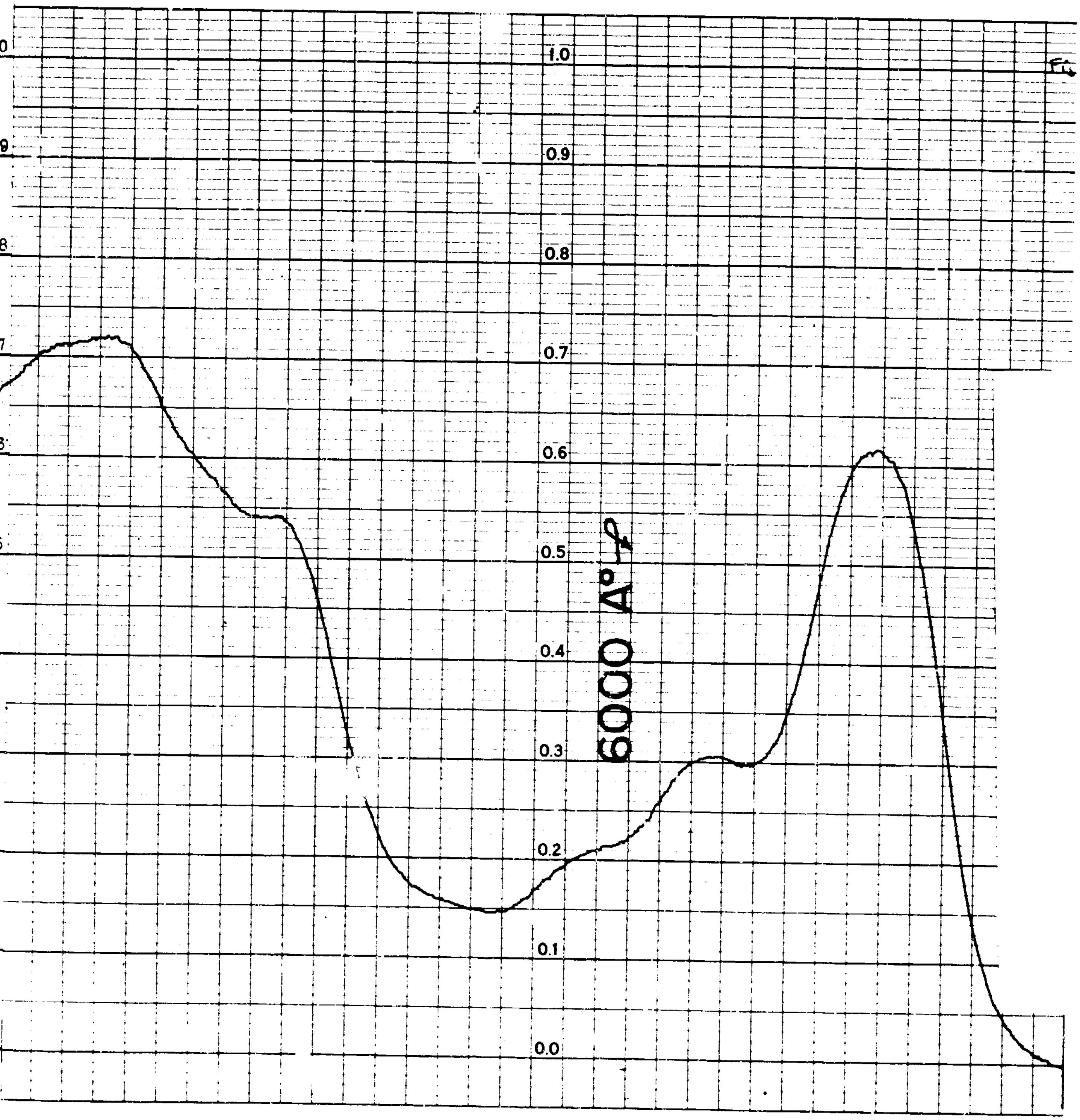
A



Cryptomonas ovata Sample Conc: 50 ul/ml Scan Speed:
ec. Scan: 100 A°/div. Solvent: 1 M Sucrose Chart
5 in/min.

B

Figure 50: Sample: Euglena gracilis Sample Conc: 3 u
25 A°/sec. Scan: 100 A°/div. Solvent: H_2
5 in/min.



__Euglena__ __gracilis__ Sample Conc: 3 ul/ml Scan Speed:
c. Scan: 100 A°/div. Solvent: H_2O Chart Speed:
.

B

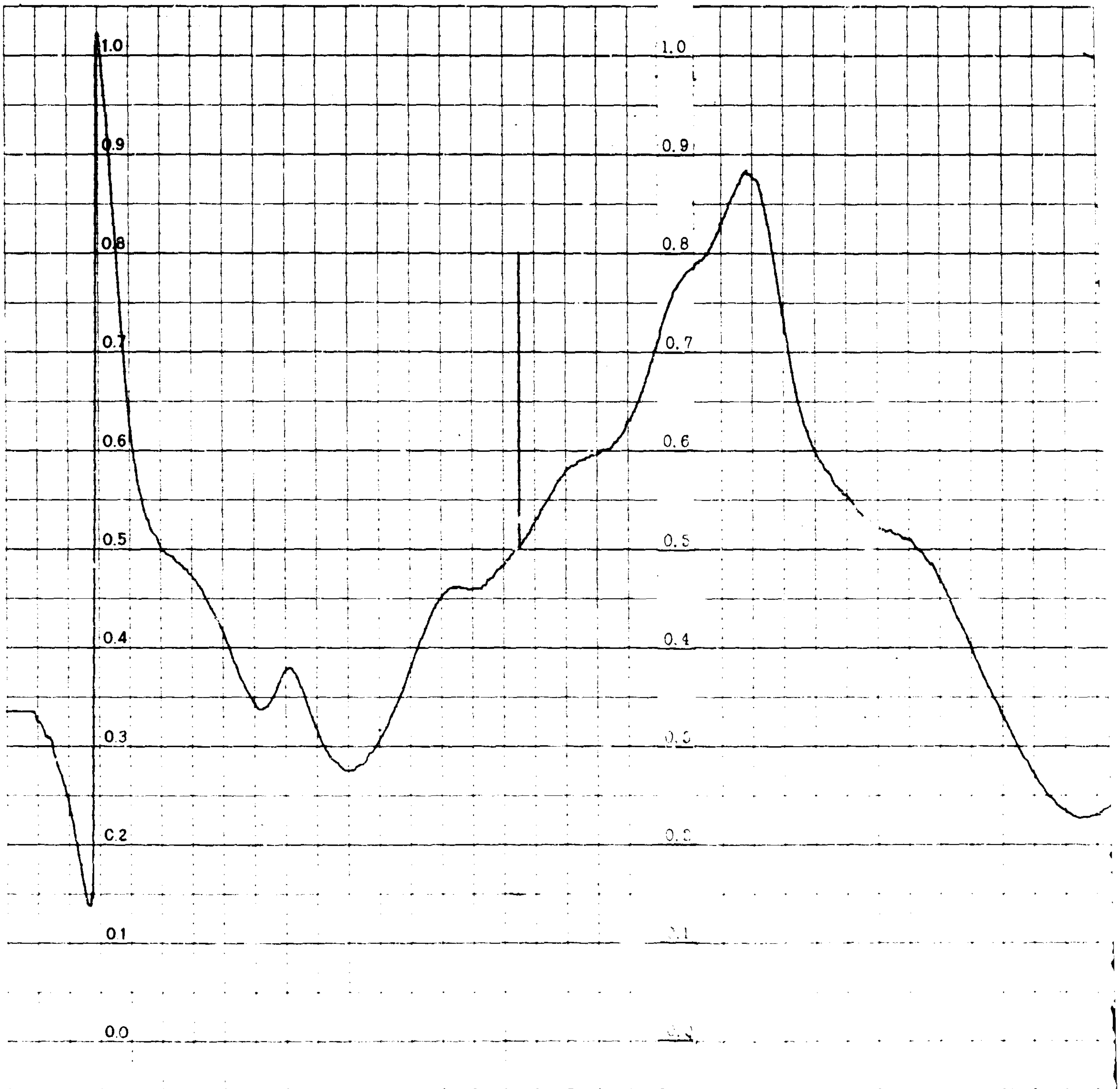
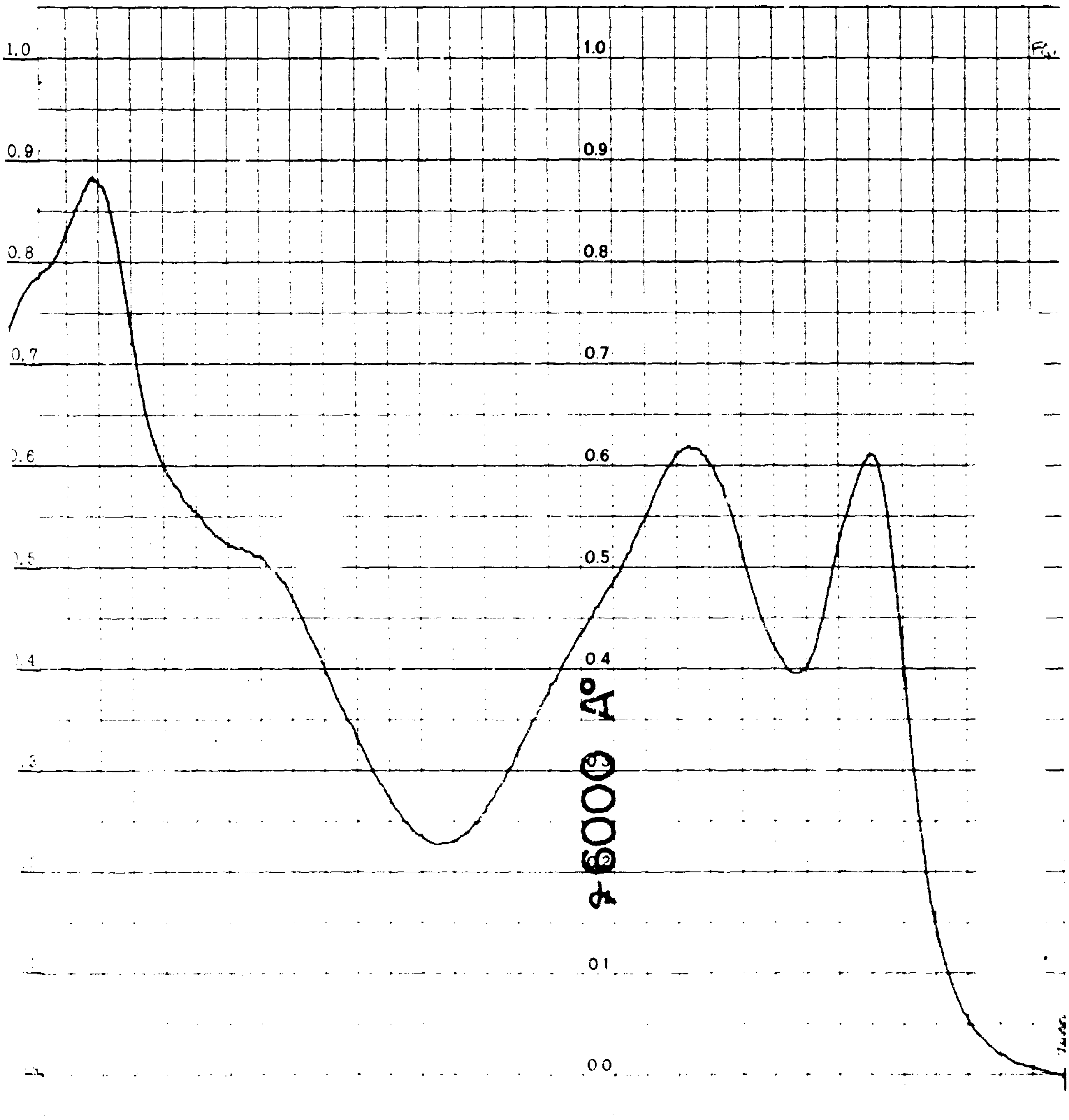


Figure 51: Sample: Gloeocapsa alpicola Sample Con : 6
Speed: 25 A°/sec. Scan: 100 A°/div. Solve
Speed: 5 in/min.



: Gloeocapsa alpicola Sample Conc: 6 ul/ml Scan 25 A°/sec. Scan: 100 A°/div. Solvent: H_2O Chart 5 in/min.

B

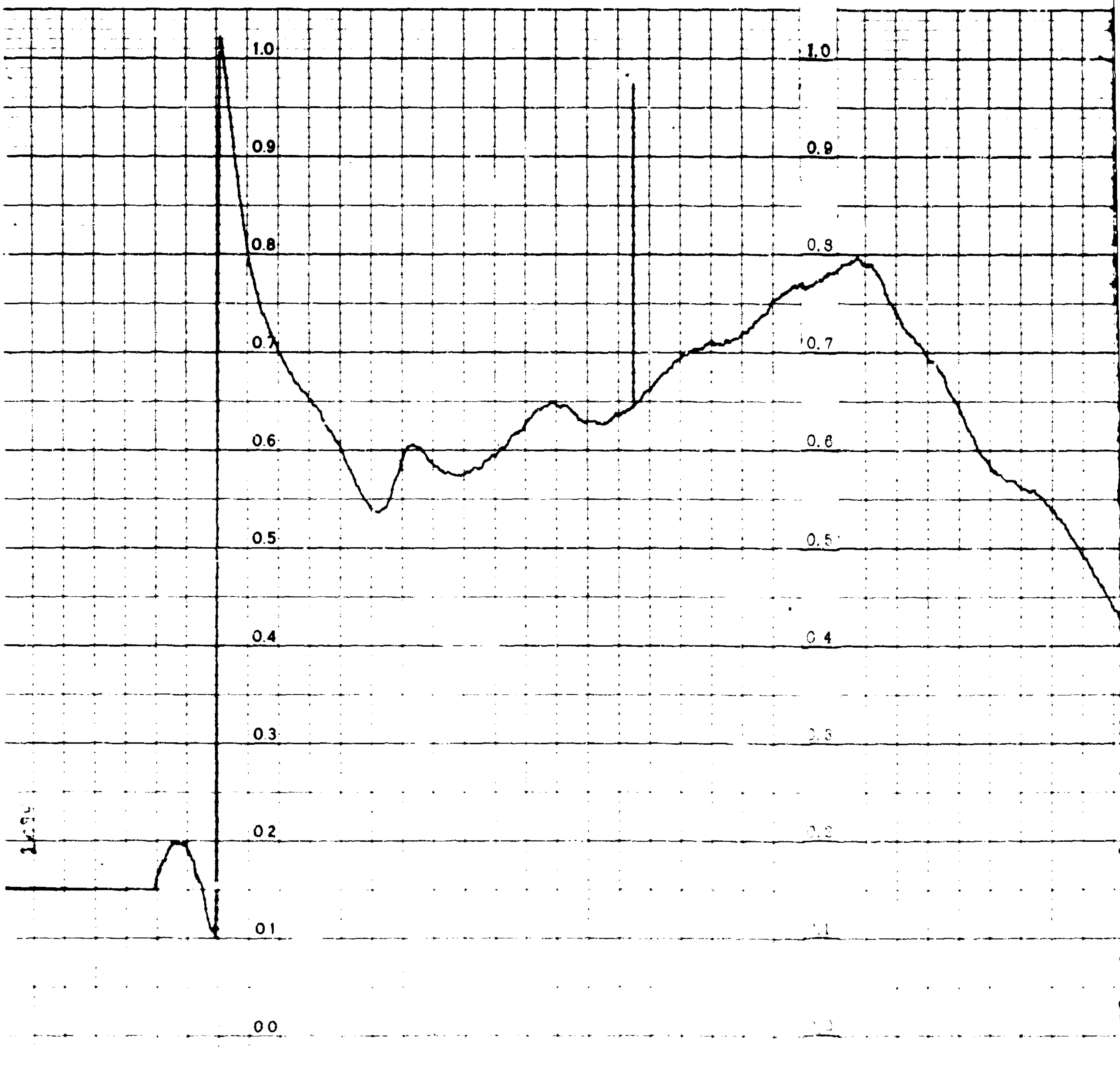
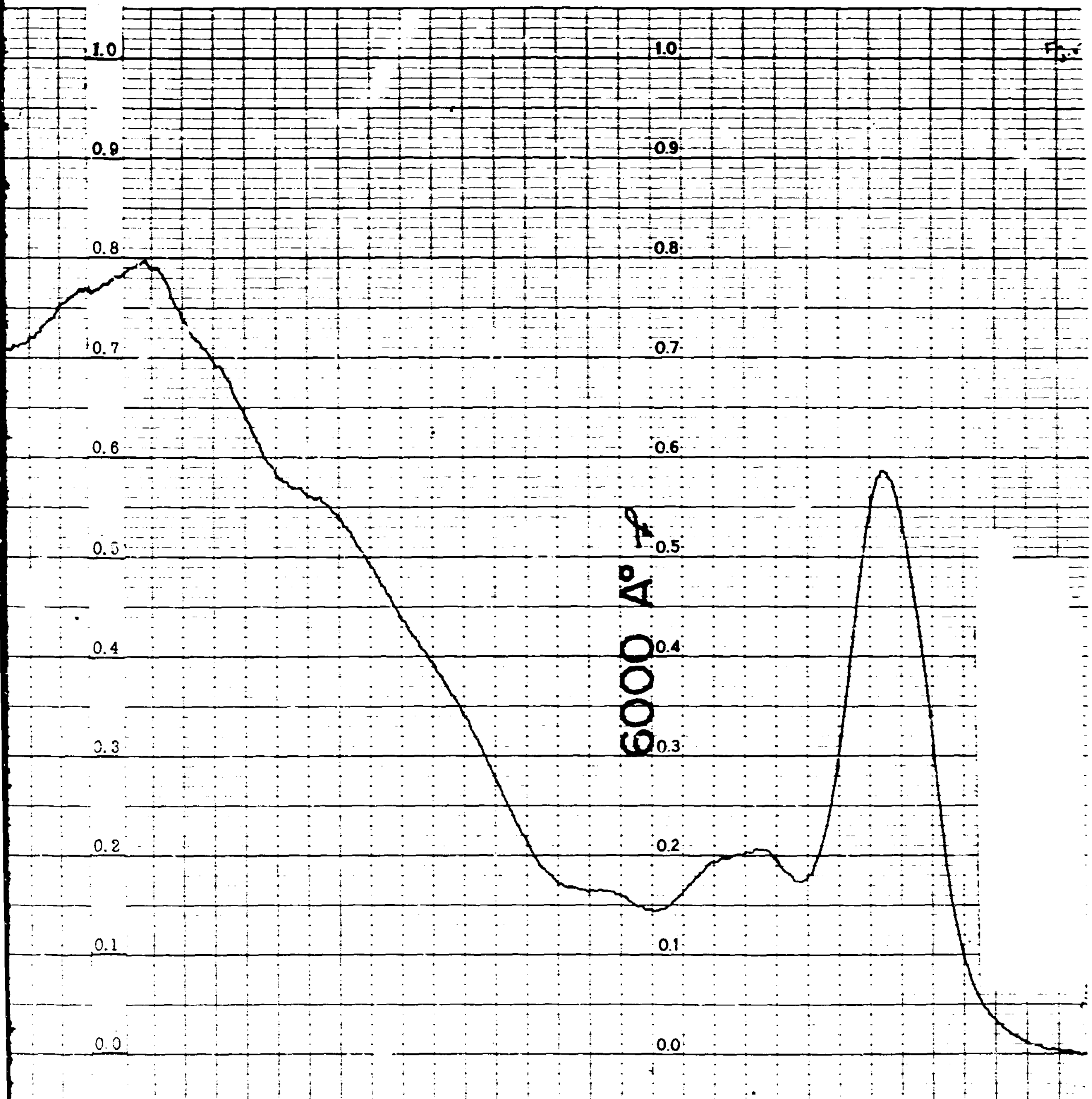


Figure 52: Sample: Nitzschia closterium Sample Conc
Speed: 25 A°/sec. Scan: 100 A°/div. So
Chart Speed: 5 in/min.



: Nitzschia closterium Sample Conc: 5 ul/ml Scan
25 A°/sec. Scan: 100 A°/div. Solvent: 1 M Sucrose
Speed: 5 in/min.

B

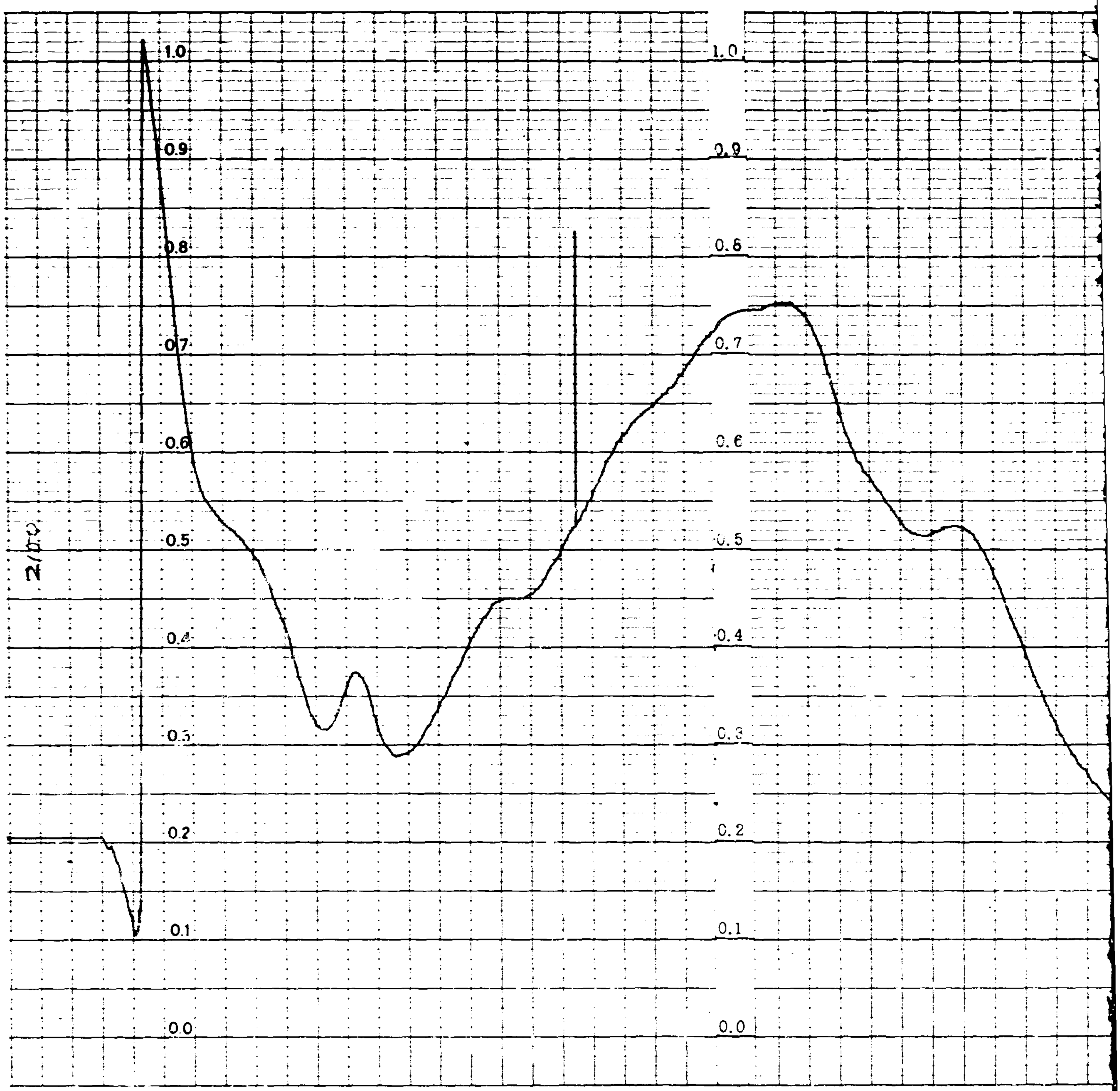
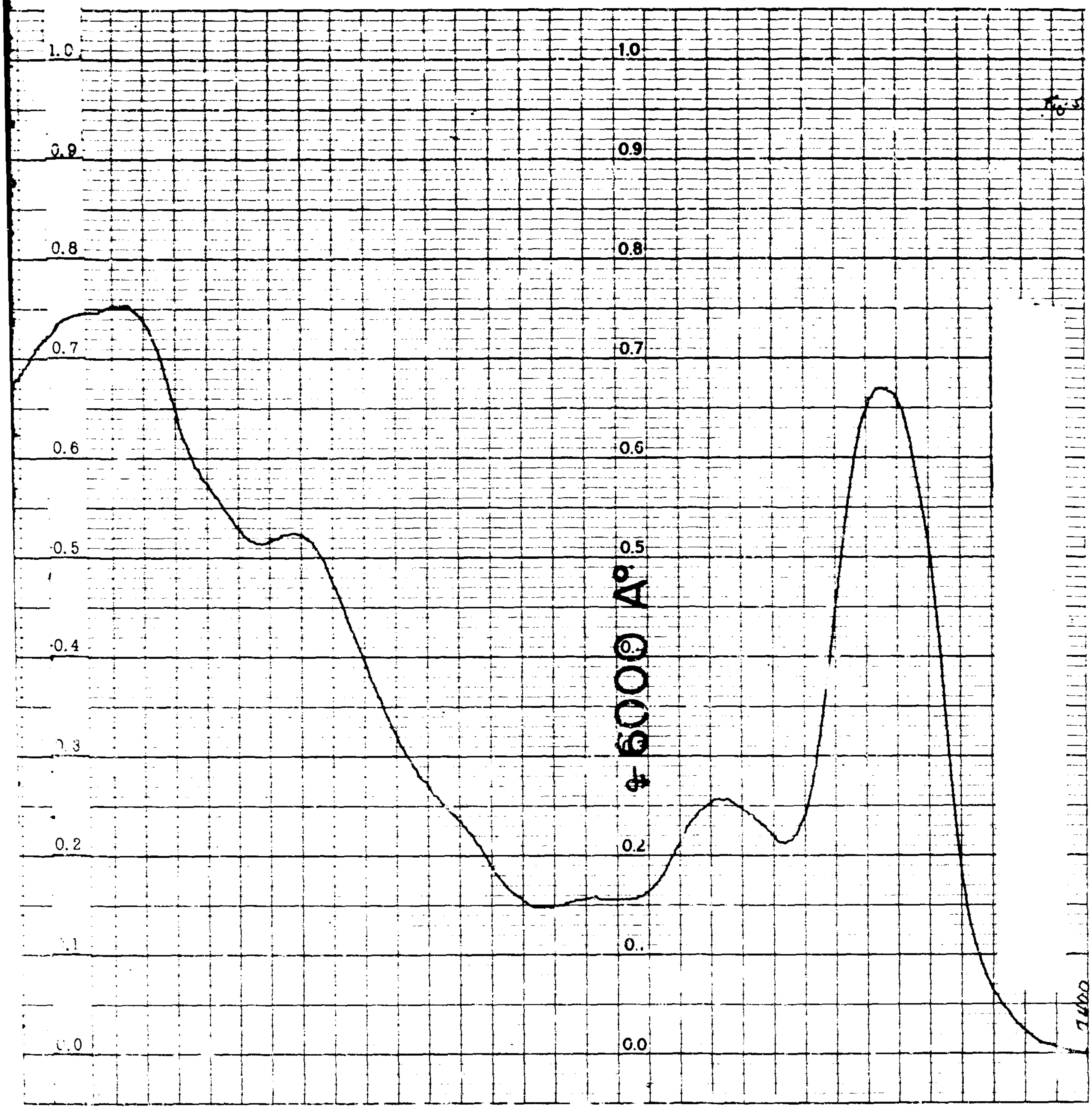


Figure 53: Sample: <u>Ochromonas danica</u> Sample Conc: 4 u
25 A°/sec. Scan: 100 A°/div. Solvent: H_2O
5 in/min.



: Ochromonas danica Sample Conc: 4 ul/ml Scan Speed:
sec. Scan: 100 A°/div. Solvent: H_2O Chart Speed:
in.

B

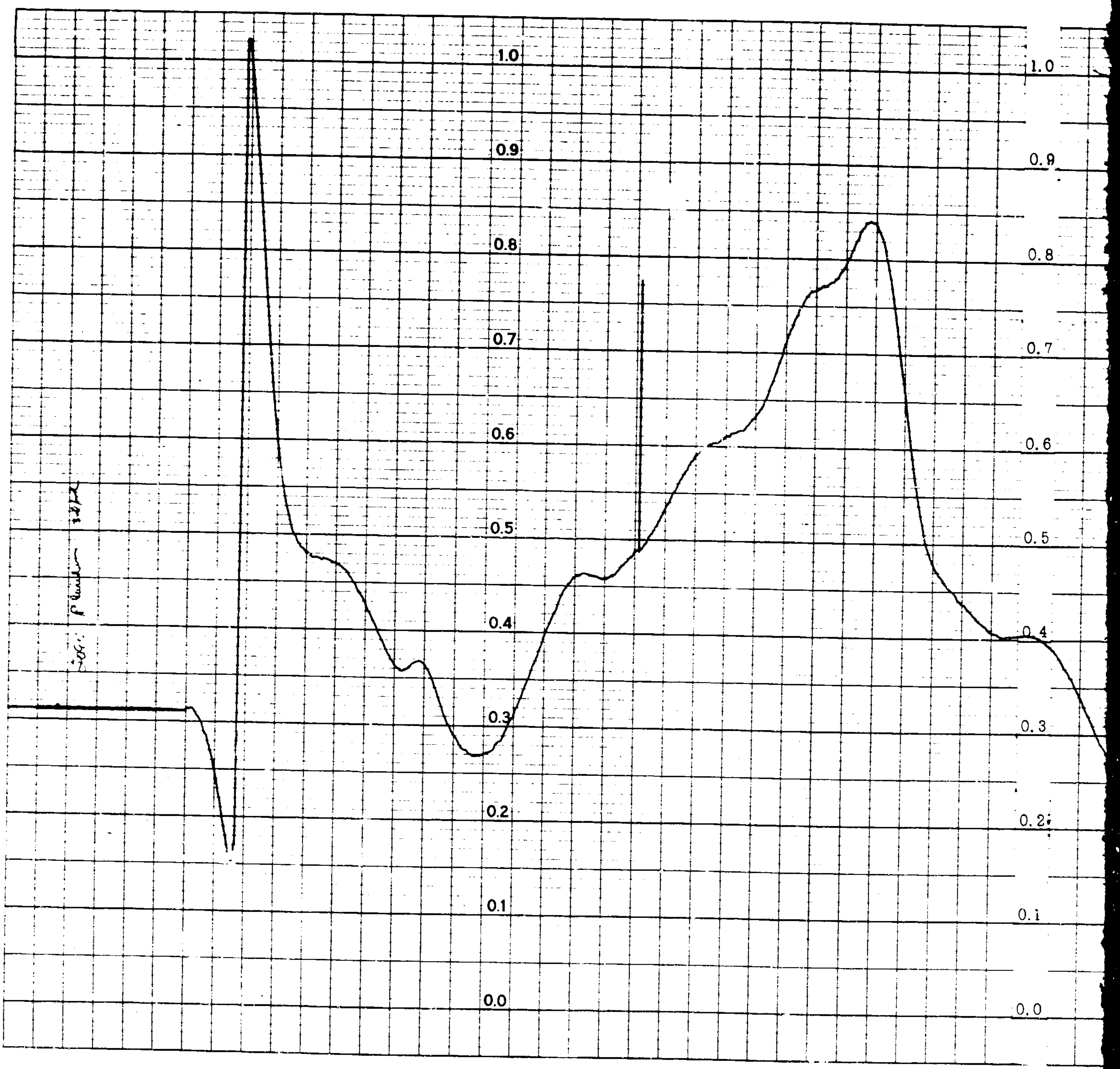
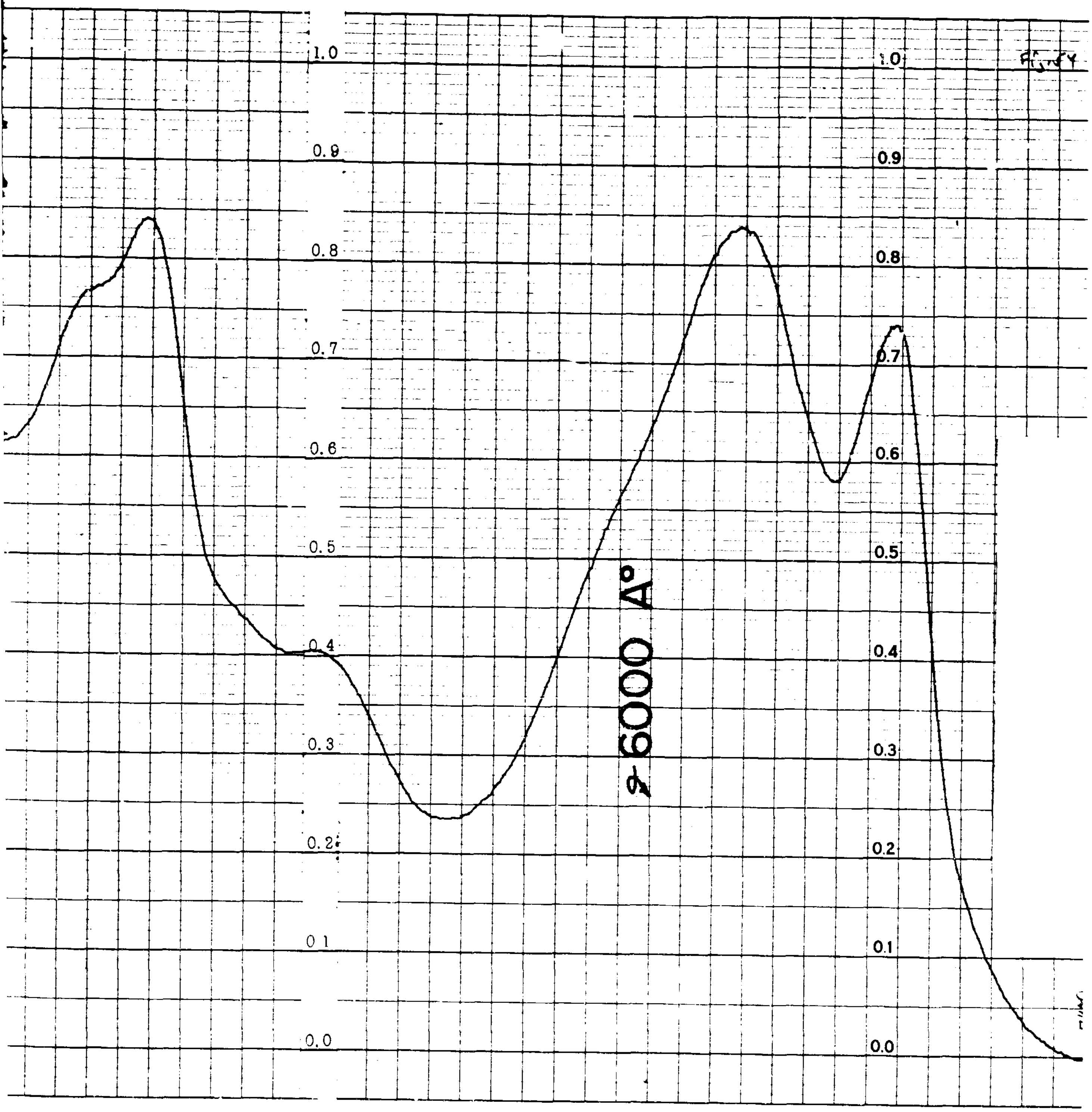


Figure 54: Sample: Phormidium luridum Sample Conc: 3 u
25 A°/sec. Scan: 100 A°/div. Solvent: H_2O
5 in/min.



ormidium luridum Sample Conc: 3 ul/ml Scan Speed:
Scan: 100 A°/div. Solvent: H_2O Chart Speed:

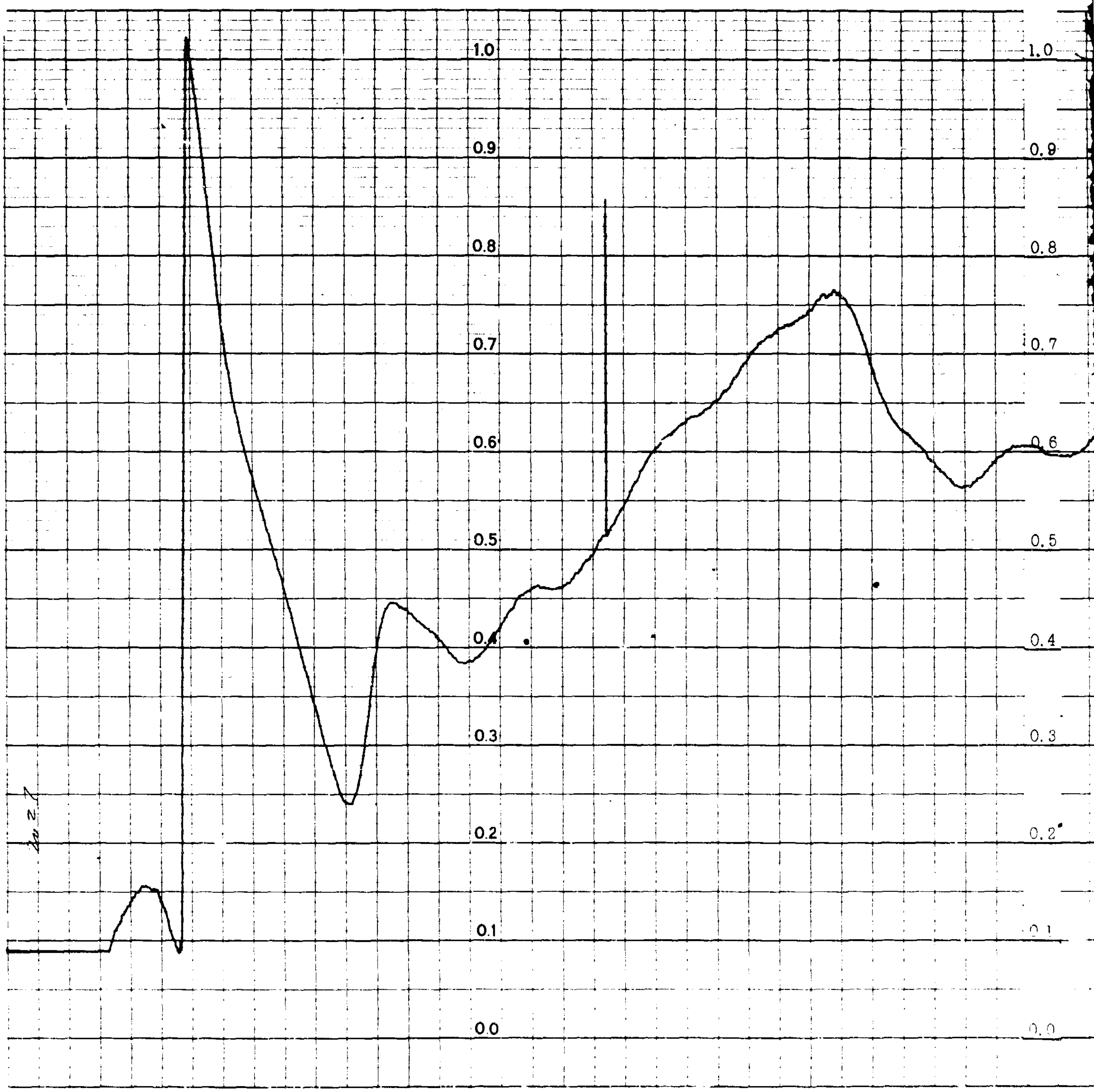
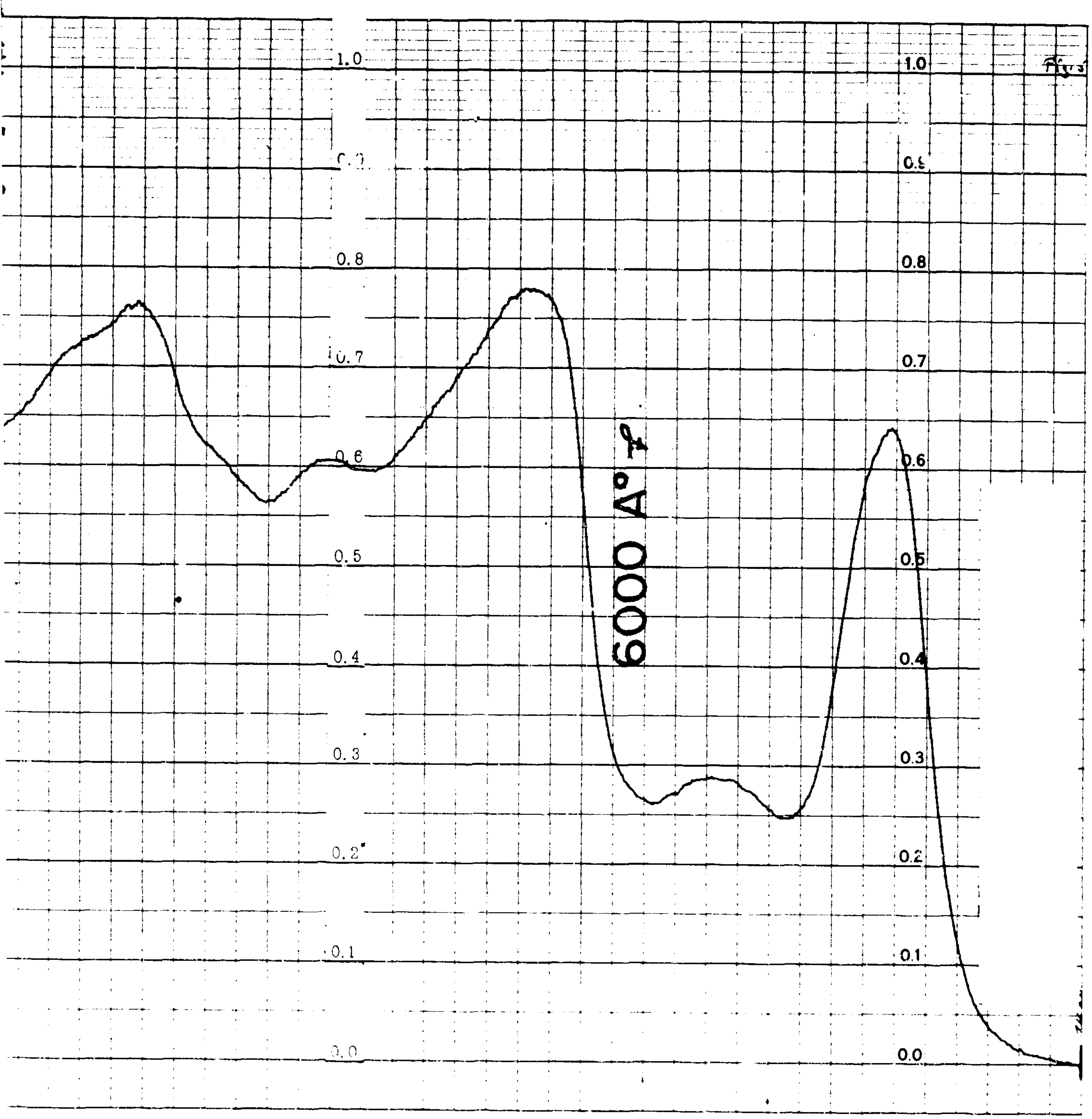


Figure 55: Sample: Phormidium persicinum Sample Conc
Speed: 25 A°/sec. Scan: 100 A°/div. Sol
Speed: 5 in/min.



<u>Phormidium</u> <u>persicinum</u> Sample Conc: 6 ul/ml Scan 25 A°/sec. Scan: 100 A°/div. Solvent: H_2O Chart 5 in/min.

B

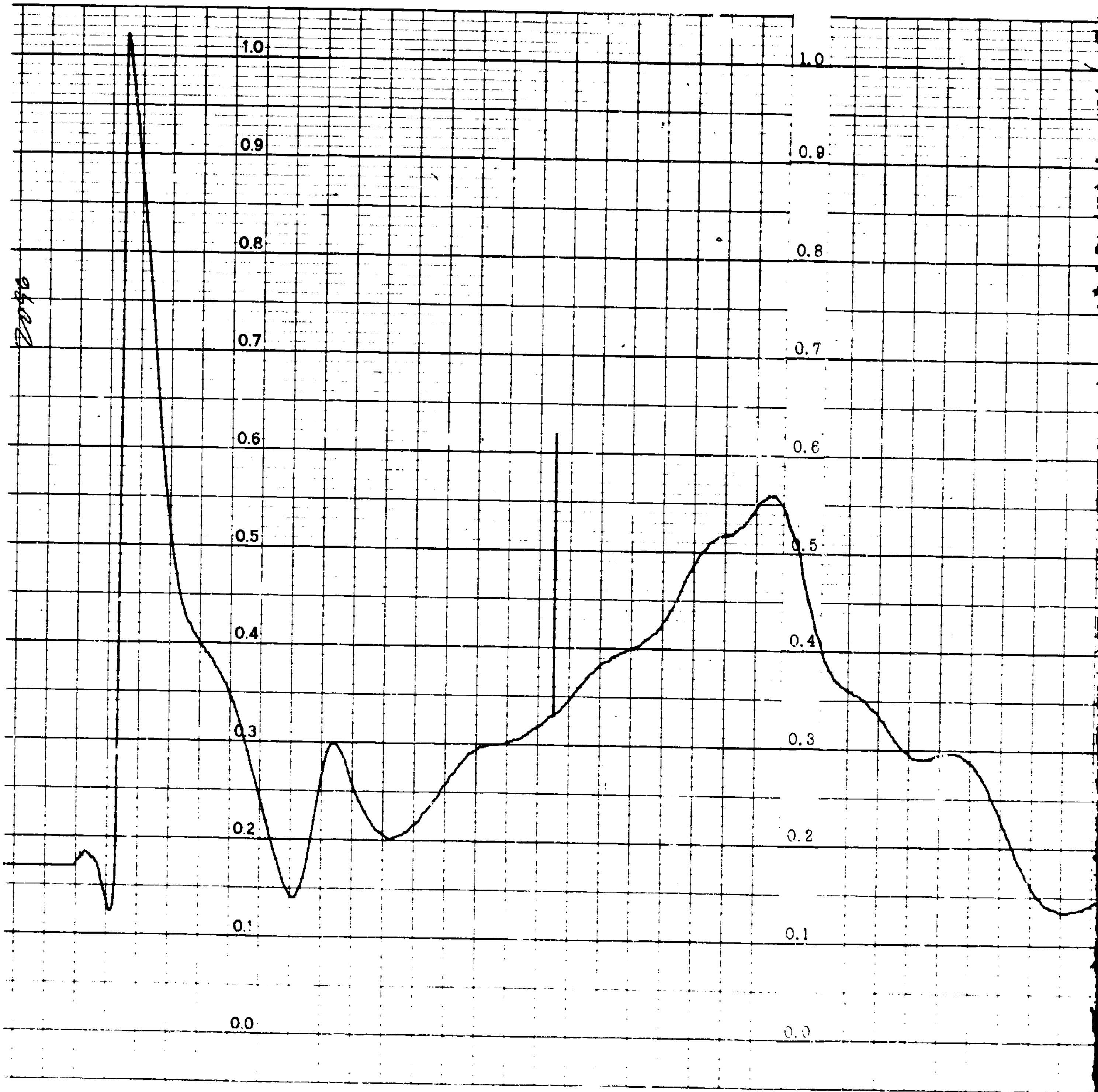
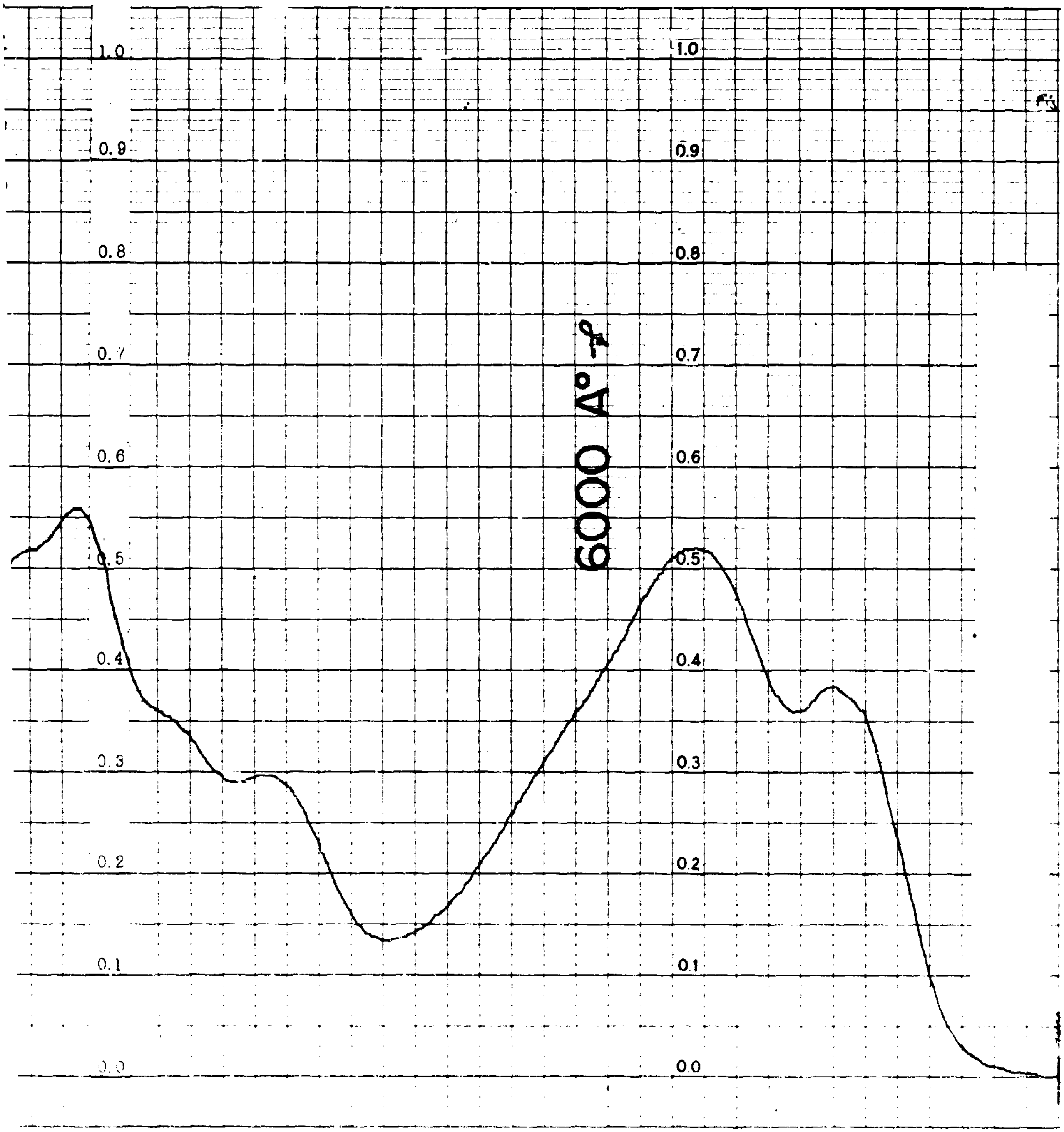


Figure 56: Sample: Porphyridium aerugineum Sample
Speed: 25 A°/sec. Scan: 100 A°/div. S
Speed: 5 in/min.



: *Porphyridium aerugineum* Sample Conc: 15 ul/ml Scan 25 A°/sec. Scan: 100 A°/div. Solvent: H_2O Chart 5 in/min.

B

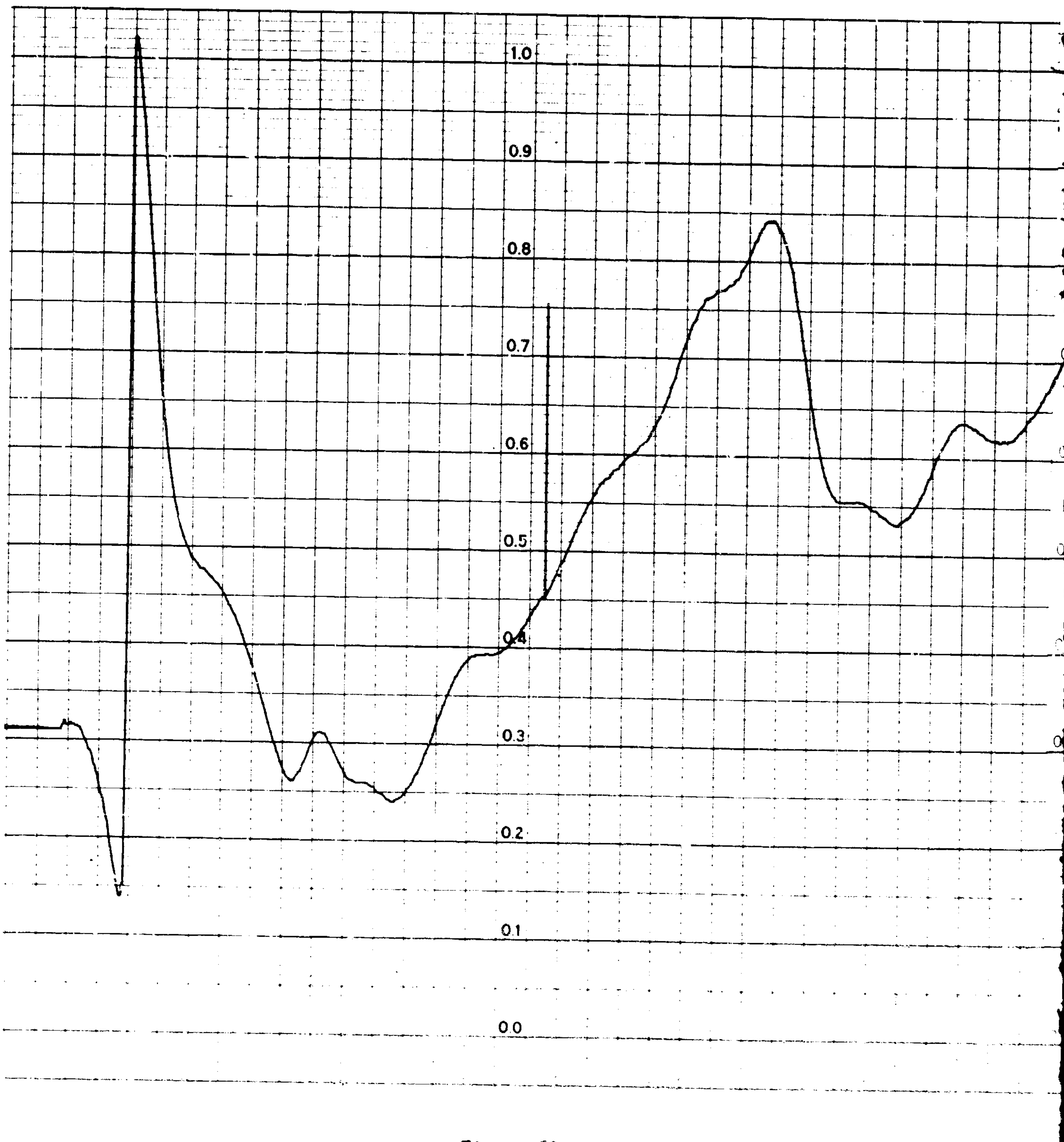
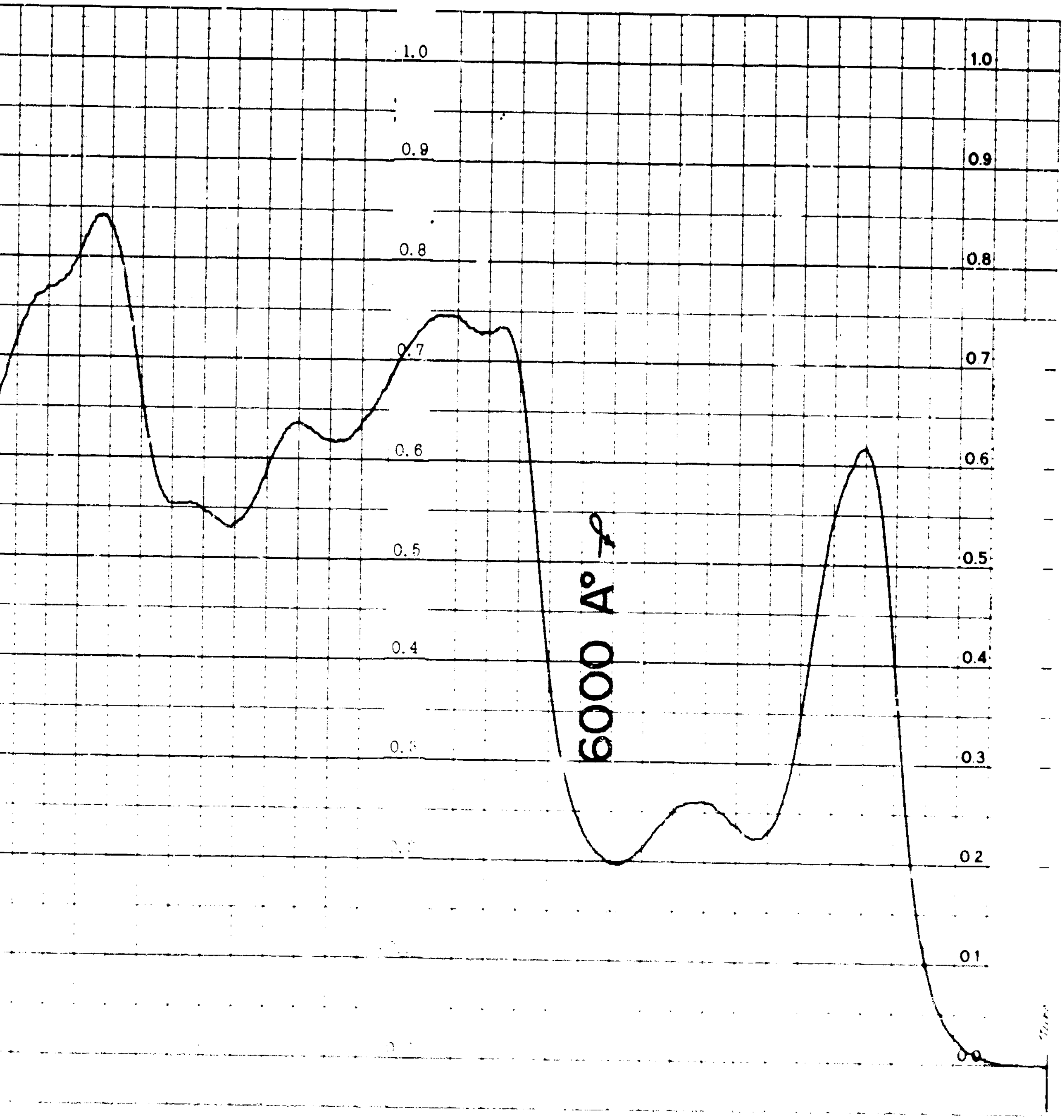
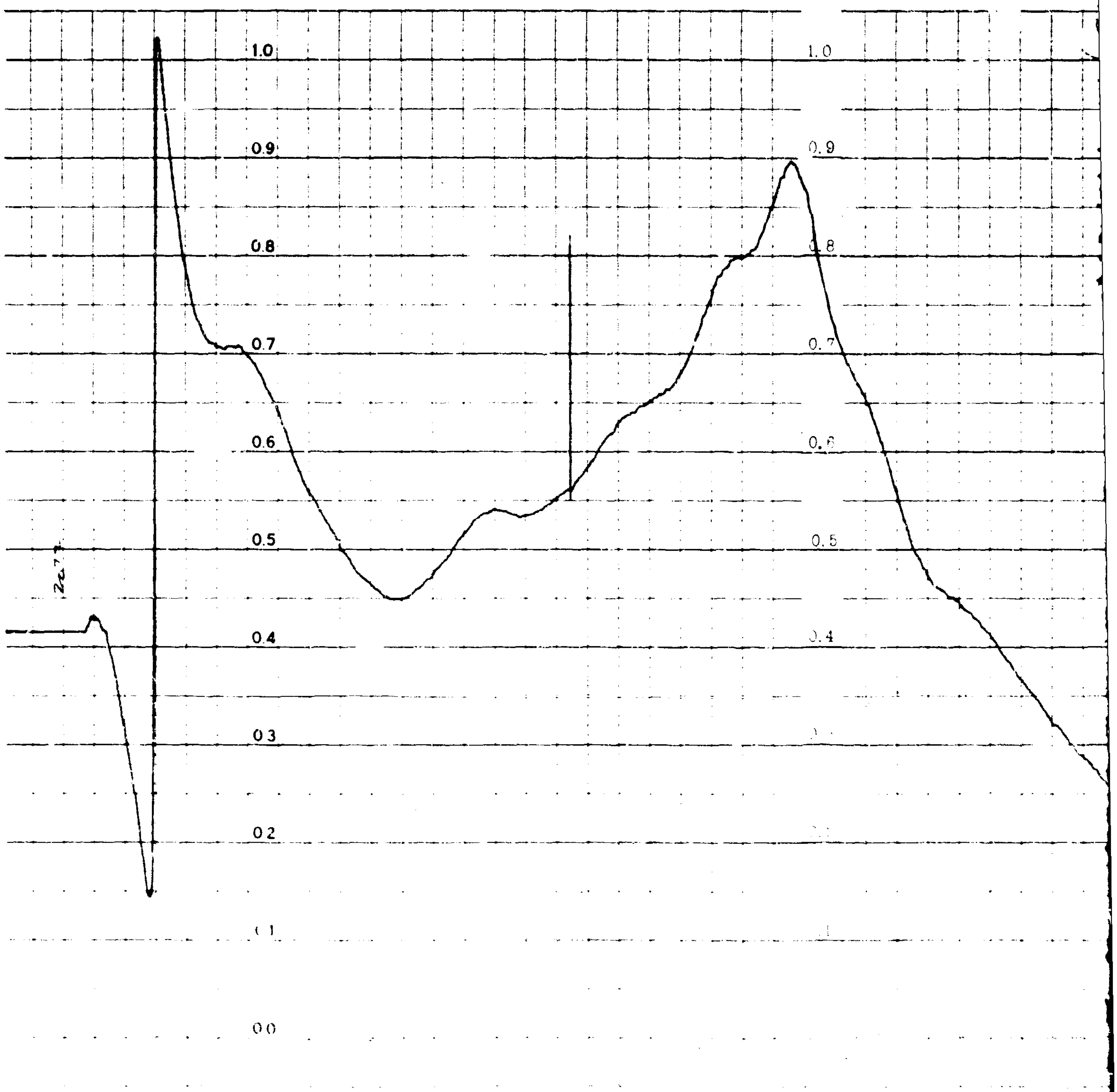


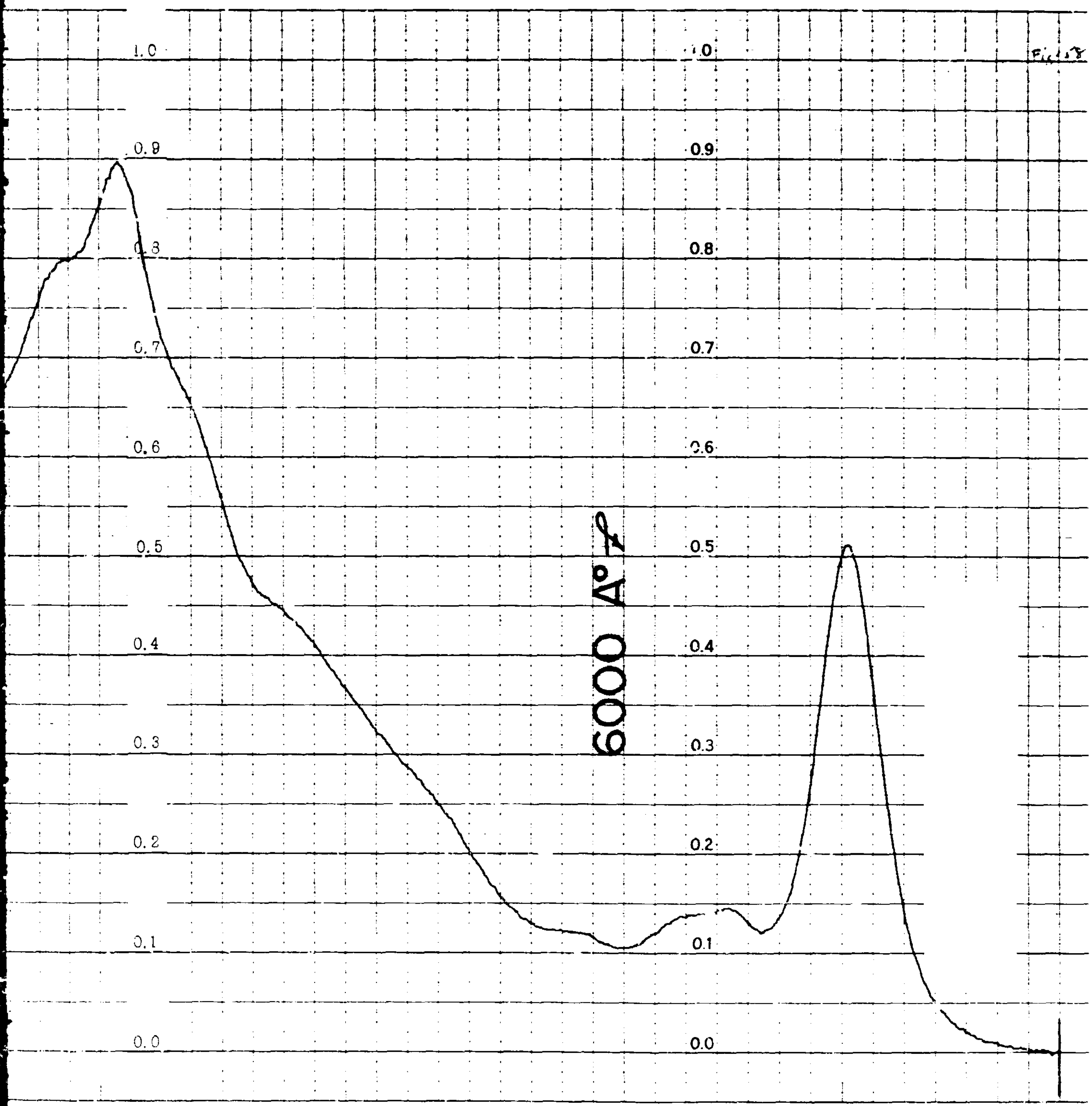
Figure 57: Sample: Porphyridium cruentum Sample Conc
Speed: 25 A°/sec. Scan: 100 A°/div. So
Speed: 5 in/min.



Porphyridium cruentum Sample Conc: 6 ul/ml Scan 25 A°/sec. Scan· 100 A°/div. Solvent: H_2O Chart 5 in/min.



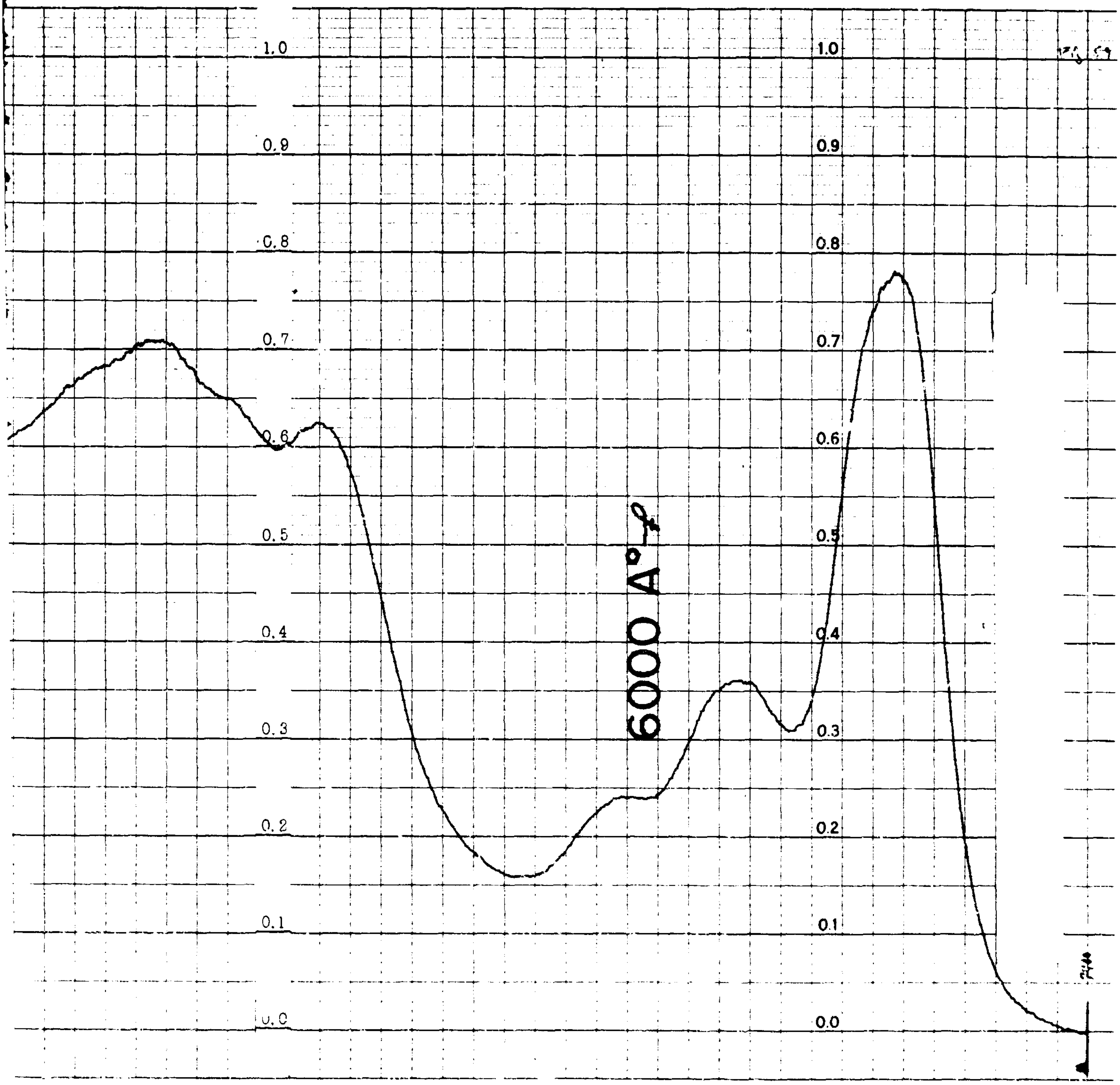
F'gure 58: Sample: Sphacelaria sp. Sample Conc: 25 ul/
25 A°/sec. Scan: 100 A°/div. Solvent: 2 M
Speed: 5 in/min.



Sphacelaria sp. Sample Conc: 25 ul/ml Scan Speed:
ec. Scan: 100 A°/div. Solvent: 2 M Sucrose Chart
5 in/min.

B

Figure 59: Sample: Tribonema aequale Sample Conc: 10
25 A°/sec. Scan: 100 A°/div. Solvent: H
5 in/min.



:ibonema aequale Sample Conc: 10 ul/ml Scan Speed:
Scan: 100 A°/div. Solvent: H_2O Chart Speed:

B

Appendix C

This section includes tables for preparation of culture media for growth of the individual alage.

Preparation of soil extract.

1. Suspend average grade, clean topsoil in water at approx. 1g./ml.
2. Autoclave 15 min. at 15 psi.
3. Allow to stand overnight.
4. Centrifuge for 15 min. at approx. 2,000 x g.
5. Pour supernatant through a Millipore (or similar) pre-filter (AP2504700, glass fiber with starch binder, 0.035").
6. Autoclave for 15 min. at 15 psi.
7. Cold store until used.

Tab. XIX	*Amphidinium sp.*
Tab. XX	*Botrydiopsis alpina*
Tab. XXI	*Chlamydomonas reinhardi*
Tab. XXII	*Chlorella pyrenoidosa*
Tab. XXIII	*Chlorella sorokiniana* (7-11-05)
Tab. XXIV	*Chlorococcum wimmeri*
Tab. XXV	*Cryptomonas ovata*
Tab. XXVI	*Euglena gracilis*
Tab. XXVII	*Gloeocapsa alpicola*
Tab. XXVIII	*Nitzschia closterium*
Tab. XXIX	*Ochromonas danica*
Tab. XXX	*Phormidium luridum*
Tab. XXXI	*Phormidium persicinum*
Tab. XXXII	*Porphyridium aerugineum*
Tab. XXXIII	*Porphyridium cruentum*
Tab. XXXIV	*Sphacelaria sp.*
Tab. XXXV	*Tribonema aequale*

Table XIX

Name: Amphidinium sp.

Source: Culture Collection of Algae at Indiana University (#1002)

Isolator: Parke

Atmosphere: Air

Sample Density (µl/ml): Abs. spec. 3, Ps & Res. 2

Culture Medium

Ingredients	Grams/liter
$NaNO_3$	.15
$NaH_2PO_4 \cdot H_2O$	.01
Fe sequestrene	.01
$Na_2SiO_3 \cdot 9H_2O$	.04
Thiamin HCl	.0002
Biotin	1 µg
B_{12}	1 µg
Sea Water	1 liter
10% Tris	5 ml (.5 grams)
$CuSO_4 \cdot 5H_2O$	.0000196
$ZnSO_4 \cdot 7H_2O$	.000044
$CaCl_2 \cdot 6H_2O$	.00002
$MnCl_2 \cdot 4H_2O$	.00036
$Na_2MoO_4 \cdot 2H_2O$	.0000126
Soil Extract	40 ml

Table XX

Name: Botrydiopsis alpina vischer

Source: Culture Collection of Algae at Indiana University (#295)

Isolator: Vischer

Atomosphere: 5% CO_2 in air

Sample density (µl/ml): Abs. spec. 10, Ps. & Res. 5

Culture Medium

Ingredients	Grams/Liter
$NaNO_3$	.25
$CaCl_2 \cdot 2H_2O$	.025
$MgSO_4 \cdot 7H_2O$	.075
K_2HPO_4	.075
KH_2PO_4	.175
NaCl	.025
H_3BO_3	.006184
$ZnSO_4 \cdot 7H_2O$	.001024
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	.012360
$CuSO_4$	.000158
$MnCl_2 \cdot 4H_2O$	.00362
$FeCl_3 \cdot 6H_2O$	.00388

Table XXI

Name: *Chlamydomonas reinhardii* Dangeard (+ s^+r.)

Source: Culture Collection of Algae at Indiana University (#89)

Isolator: G.M. Smith

Atmosphere: 5% CO_2 in air

Sample density (µl/ml): Abs. spec. 3, Ps & Res. 3

Culture Medium

Ingredients	Grams/liter
$Ca(NO_3)_2 \cdot 4H_2O$	1.0
K_2HPO_4	0.2
$MgSO_4 \cdot 7H_2O$	0.2
Na Citrate (25% H_2O)	0.375
$FeSO_4 \cdot 7H_2O$	0.02
H_3BO_3	.003092
$ZnSO_4 \cdot 7H_2O$	.000512
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	.00618
$CuSO_4$	.000079
$MnCl_2 \cdot 4H_2O$	.00181

Table XXII

Name: **<u>Chlorella</u> <u>pyrenoidosa</u> Emerson's str.**

Source: **Robert Emerson, Univ. Illinois**

Isolator: **Emerson**

Atmosphere: **5% CO_2 in air**

Sample density (µl/ml): **Abs. spec. 1, Ps & Res. 3**

Culture Medium

Ingredients	Grams/liter
KNO_3	2.5
KH_2PO_4	2.5
$MgSO_4 \cdot 7H_2O$	5.0
NaCl	2.0
H_3BO_3	.003092
$MnCl_2 \cdot 4H_2O$	.001810
$ZnSO_4 \cdot 7H_2O$	.000512
$CuSO_4 \cdot 5H_2O$	.000079
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	.000618
$FeSO_4 \cdot 7H_2O$	.002

Table XXIII

Name: Chlorella pyrenoidosa 7-11-05 Hi-Temperature Strain (C. sorokiniana)

Source: C. Sorokin, Department of Botany, University of Md.

Isolator: C. Sorokin

Atmosphere: 5% CO_2 in air

Sample density (µl/ml): Abs. spec. 1, Ps. & Res. 3

Culture Medium

Ingredients	Grams/Liter
KNO_3	1.25
KH_2PO_4	1.25
$MgSO_4 \cdot 7H_2O$	1.00
$CaCl_2$	0.0835
H_3BO_3	.001142
$FeSO_4 \cdot 7H_2O$	.000498
$ZnSO_4$	.000882
$MnCl_2 \cdot 4H_2O$	.000144
MoO_3	.000071
$CuSO_4 \cdot 5H_2O$	.000157
$Co(NO_3)_2 \cdot 6H_2O$	.000049
EDTA (Na salt)	.005

Table XXIV

Name: <u>*Chlorococcum wimmeri*</u> Rabenhorst

Source: Culture Collection of Algae at Indiana University (#113)

Isolator: Mainx

Atmosphere: 5% CO_2 in air

Sample density (µl/ml): Abs. spec. 4, Ps & Res. 4

Culture Medium

Ingredients	Grams/Liter
$NaNO_3$	.25
$CaCl_2 \cdot 2H_2O$	.025
$MgSO_4 \cdot 7H_2O$	.075
K_2HPO_4	.075
KH_2PO_4	.175
NaCl	.025
EDTA (Na Salt)	.050
H_3BO_3	.00114
$Co_3(NO_3)_2 \cdot 6H_2O$	.00098
$CuSO_4$	.00050
$MnSO_4 \cdot H_2O$	.00062
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	.00258
$ZnSO_4 \cdot 7H_2O$	.00266
$FeSO_4 \cdot 7H_2O$	.005

Table XXV

Name: Cryptomonas ovata var. palustris Pringsheim

Source: Culture Collection of Algae at Indiana University (#358)

Isolator: E.G. Pringsheim

Atmosphere: 1% CO_2 in air

Sample density (µl/ml): Abs. spec. 12, Ps & Res. 5

Culture Medium

Ingredients	Grams/Liter
$NaNO_3$	.25
$CaCl_2 \cdot 2H_2O$	.025
$MgSO_4 \cdot 7H_2O$	.75
K_2HPO_4	.075
KH_2PO_4	.175
NaCl	.025
Soil Extract	40 ml
1% $FeCl_3$	1 drop

Table XXVI

Name: *Euglena gracilis* Klebs "Z" strain

Source: Culture Collection of Algae at Indiana University (#369)

Isolator: E.G. Pringsheim

Atmosphere: 5% CO_2 in air

Sample density (µl/ml): Abs. spec. 4, Ps & Res 3

Culture Medium

Ingredients	Grams/Liter
EDTA (Na salt)	.50
KH_2PO_4	.30
$MgSO_4 \cdot 7H_2O$	.50
$CaCO_3$	.06
$(NH_4)_2SO_4$	1.0
Thiamine hydrochloride	.0006
B_{12}	.000005
Metal Mix "49"	.130 g/l of below listed chemicals
$Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$	.07
$ZnSO_4 \cdot 7H_2O$	.022
$MnSO_4 \cdot H_2O$	.031
$CuSO_4 \cdot 5H_2O$	.004
$CoSO_4 \cdot 7H_2O$	.0024
H_3BO_3	.00057
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	.00072
$Na_3VO_{21} \cdot 16H_2O$	.0046

Adjust pH to 3.6-4.0 c̄ 10N H_2SO_4

Table XXVII

Name: Gloeocapsa alpicola (Lyngb.)

Source: Culture Collection of Algae at Indiana University (#B589)

Isolator: Frenkel

Atmosphere: 1% CO_2 in air

Sample Density (µl/ml): Abs. spec. 3, Ps & Res. 2

Culture Medium

Ingredients	Grams/Liter
KNO_3	1.0
$CaCl_2$	.01
$MgSO_4 \cdot 7H_2O$	.25
K_2HPO_4	.25
NaCl	.10
$FeSO_4 \cdot 7H_2O$	.02
H_3BO_3	.003092
$ZnSO_4 \cdot 7H_2O$	.000512
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	.000618
$CuSO_4$	.000079
$MnCl_2 \cdot 4H_2O$	.00181

Table XXVIII

Name: Nitzschia closterium (Ehr.) W. Smith

Source: Culture Collection of Algae at Indiana University (#640)

Isolator: Allen

Atmosphere: 1% CO_2 in air

Sample density (µl/ml): Abs. spec. 7; Ps & Res. 7

Culture Medium

Ingredients	Grams/Liter
Sea Water	1 liter
KNO_3	.125
$CaCl_2 \cdot 2H_2O$	.02649
$MgSO_4 \cdot 7H_2O$	.05
K_2HPC_4	.10
HCl (conc)	.01 ml/1
$FeCl_2$ (melted)	.01 ml/1

Table XXIX

Name: Ochromonas danica

Source: S.H. Hutner, Haskins Laboratory, New York 17, New York

Isolator: E.G. Pringsheim

Atmosphere: Air

Sample density (µl/ml): Abs. spec. 3, Ps & Res. 3

Culture Medium (can be purchased prepared from General Biochemicals)

Ingredients	Grams/Liter
Nitrilotriacetic acid	.2
KH_2PO_4	.3
$MgCO_3$	.4
$MgSO_4 \cdot 7H_2O$	1.0
$CaCO_3$	.05
L-glutamic acid	10.0
Glucose	10.0
Thiamine HCl	.001
Biotin	.00001
L-arginine HCl	.4
Glycine	.1
L-histidine HCl	.4
Metal Mix	.01 of below mixed chemicals
$Fe(NH_4)_2 \cdot (SO_4)_2 \cdot 6H_2O$	1.405
$ZuSO_4 \cdot 7H_2O$	1.4395
$MnSO_4 \cdot H_2O$	0.0154
$CuSO_4 \cdot 5H_2O$	0.00314
$Co(NO_3)_2 \cdot 6H_2O$	0.0495
H_3BO_3	0.0618
$(NH_4)_6Mo_2O_{24} \cdot 4H_2O$	0.0619
$NaVO_3 \cdot H_2O$	0.00275

Table XXX

Name: Phormidium luridum var. olivacea Boresch

Source: Culture Collection of Algae at Indiana University (#426)

Isolator: Boresch

Atmosphere: Air

Sample density (µl/ml): Abs. spec. 5, Ps & Res. 5

Culture Medium

Ingredients	Grams/Liter
KNO_3	1.0
$CaCl_2$	.01
$MgSO_4 \cdot 7H_2O$	.25
K_2HPO_4	.25
NaCl	.10
$FeSO_4 \cdot 7H_2O$	.02
H_3BO_3	.003092
$ZnSO_4 \cdot 7H_2O$	.000512
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	.000618
$CuSO_4$	.000079
$MnCl_2 \cdot 4H_2O$	.00181

Table XXXI

Name: <u>Phormidium persicinum</u>

Source: L. Provasoli, Haskins Laboratory, New York 17, New York

Isolator: L. Provasoli

Atmosphere: Air

Sample density (µl/ml): Abs. spec. 3, Ps & Res. 3

Culture Medium

Ingredients	Grams/Liter
EDTA (Na salt)	.2
NaCl	25.0
KCl	.38
$CaCl_2$	.294
$MgSO_4 \cdot 7H_2O$	5.0
$NaNO_3$	.1
dL-asparagine	.2
K_3PO_4	.05
Tris	1.0
$FeCl_3 \cdot 6H_2O$	.00194
B_{12}	.000002
$MnCl_2 \cdot 4H_2O$	.000576
$ZnCl_2$	.000332
CuCl	.0000064
$CoCl_2 \cdot 6H_2O$	.000008
$FeCl_3 \cdot 6H_2O$	.0000388
Citric acid	.00012

Table XXXII

Name: Porphyridium aerugineum, Geitler

Source: Culture Collection of Algae and Protozoa at University of Cambridge (#1380/2)

Isolator: E.G. Pringsheim

Atmosphere: 5% CO_2 in air

Sample density (µl/ml): Abs. spec. 8, Ps & Res. 5

Culture Medium

Ingredients	Grams/Liter
$NaNo_3$	.25
$CaCl_2 \cdot 2H_2O$	.025
$MgSO_4 \cdot 7H_2O$	.75
KH_2PO_4	.175
NaCl	.075
Soil Extract	40 ml
1% $FeCl_2$	1 drop
K_2HPO_4	.075

Table XXXIII

Name: Porphyridium cruentum (Ag.) Naeg.

Source: Culture Collection of Algae at Indiana University (#161)

Isolator: Vischer

Atmosphere: 5% CO_2 in air

Sample density (µl/ml): Abs. spec. 5, Ps & Res. 4

Culture Medium

Ingredients	Grams/Liter
KCl	16.03
NaCl	12.61
KNO_3	1.24
K_2HPO_4	.50
$MgSO_4 \cdot 7H_2O$	2.49
$Ca(NO_3)_2 \cdot 4H_2O$	.25
KI	.04997
H_3BO_3	.003092
$MnCl_2 \cdot 4H_2O$	.001810
$ZnSO_4 \cdot 7H_2O$	.000512
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	.000618
$CuSO_4$	.0000798
$K_2Al_2(SO_4)_4 \cdot 2H_2O$	.0009488
KBr	.000099
$Cd(NO_3)_2 \cdot 4H_2O$	.0001542
$Co(NO_3)_2 \cdot 6H_2O$	.0001455
$NiCl_2 \cdot 6H_2O$	.0001189
$Cr(NO_3)_3 \cdot 7H_2O$	.000373
NH_4VO_3	.000002
$NaNO_3 \cdot 2H_2O$	.000033
$FeSO_4 \cdot 7H_2O$	.0055602

Table XXXIV

Name: Sphacelaria sp.

Source: Culture Collection of Algae at Indiana University (#LB 800)

Isolator: Norris

Atmosphere: Air

Sample density (μl/ml): Abs. spec. 25, Ps & Res. 10

Culture Medium

Ingredients	Grams/Liter
$NaNO_3$	.15
$NaH_2PO_4 \cdot H_2O$	.01
Fe sequestrene	.01
$Na_2SiO_3 \cdot 9H_2O$	.04
Thiamine HCl	.0002
Biotin	1 μg
B_{12}	1 μg
Sea H_2O	1 liter
$CuSO_4 \cdot 5H_2O$	.0000196
$ZnSO_4 \cdot 7H_2O$	.000044
$CaCl_2 \cdot 6H_2O$	.00002
$MnCl_2 \cdot 4H_2O$	.00036
$Na_2MoO_4 \cdot 2H_2O$	.0000126
Tris 10%	5 ml

Table XXXV

Name: *Tribonema aequale* Pascher

Source: Culture Collection of Algae at Indiana University (#50)

Isolator: E.G. Pringsheim

Atmosphere: 5% CO_2 in air

Sample density (µl/ml): Abs. spec. 15, Ps & Res. 10

Culture Medium

Ingredients	Grams/Liter
KNO_3	1.0
$CaCl_2$	.01
$MgSO_4 \cdot 7H_2O$	.25
K_2HPO_4	.25
NaCl	.10
$FeSO_4 \cdot 7H_2O$	.02
Trace Elements	
H_3BO_3	.003092
$ZnSO_4 \cdot 7H_2O$	.000512
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	.000618
$CuSO_4$	.000079
$MnCl_2 \cdot 4H_2O$	.00181

Appendix D

This section includes tables of all rates of photosynthesis measured, uncorrected for respiration.

Tab. XXXVI	Amphidinium sp.
Tab. XXXVII	Botrydiopsis alpina
Tab. XXXVIII	Chlamydomonas reinhardi
Tab. XXXIX	Chlorella pyrenoidosa
Tab. XL	Chlorella sorokiniana (7-11-05)
Tab. XLI	Chlorococcum wimmeri
Tab. XLII	Cryptomonas ovata
Tab. XLIII	Euglena gracilis
Tab. XLIV	Gloeocapsa alpicola
Tab. XLV	Nitzschia closterium
Tab. XLVI	Ochromonas danica
Tab. XLVII	Phormidium luridum
Tab. XLVIII	Phormidium persicinum
Tab. XLIX	Porphyridium aerugineum
Tab. L	Porphyridium cruentum
Tab. LI	Sphacelaria sp.
Tab. LII	Tribonema aequale

Table XXXVI

Process: Photosynthesis (not corrected for respiration)

Alga: ***Amphidinium* sp.**

Dates: 6/13-23/66; 6/28-7/8/66

Pr or actual measurement illum. (nm)	ml Oxygen/hour/ml packed cells									
White	-0.41	-0.17	-0.62	-0.37	-0.21	-0.12	-0.70	-0.82	-1.15	-0.17
680	0.49	-0.17	0.16	0.37	0.74	0.50	-0.86	-0.82	-0.99	0.29
650	-0.29	-0.62	-0.29	-0.08	0.00	-0.16	-0.95	-1.65	-1.48	-0.08
640	-0.17	-0.25	-0.12	-0.13	0.29	0.17	-0.74	-1.52	-1.40	0.16
630	-0.21	-0.66	-0.17	0.08	0.37	0.12	-0.78	-1.40	-1.24	0.16
620	-0.25	-0.25	-0.25	-0.21	0.00	0.05	-0.86	-1.40	-1.52	0.04
560	-0.29	-0.54	-0.29	0.00	0.08	-0.08	-0.78	-1.28	-1.24	-0.12
540	-0.08	0.41	-0.08	0.08	0.21	0.25	-0.66	-0.86	-1.11	0.29
440	-0.50	0.00	-0.46	-0.12	0.33	-0.16	-0.62	-0.91	-1.28	-0.33
405	-1.16	-0.83	-0.99	-0.91	-0.91	-1.07	-1.11	-1.65	-1.73	-1.07
	405	440	540	560	620	630	640	650	680	White

Growth illumination (nm)

Table XXXVII

Process: Photosynthesis (not corrected for respiration)

Alga: *Botrydiopsis alpina*

Dates: 1/13-21; 2/3-11/66

Prior or actual measurement illum. (nm)	ml Oxygen/hour/ml packed cells									
white	-0.2	-0.3	-0.2	-0.3	0.0	0.1	0.0	0.1	-0.3	-0.6
710	-0.7	-1.0	-1.0	-1.1	-1.2	-1.3	-1.6	-1.2	-1.1	-1.0
680	1.2	1.8	1.2	1.4	0.9	0.6	0.2	0.0	2.1	2.5
650	-0.3	-0.3	-0.4	-0.4	0.2	0.7	0.7	0.6	-0.2	-0.8
640	-0.2	-0.4	-0.2	-0.2	0.0	0.6	0.8	0.7	-0.2	-0.7
620	0.2	0.5	0.2	0.4	-0.1	-0.4	-0.5	-0.6	0.3	0.8
560	-0.4	-0.3	-0.5	-0.4	-0.7	-0.9	-0.6	-0.6	-0.5	-0.6
540	-0.3	-0.3	-0.8	-0.3	-0.6	-0.7	-0.6	-0.5	-0.4	-0.3
440	0.7	0.8	0.3	0.9	0.4	0.2	0.1	-0.1	0.7	1.0
405	0.3	0.4	0.2	0.4	0.1	-0.1	-0.1	-0.2	0.2	0.5
Growth illumination (nm)	405	440	540	560	620	640	650	680	710	White

Table XXXVIII

Process: Photosynthesis (not corrected for respiration)

Alga: Chlamydomonas reinhardi

Dates: 9/8-20/66; 10/27-11/8/66

Prior or actual measurement illum. (nm)	405	440	540	560	620	640	650	680	710	White
	ml Oxygen/hour/ml packed cells									
White	0.9	1.3	-1.4	-1.1	0.8	0.5	0.5	-0.1	-4.9	0.7
710	-1.3	-1.5	-3.5	-3.3	-1.6	-1.7	-1.4	-2.0	-5.3	-1.4
680	6.0	7.9	0.3	1.1	6.5	5.9	5.7	5.3	-4.0	5.4
650	4.5	5.5	1.0	0.1	4.4	3.9	3.3	2.6	-4.0	2.5
640	3.6	4.6	1.3	-0.2	3.4	2.9	2.3	1.8	-4.2	3.0
620	2.3	3.4	-1.8	-1.0	2.2	1.9	1.4	1.0	-4.2	2.0
560	-0.4	0.3	-3.0	-2.5	-0.9	-0.9	-1.1	-1.3	-4.2	-0.7
540	-0.7	-0.2	-3.1	-2.9	-1.4	-1.1	-1.4	-1.8	-4.6	-1.1
440	2.5	3.3	-1.8	-0.7	2.1	2.3	1.6	1.5	-4.7	1.9
405	1.4	1.6	-2.0	-1.5	1.0	1.5	0.4	0.2	-5.4	1.1

Growth illumination (nm)

Table XXXIX

Process: Photosynthesis (not corrected for respiration)

Alga: Chlorella pyrenoidosa

Dates: 8/27-9/7; 9/30-10/12/65

Prior or actual measurement illum. (nm)	ml Oxygen/hour/ml packed cells									
White	6.1	8.6	3.9	3.2	6.0	5.8	7.5	6.8	4.1	6.7
710	-1.6	-1.3	-1.7	-1.5	-1.8	-2.0	-1.6	-2.0	-1.4	-0.9
680	14.0	16.0	9.2	10.6	12.1	10.5	13.1	11.1	6.5	12.6
650	12.3	14.4	8.5	9.3	11.1	10.4	13.1	10.1	6.0	8.5
640	10.1	12.9	6.4	7.0	9.7	9.1	12.1	10.5	4.8	6.5
620	8.0	9.5	4.4	5.5	7.5	7.0	10.1	8.1	3.8	4.[illegible]
560	1.8	2.9	0.3	2.9	2.0	1.3	2.6	1.7	0.4	-0.5
540	1.3	2.2	0.3	0.4	1.5	0.9	2.0	1.1	0.1	0.6
440	4.5	4.5	3.4	3.7	4.1	3.7	4.8	4.0	3.3	2.6
405	3.3	2.5	1.4	1.7	2.3	1.7	2.6	1.5	1.5	0.8
	405	440	540	560	620	640	650	680	710	White

Growth illumination (nm)

Table XL

Process: Photosynthesis (not corrected for respiration)

Alga: *Chlorella sorokiniana* (7-11-05)

Dates: 12/28/66-1/6; 1/16-26/67

ml Oxygen/hour/ml packed cells

Prior or actual measurement illum. (nm)	405	440	540	560	620	640	650	680	710	White
White	3.0	1.9	3.0	0.9	1.9	1.3	0.1	-1.0	0.1	1.9
710	-1.4	-2.9	-1.3	-2.3	-1.7	-1.6	-4.1	-3.6	-3.1	-2.8
680	9.1	11.1	11.0	9.6	8.6	8.1	5.9	5.2	5.9	11.1
650	7.3	9.1	8.4	7.0	6.9	6.3	3.8	2.1	3.2	8.2
640	7.2	9.0	8.2	6.5	6.9	5.0	3.4	2.3	3.4	9.1
620	5.4	7.6	6.3	3.7	4.9	4.9	2.5	1.3	2.1	6.8
560	1.8	1.4	1.4	0.0	1.3	0.8	-1.6	-1.9	-0.7	2.1
540	0.9	1.0	0.3	-0.8	0.4	0.1	-2.6	-2.6	-1.9	0.3
440	3.2	2.7	3.5	1.5	1.5	1.4	-1.2	-0.9	0.0	1.6
405	1.6	1.1	1.6	0.3	0.3	0.6	-1.8	-2.1	-1.5	0.7

Growth illumination (nm)

Table XLI

Process: Photosynthesis (not corrected for respiration)

Alga: <u>Chlorococcum</u> <u>wimmeri</u>

Dates: 5/12-20/66; 5/24-6/1/66

ml Oxygen/hour/ml packed cells

Prior or actual measurement illum. (nm)	405	440	540	560	620	640	650	680	710	White
White	0.22	0.44	0.36	0.48	-0.22	-0.70	-0.97	-0.88	-0.04	0.26
710	-0.75	-0.70	-0.88	-0.97	-1.72	-2.38	-2.47	-2.33	-0.44	-0.83
680	2.54	2.90	2.11	1.98	1.81	1.99	1.98	1.45	1.19	2.11
650	1.09	1.36	1.19	1.05	0.80	0.62	0.22	-0.26	0.26	1.09
640	0.92	1.15	1.06	1.14	0.58	0.39	-0.01	-0.53	0.14	1.06
620	0.74	0.79	0.79	0.83	0.27	0.05	-0.36	-0.71	0.00	0.16
560	-0.39	-0.44	-0.26	-0.22	-0.62	-1.23	-1.41	-1.93	-0.53	-0.31
540	-0.35	-0.48	-0.31	-0.26	-0.70	-1.23	-1.51	-1.63	-0.53	-0.27
440	0.31	0.08	0.52	0.35	-0.17	-0.44	-0.62	-0.97	0.00	0.17
405	0.0	-0.04	-0.08	0.14	-0.57	-1.06	-1.15	-1.23	-0.31	0.00

Growth illumination (nm)

Table XLII

Process: Photosynthesis (not corrected for respiration)

Alga: <u>Cryptomonas</u> <u>ovata</u>

Dates: 11/9-18; 12/5-14/66

ml Oxygen/hour/ml packed cells

Prior or actual measurement illum. (nm)	405	440	540	560	620	630	640	650	680	White
White	0.3	-0.8	-0.4	-0.4	0.2	0.3	-0.3	-0.6	-0.8	-0.2
680	1.8	-0.2	-0.4	-0.1	1.5	1.6	0.3	-0.1	-0.5	0.7
650	1.0	-0.6	-0.5	-0.5	-0.6	0.6	-0.4	-0.7	-0.9	-0.1
640	0.9	-0.6	-0.5	-0.5	-0.6	0.5	-0.4	-0.6	-1.0	-0.1
630	0.9	-0.6	-0.4	-0.5	-0.5	0.1	-0.4	-0.6	-0.9	-0.1
620	0.9	-0.6	-0.4	-0.5	-0.7	0.6	-0.2	-0.5	-0.9	0.0
560	0.9	-0.3	-0.3	-0.4	-0.8	0.8	-0.2	-0.6	-0.9	0.1
540	0.7	-0.8	-0.5	-0.5	-0.3	0.4	-0.4	-0.7	-1.0	-0.2
440	0.7	-0.6	-0.4	-0.4	-0.6	0.6	-0.2	-0.4	-1.0	-0.1
405	0.6	-0.7	-0.6	-0.5	-0.5	-0.5	-0.4	-0.5	-1.0	-0.2

Growth illumination (nm)

Table XLIII

Process: Photosynthesis (not corrected for respiration)

Alga: *Euglena gracilis*

Dates: 8/2-9; 8/24-31/66

ml Oxygen/hour/ml packed cells

Prior or actual measurement illum.(nm)	405	440	540	560	620	640	650	680	710	White
White	1.4	1.7	1.9	1.3	2.0	1.8	1.5	2.0	1.7	1.2
710	1.4	1.0	2.0	1.1	2.1	2.4	1.9	4.0	2.4	0.8
680	7.2	8.2	7.9	6.1	6.8	7.2	7.1	6.6	7.4	5.9
650	5.2	5.6	5.8	4.1	5.2	4.6	5.4	5.8	5.1	3.9
640	5.0	5.5	5.9	4.0	5.2	5.4	5.7	5.4	4.9	3.7
620	4.2	4.7	4.9	3.4	4.8	5.2	4.9	5.3	4.2	3.5
560	0.7	0.9	1.3	0.2	1.2	0.5	1.0	1.2	0.0	0.0
540	-0.1	0.0	0.5	-0.3	0.4	0.4	0.1	0.2	-0.9	-0.6
440	2.8	3.2	3.1	1.9	3.1	3.3	2.7	2.9	2.6	2.0
405	2.1	1.6	2.3	1.3	1.9	2.0	1.7	1.8	1.2	0.9

Growth illumination (nm)

Table XLIV

Process: Photosynthesis (not corrected for respiration)

Alga: Gloeocapsa alpicola

Dates: 4/26-5/3/66; 5/4-11/66

Prior or actual measurement illum. (nm)	ml Oxygen/hour/ml packed cells									
White	0.33	0.23	0.28	0.34	0.26	0.32	0.28	0.29	0.33	0.32
680	0.63	0.43	0.31	0.42	0.32	0.39	0.40	0.40	0.68	0.44
650	0.39	0.57	0.48	0.93	0.65	0.89	0.81	0.81	0.86	0.79
640	0.92	0.62	0.79	1.07	0.75	0.93	0.87	0.86	0.95	0.89
630	0.91	0.67	0.88	1.11	0.82	0.97	0.91	0.94	1.00	0.92
620	0.90	0.64	0.74	1.06	0.78	0.97	0.88	0.90	0.97	1.05
560	0.08	-0.18	0.24	0.40	0.18	0.28	0.22	0.22	0.27	0.27
540	0.03	-0.08	0.04	0.09	0.00	0.03	0.03	0.01	0.02	0.02
440	0.04	-0.03	0.00	0.03	-0.01	0.00	-0.02	0.00	0.08	-0.03
405	0.17	-0.07	-0.05	-0.04	-0.02	-0.04	-0.07	0.16	-0.02	-0.05
	405	440	540	560	620	630	640	650	680	White

Growth illumination (nm)

Table XLV

Process: Photosynthesis (not corrected for respiration)

Alga: Nitzschia closterium

Dates: 1/31-2/10/67; 2/28-3/10/67

ml Oxygen/hour/ml packed cells

Prior or actual measurement illum. (nm)	405	440	540	560	620	630	640	650	680	White
White	0.9	1.0	-0.1	0.0	0.6	0.8	0.5	0.7	0.5	0.1
680	2.6	3.3	1.0	1.7	2.4	3.7	2.9	4.5	3.4	1.9
650	1.4	1.8	0.1	0.5	0.9	1.8	1.0	2.0	1.2	0.4
640	1.3	2.0	0.2	0.6	1.0	2.1	1.1	2.1	1.4	0.6
630	1.3	2.1	0.2	0.6	1.0	2.1	1.1	1.9	1.3	0.6
620	1.2	1.7	0.0	0.4	0.9	1.7	0.9	1.8	0.9	0.4
560	0.7	1.0	-0.2	-0.1	0.3	0.9	0.4	0.8	0.1	0.0
540	1.1	1.4	-0.1	0.1	0.6	1.3	0.6	1.3	0.6	0.3
440	1.4	1.4	0.1	0.3	0.9	1.0	0.9	1.1	0.8	0.2
405	0.6	0.5	-0.4	-0.1	0.2	0.4	0.0	0.0	-0.3	0.2

Growth illumination (nm)

Table XLVI

Process: Photosynthesis (not corrected for respiration)

Alga: Ochromonas danica

Dates: 7/7-15; 11/30-12/7/65; 2/15-22/66

ml Oxygen/hour/ml packed cells

Prior or actual measurement illum. (nm) \ Growth illumination (nm)	405	440	540	560	620	640	650	680	710	White
White	-0.8	-1.6	-2.6	-3.1	-0.3	-3.5	0.0	0.5	-1.9	-3.3
710	-3.3	-4.7	-6.0	-5.0	-2.3	-5.1	-0.7	-0.4	-3.2	-5.4
680	-0.7	0.6	-6.0	-4.7	0.5	-3.6	0.3	-2.8	-1.9	-2.3
650	-0.6	-0.7	-6.4	-5.4	-0.2	-3.7	-0.8	-3.8	-2.2	-0.9
640	-1.4	0.2	-6.7	-4.7	0.6	-3.4	-0.4	-3.5	-1.2	-3.0
620	-1.0	-1.4	-5.0	-6.2	-1.5	-2.3	-0.9	-2.8	-2.4	-1.6
560	-2.6	-2.0	-4.3	-5.3	-0.7	-2.7	-1.4	-3.2	-2.5	-2.7
540	-2.2	-3.6	-3.4	-4.4	-0.9	-4.3	-2.0	-2.8	-2.7	-4.2
440	-3.1	-2.6	-2.6	-4.0	-0.8	-1.9	-1.1	-1.7	-1.9	-3.6
405	-4.7	-3.2	-4.5	-4.5	-2.7	-2.9	-1.6	-1.8	-2.7	-6.9

Table XLVII

Process: Photosynthesis (not corrected for respiration)

Alga: Phormidium luridum

Dates: 1/5-12; 1/26-2/2/66

ml Oxygen/hour/ml packed cells

Prior or actual measurement illum. (nm)	405	440	540	560	620	640	650	680	7.0	White
White	0.6	0.5	0.7	0.6	1.8	1.9	.6	1.3	-0.2	1.1
710	-0.9	-0.9	-1.0	-1.0	-0.8	-1.2	-1.3	-0.9	-1.0	-1.2
680	0.9	0.5	0.3	0.2	0.9	0.9	1.1	1.0	-0.8	0.7
650	2.1	1.2	2.0	2.2	4.0	4.8	3.4	2.9	-0.5	3.7
640	2.4	1.7	2.5	2.7	4.5	5.2	3.7	3.1	-0.4	3.4
620	2.3	1.7	2.7	2.6	4.7	5.0	3.4	2.9	-0.3	3.1
560	0.3	0.0	0.6	0.5	2.0	1.8	1.0	0.5	-0.3	0.4
540	-0.3	-0.4	-0.1	-0.3	1.5	0.4	0.1	-0.2	-0.3	-0.2
440	-0.2	-0.6	-0.2	-0.7	-0.3	-0.7	-0.5	-0.1	-0.3	0.1
405	-0.4	-0.3	-0.3	-0.7	-0.5	-0.9	-0.6	-0.3	-0.3	-0.3

Growth illumination (nm)

Table XLVIII

Process: Photosynthesis (not corrected for respiration)

Alga: Phormidium persicinum

Dates: 7/12-19; 7/20-27/66

ml Oxygen/hour/ml packed cells

Prior or actual measurement illum. (nm)	405	440	540	560	620	630	640	650	680	White
White	0.74	0.49	0.66	0.73	1.90	1.89	1.34	0.92	1.34	1.00
680	0.45	-0.09	-0.19	-0.23	1.03	0.80	0.75	-0.47	0.67	-0.08
650	1.26	0.45	0.78	0.35	2.76	2.17	2.81	0.30	1.04	0.88
640	1.27	0.73	0.80	0.69	3.49	3.00	2.76	1.46	1.24	1.00
630	1.12	0.89	0.93	0.85	3.27	2.63	2.46	0.80	1.37	1.01
620	1.24	0.97	0.77	0.60	2.93	2.59	2.34	0.51	1.29	1.03
560	3.53	3.65	3.36	3.15	4.47	5.26	4.79	1.78	4.10	4.20
540	2.88	2.67	2.90	2.71	4.18	4.47	3.93	2.89	3.00	3.19
440	-0.62	-1.08	-0.52	-0.42	0.08	-0.07	-0.42	-0.68	-0.94	-0.19
405	-0.35	-1.06	-0.57	-0.56	-0.23	-0.38	-0.56	-1.13	-0.87	-0.06

Growth illumination (nm)

T... IX

Process: Photo...nesis (not corrected for respira...

Alga: Porphyridium ...

Dates: 11/17-24; 5

ml Oxygen/hour/ml packed c...s

Prior or actual measurement illum. (nm)	405	440	540	560	620	640	650	680	710	white
White	0.3	0.4	0.9	0.7	1.[illegible]	.1	0.9	1.5	1.6	1.0
710	-3.1	-1.5	-1.6	-1.6	-3.[illegible]	-2.4	-3.3	-3.2	-3.7	-1.6
680	2.2	1.6	1.1	1.6	1.8	1.4	2.6	4.2	4.6	1.1
650	2.2	1.6	3.6	3.4	3.9	4.5	4.7	5.3	4.6	3.1
640	2.5	1.9	4.2	4.2	3.8	4.9	6.5	6.0	6.9	3.5
620	2.7	2.0	3.7	4.0	5.3	4.9	5.6	6.5	6.4	3.5
560	0.0	0.2	1.2	1.1	0.6	1.1	0.9	1.8	1.4	1.3
540	-0.7	-0.3	0.2	0.2	0.4	0.2	-0.8	0.1	-0.2	-0.1
440	-0.8	-0.2	-0.8	-0.6	-0.9	-0.9	-1.5	-0.7	-0.5	-0.7
405	-0.5	-0.1	-0.6	-0.4	-0.7	-0.7	-1.1	-0.3	-0.1	-0.4

Growth illumination (nm)

Table L

Process: Photosynthesis (not corrected for respiration)

Alga: <u>Porphyridium cruentum</u>

Dates: 3/4-11/66; 3/15-22/66

ml Oxygen/hour/ml packed cells

Prior or actual measurement illum. (nm)	405	440	540	560	620	630	640	650	680	White
White	1.19	1.10	0.12	-0.06	0.39	0.96	0.56	0.21	0.15	0.79
680	2.03	2.15	0.29	0.08	0.11	0.94	0.76	0.71	1.21	1.22
650	2.26	1.83	0.31	-0.03	0.41	0.97	0.64	0.44	0.62	1.17
640	2.21	1.88	0.35	0.05	1.23	0.68	0.54	0.55	0.62	1.25
630	2.17	1.65	0.41	0.05	1.23	1.18	0.79	0.50	1.33	1.39
620	2.04	1.77	0.35	0.08	0.97	1.09	0.64	0.76	0.62	1.19
560	2.60	2.64	1.05	0.69	1.46	2.37	0.56	1.31	1.47	1.68
540	2.35	2.47	1.17	0.67	1.37	2.28	1.59	1.23	0.44	1.80
440	0.58	0.42	-0.24	-0.3[illegible]	0.56	-0.02	0.00	0.03	0.12	0.12
405	0.26	0.09	-0.18	-0.43	0.38	-0.01	-0.14	-0.20	-0.15	0.06

Growth illumination (nm)

Table LI

Process: Photosynthesis (not corrected for respiration)

Alga: Sphacelaria sp.

Dates: 4/12-25; 9/27-10/11; 10/13-25/66

ml Oxygen/hour/ml packed cells

Prior or actual measurement illum. (nm)	405	440	540	560	620	640	650	680	710	White
White	-0.098	-0.128	-0.187	-0.251	-0.584	-0.619	-0.538	-0.161	-0.150	-0.325
710	-0.110	-0.267	-0.338	-0.348	-0.759	-0.760	-0.667	-0.220	-0.150	-0.406
680	-0.115	-0.209	-0.256	-0.336	-0.693	-0.771	-0.561	-0.197	-0.208	-0.406
650	-0.116	-0.197	-0.231	-0.336	-0.737	-0.854	-0.561	-0.207	-0.231	-0.383
640	-0.145	-0.185	-0.244	-0.336	-0.831	-0.748	-0.585	-0.185	[illegible]	-0.359
620	-0.133	-0.185	-0.232	-0.337	-0.787	-0.807	-0.584	-0.185	-0.231	-0.313
560	-0.162	-0.220	-0.220	-0.349	-0.807	-0.783	-0.620	-0.197	-0.243	-0.324
540	-0.163	-0.185	-0.256	-0.348	-0.760	-0.831	-0.549	-0.196	-0.254	-0.289
440	-0.174	-0.197	-0.290	-0.395	-0.819	-0.865	-0.679	-0.243	-0.242	-0.500
405	-0.151	-0.208	-0.302	-0.384	-0.807	-0.807	-0.620	-0.208	-0.242	-0.418

Growth illumination (nm)

Table LII

Process: Photosynthesis (not corrected for respiration)

Alga: Tribonema aequale

Dates: 3/23-30/66; 3/31-4/7/66

Prior or actual measurement illum. (nm)	ml Oxygen/hour/ml packed cells									
White	-0.22	-0.28	-0.11	-0.24	-0.29	-0.44	-0.39	-0.33	-0.25	-0.26
710	-0.64	-0.89	-0.64	-0.81	-0.71	-0.80	-0.57	-0.85	-0.67	-0.71
680	-0.17	-0.44	0.02	-0.24	-0.26	-0.50	-0.43	-0.57	-0.17	-0.30
650	-0.38	-0.58	-0.25	-0.47	-0.47	-0.64	-0.57	-0.67	-0.42	-0.54
640	-0.36	-0.50	-0.20	-0.37	-0.42	-0.55	-0.54	-0.64	-0.33	-0.40
620	-0.30	-0.47	-0.15	-0.32	-0.37	-0.42	-0.47	-0.45	-0.32	-0.49
560	-0.41	-0.43	-0.30	-0.37	-0.45	-0.43	-0.48	-0.48	-0.41	-0.40
540	-0.35	-0.46	-0.22	-0.33	-0.40	-0.43	-0.50	-0.41	-0.38	-0.36
440	-0.17	-0.28	-0.06	-0.14	-0.26	-0.31	-0.40	-0.34	-0.19	-0.28
405	-0.08	-0.32	-0.15	-0.28	-0.26	-0.39	-0.39	-0.33	-0.28	-0.25
	405	440	540	560	620	640	650	680	710	White

Growth illumination (nm)

Appendix E

This section includes "contour" plots of photosynthesis and respiration capacities of 17 algal species following growth and measurements in monochromatic and white illuminations. The maps were created and supplied by the U.S. Army Natick Laboratories (Mr. Robert Matthern). Plots for Chlorella sorokiniana and Nitzschia closterium are absent due to initial inclusion of data in the current report.

Figs. 60 & 61:	Amphidinium sp.
Figs. 62 & 63:	Botrydiopsis alpina
Figs. 64 & 65:	Chlamydomonas reinhardi
Figs. 66 & 67:	Chlorella pyrenoidosa
Figs. 68 & 69:	Chlorella sorokiniana (7-11-05)
Figs. 70 & 71:	Chlorococcum wimmeri
Figs. 72 & 73:	Cryptomonas ovata
Figs. 74 & 75:	Euglena gracilis
Figs. 76 & 77:	Gloeocapsa alpicola
Figs. 78 & 79:	Nitzschia closterium
Figs. 80 & 81:	Ochromonas danica
Figs. 82 & 83:	Phormidium luridum
Figs. 84 & 85:	Phormidium persicinum
Figs. 86 & 87:	Porphyridium aerugineum
Figs. 88 & 89:	Porphyridium cruentum
Figs. 90 & 91:	Sphacelaria sp.
Figs. 92 & 93:	Tribonema aequale

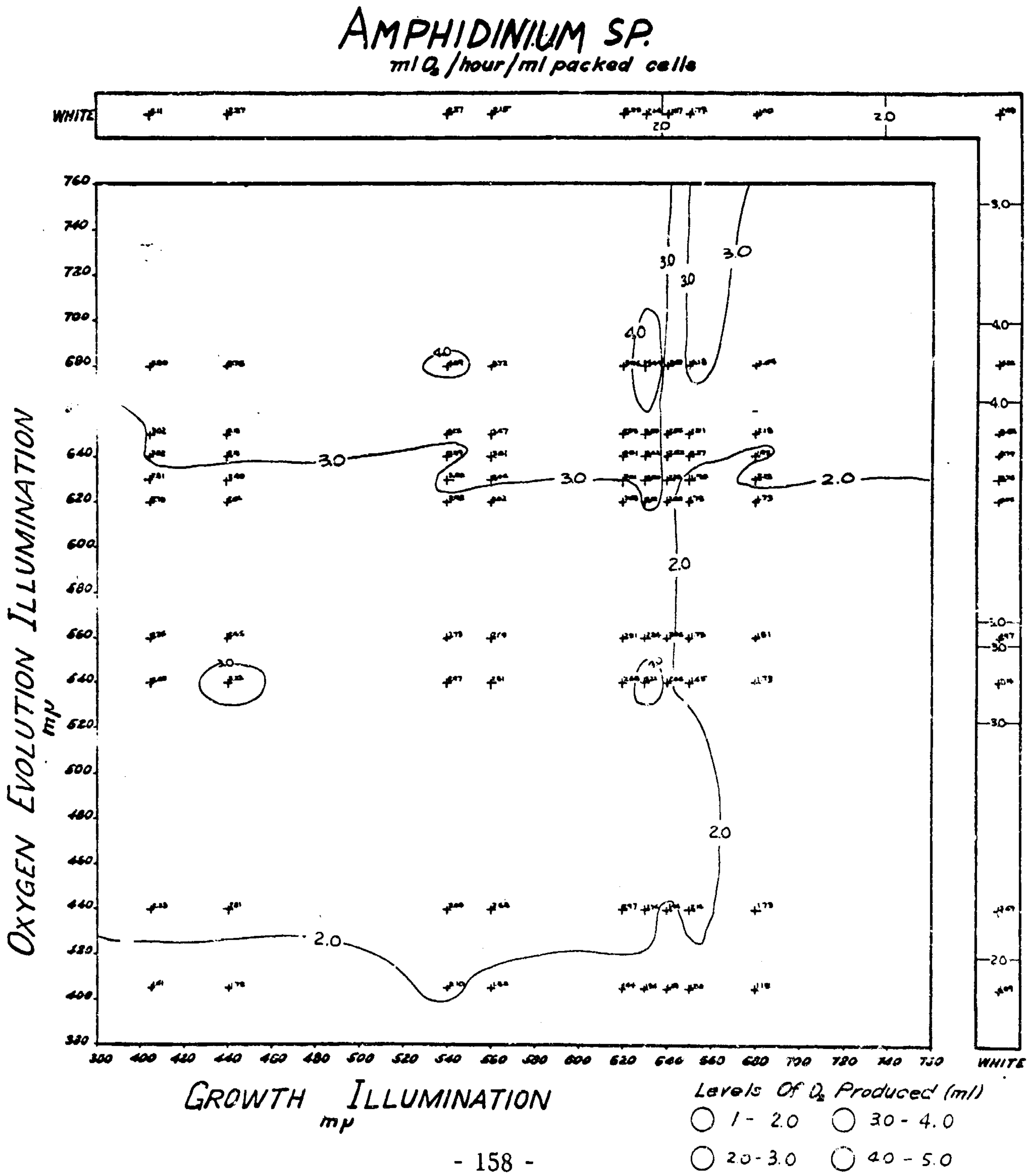

AMPHIDINIUM SP.
ml O2 /hour/ml packed cells
WHITE
OXYGEN EVOLUTION ILLUMINATION
mμ
GROWTH ILLUMINATION
mμ
Levels Of O2 Produced (ml)
1 - 2.0
3.0 - 4.0
2.0 - 3.0
4.0 - 5.0

Fig. 61 Respiration

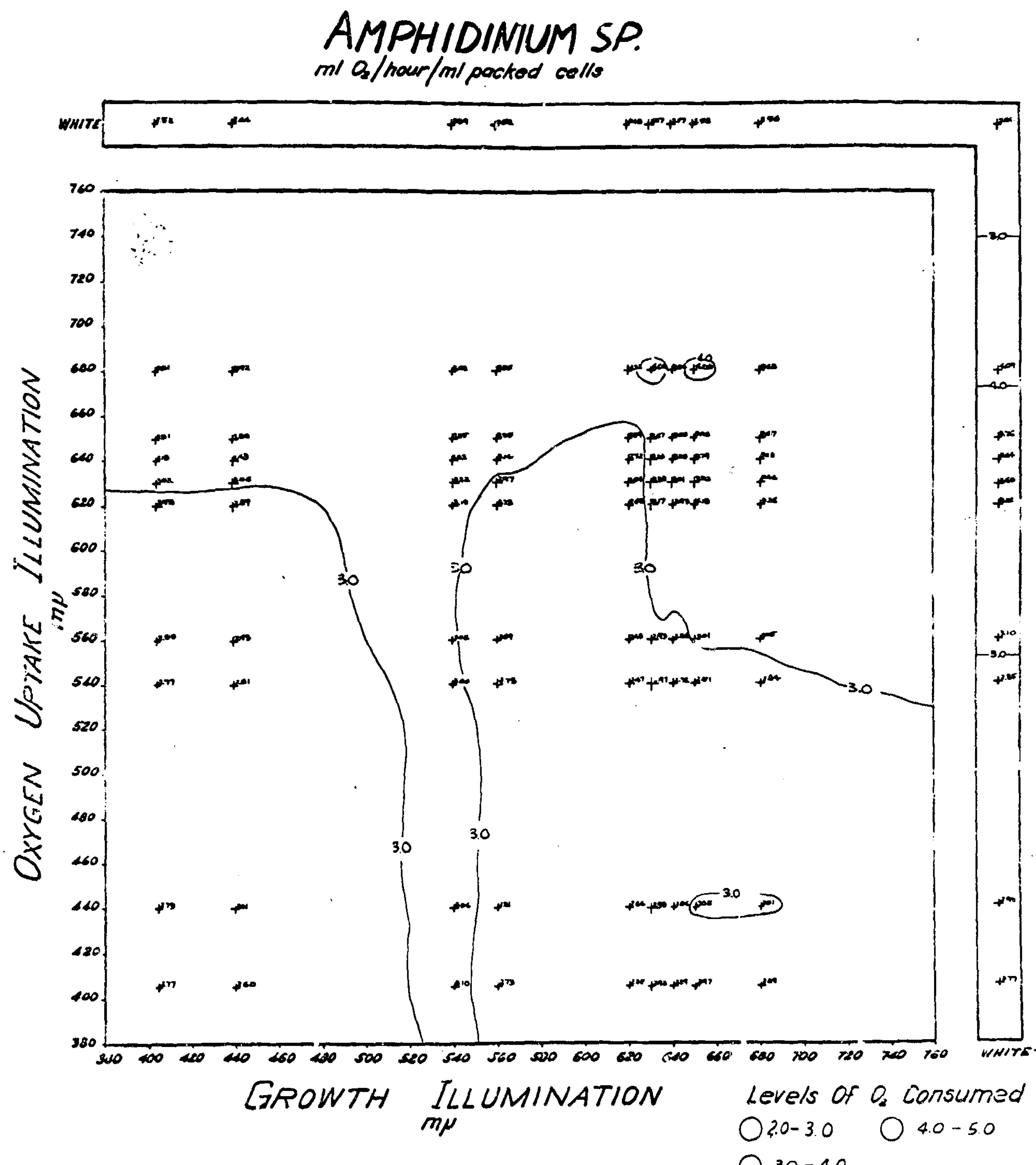


Fig. 62 Photosynthesis

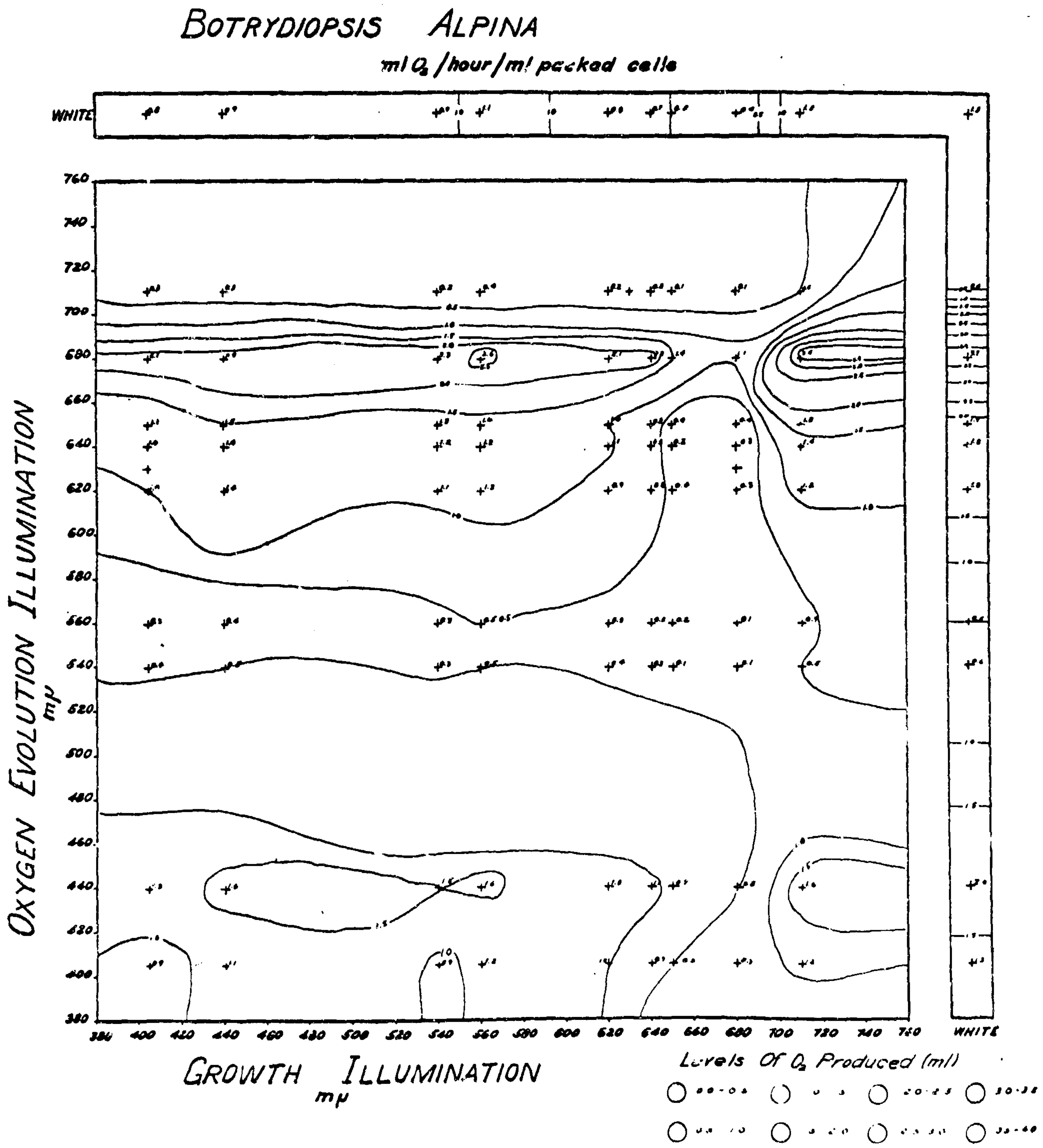


Fig. 63 Respiration

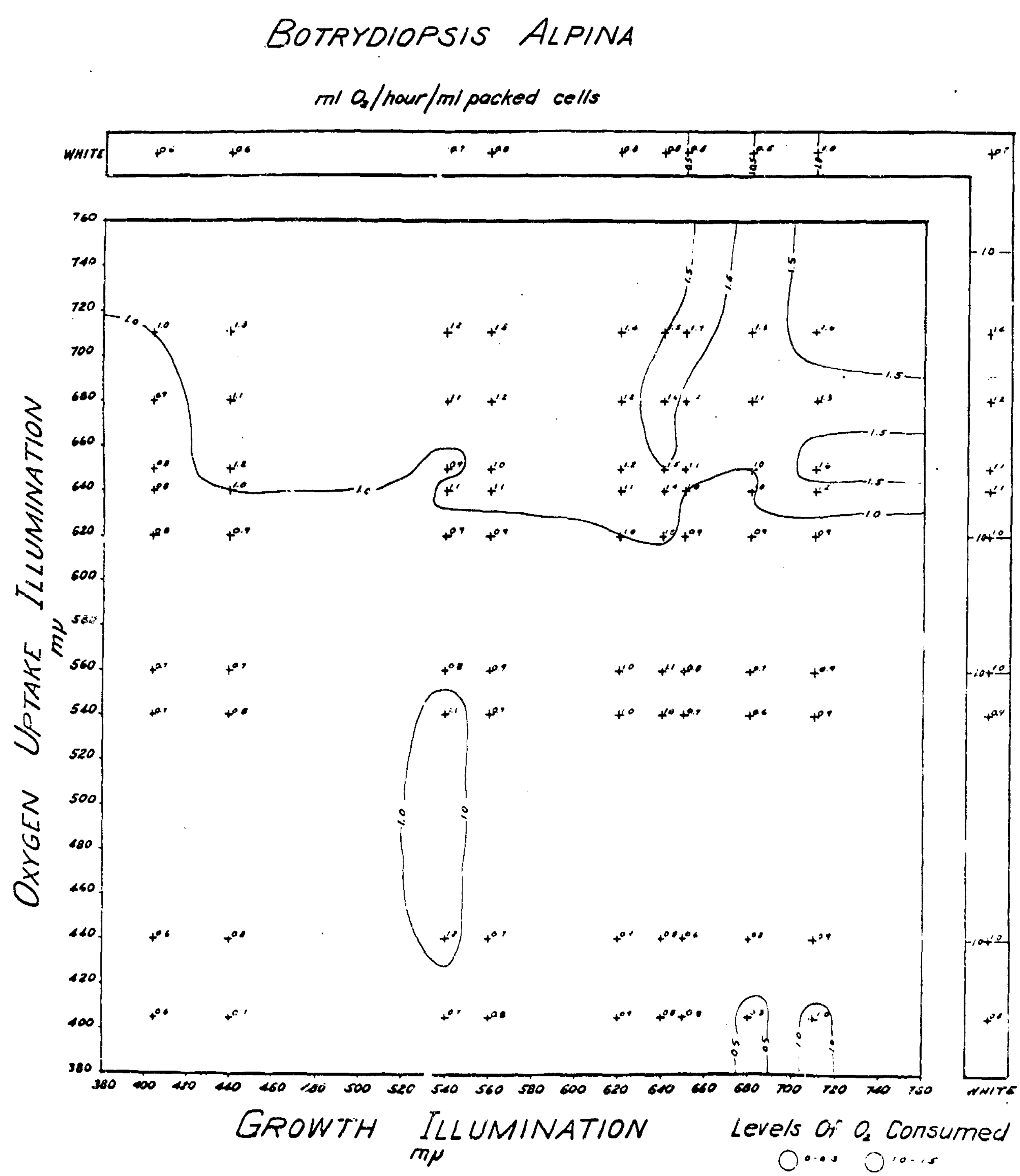


Fig. 64 Photosynthesis

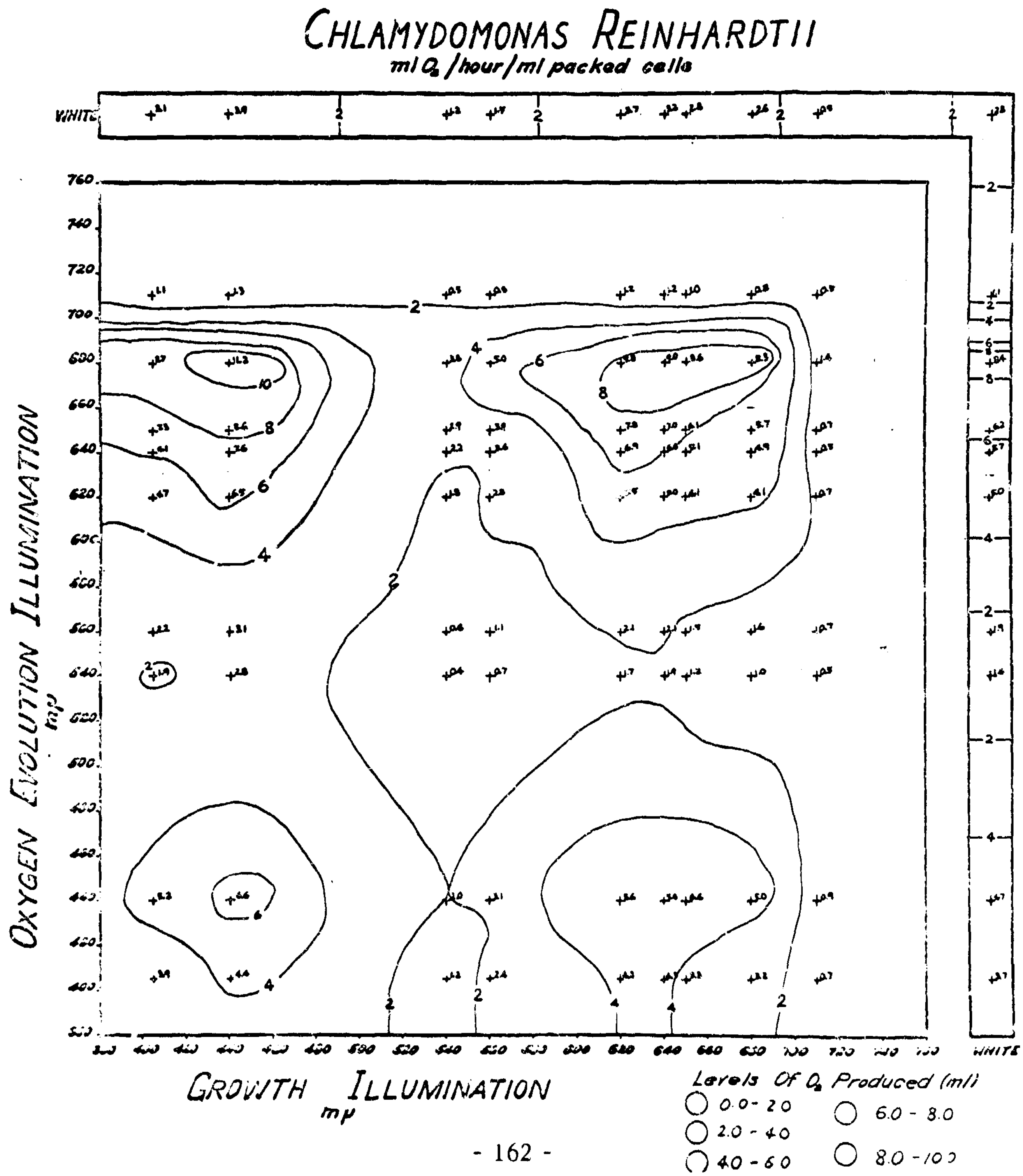


Fig. 65 Respiration

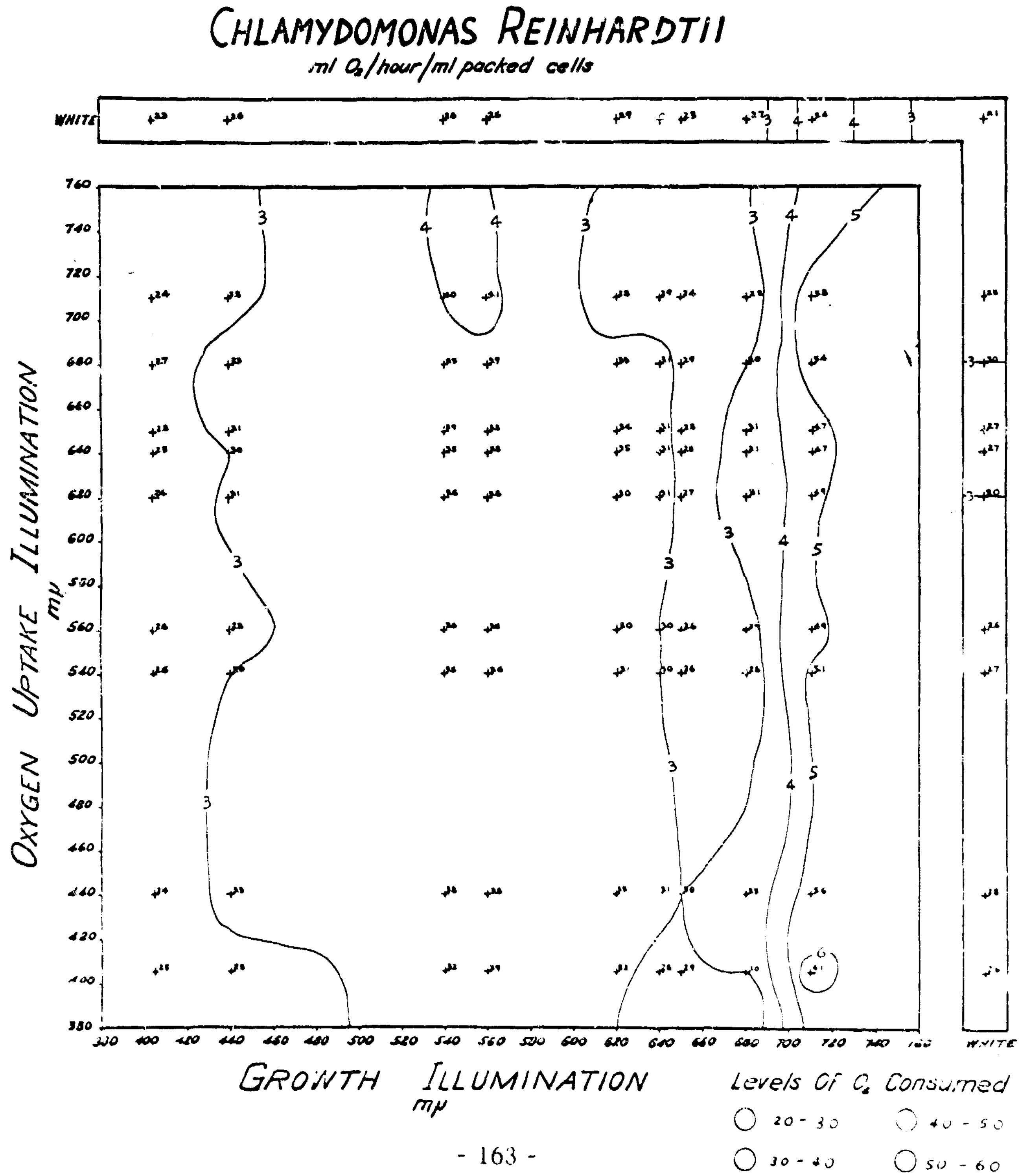
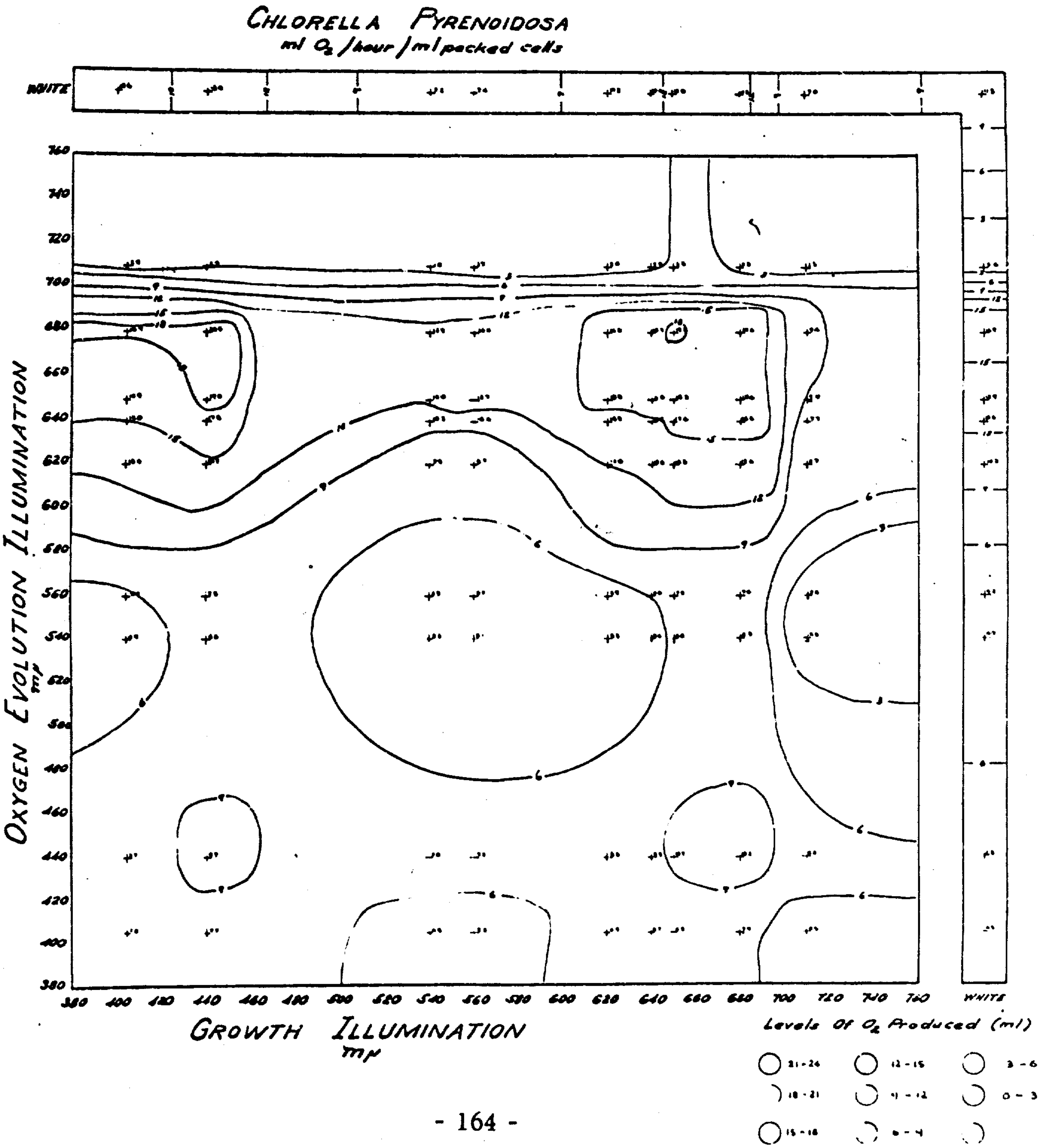


Fig. 66 Photosynthesis



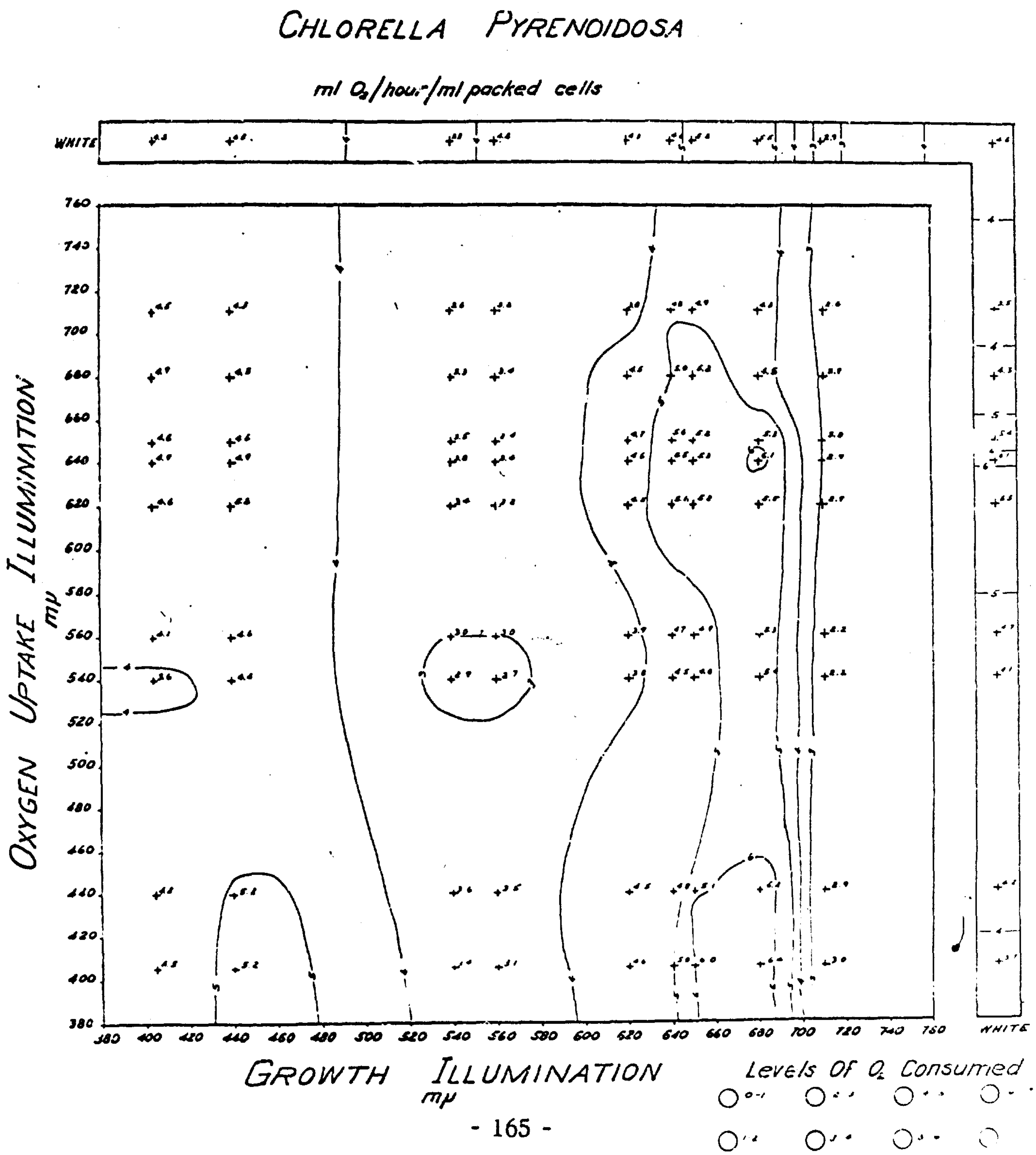

CHLORELLA PYRENOIDOSA
ml O2/hour/ml packed cells
WHITE
OXYGEN UPTAKE ILLUMINATION
mμ
760
740
720
700
680
660
640
620
600
580
560
540
520
500
480
460
440
420
400
380
GROWTH ILLUMINATION
mμ
380 400 420 440 460 480 500 520 540 560 580 600 620 640 660 680 700 720 740 760
WHITE
Levels Of O2 Consumed

Fig. 68 Photosynthesis

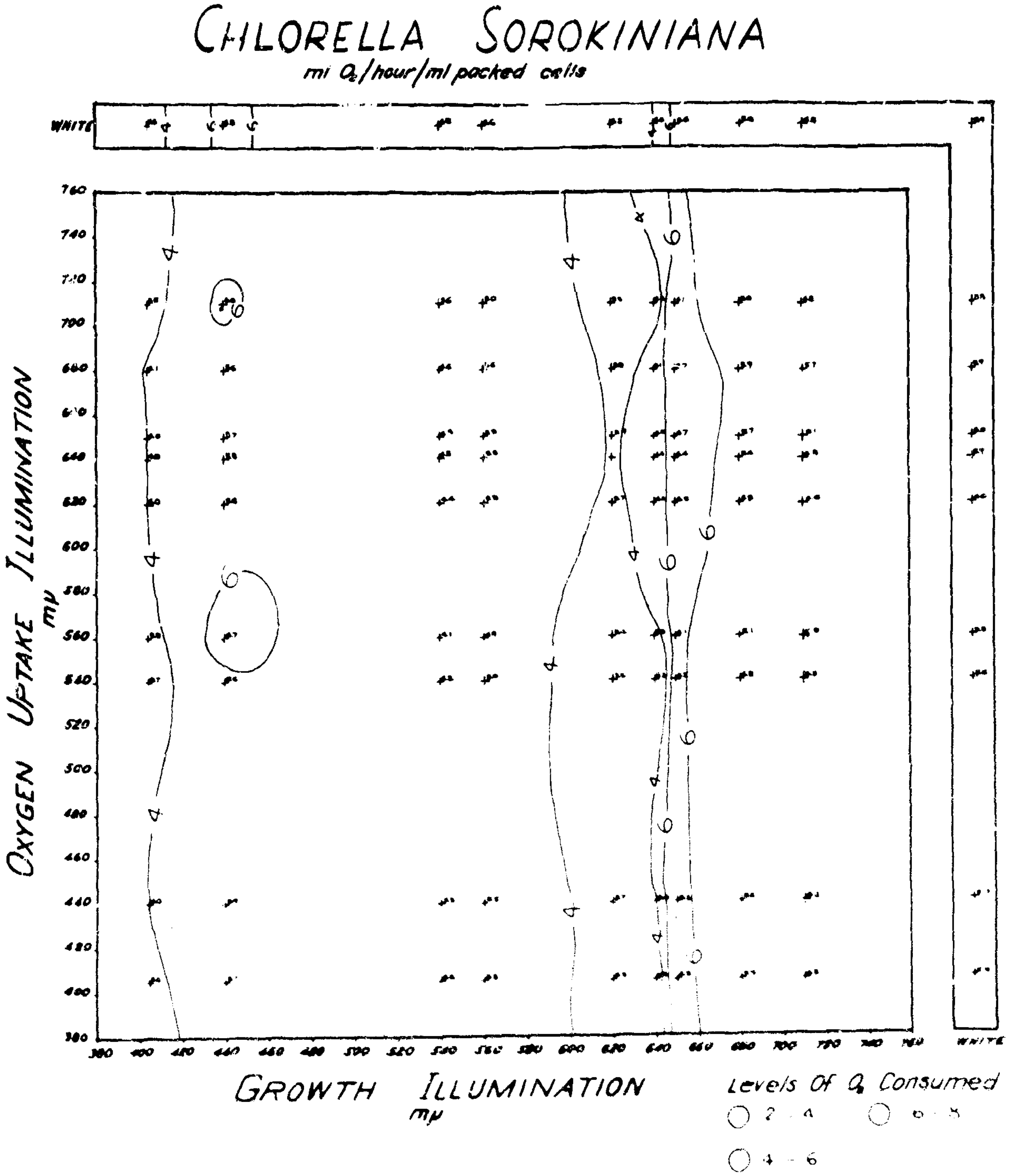


Fig. 39 Respiration

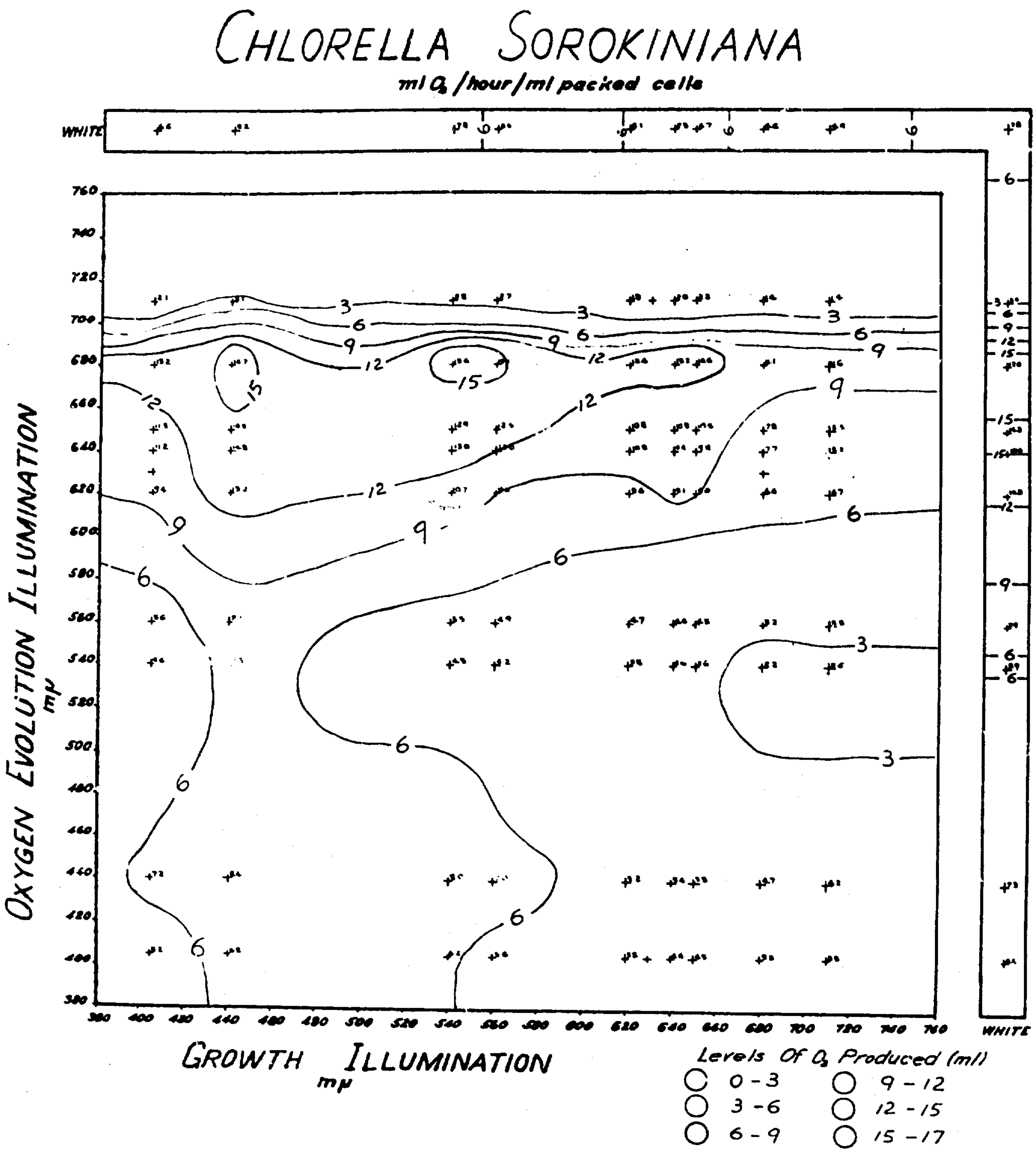


Fig. 70 Photosynthesis

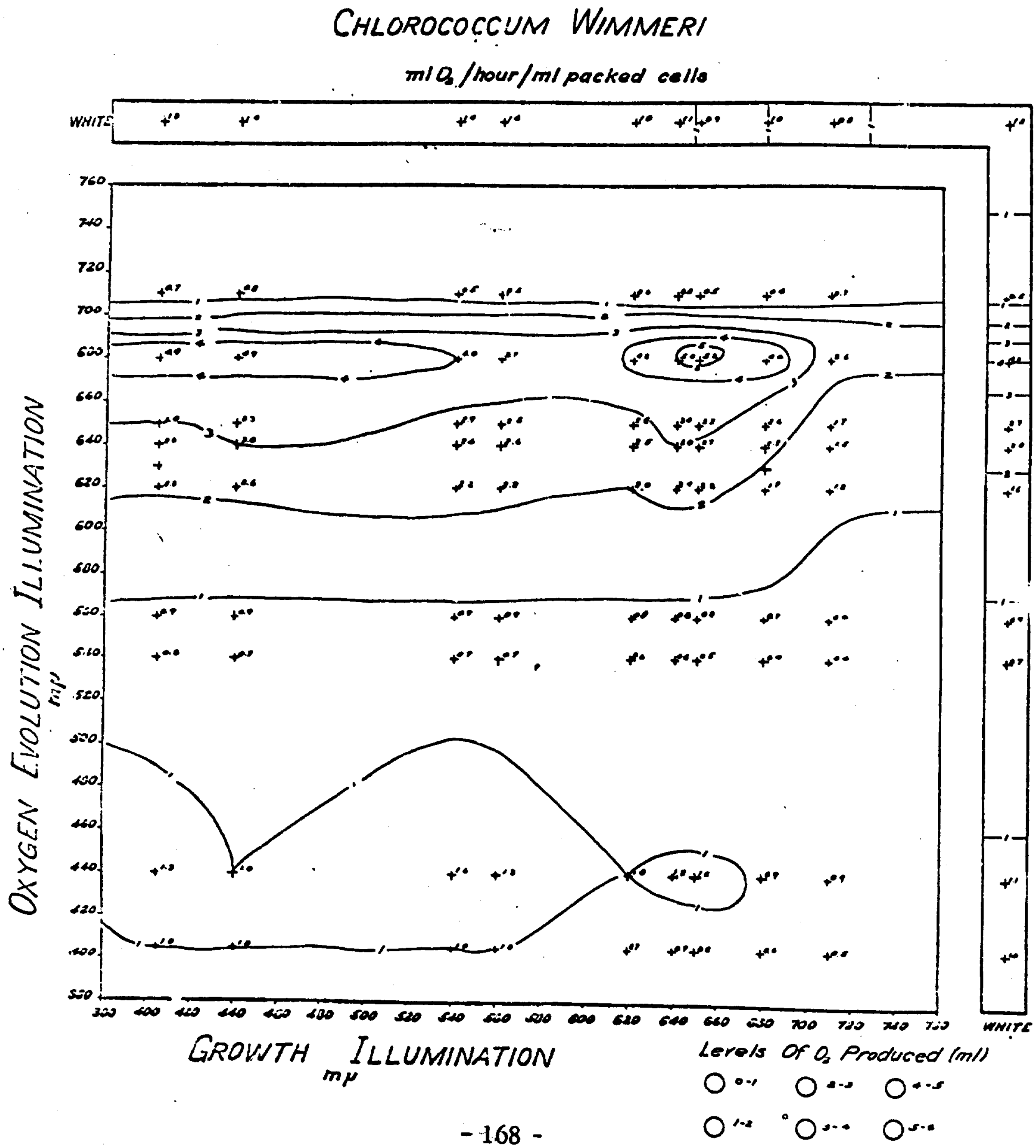


Fig. 71 Respiration

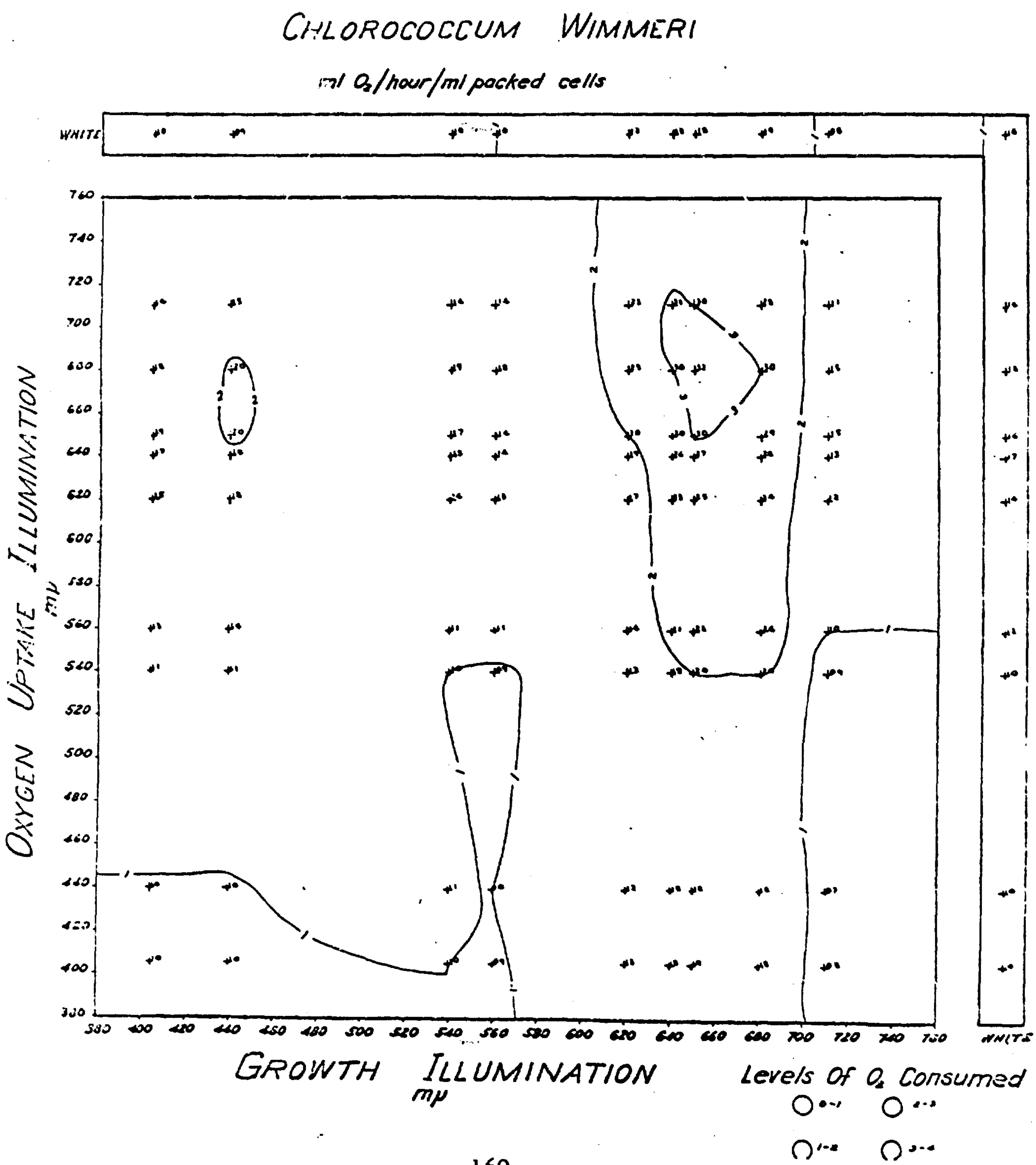


Fig. 72 Photosynthesis

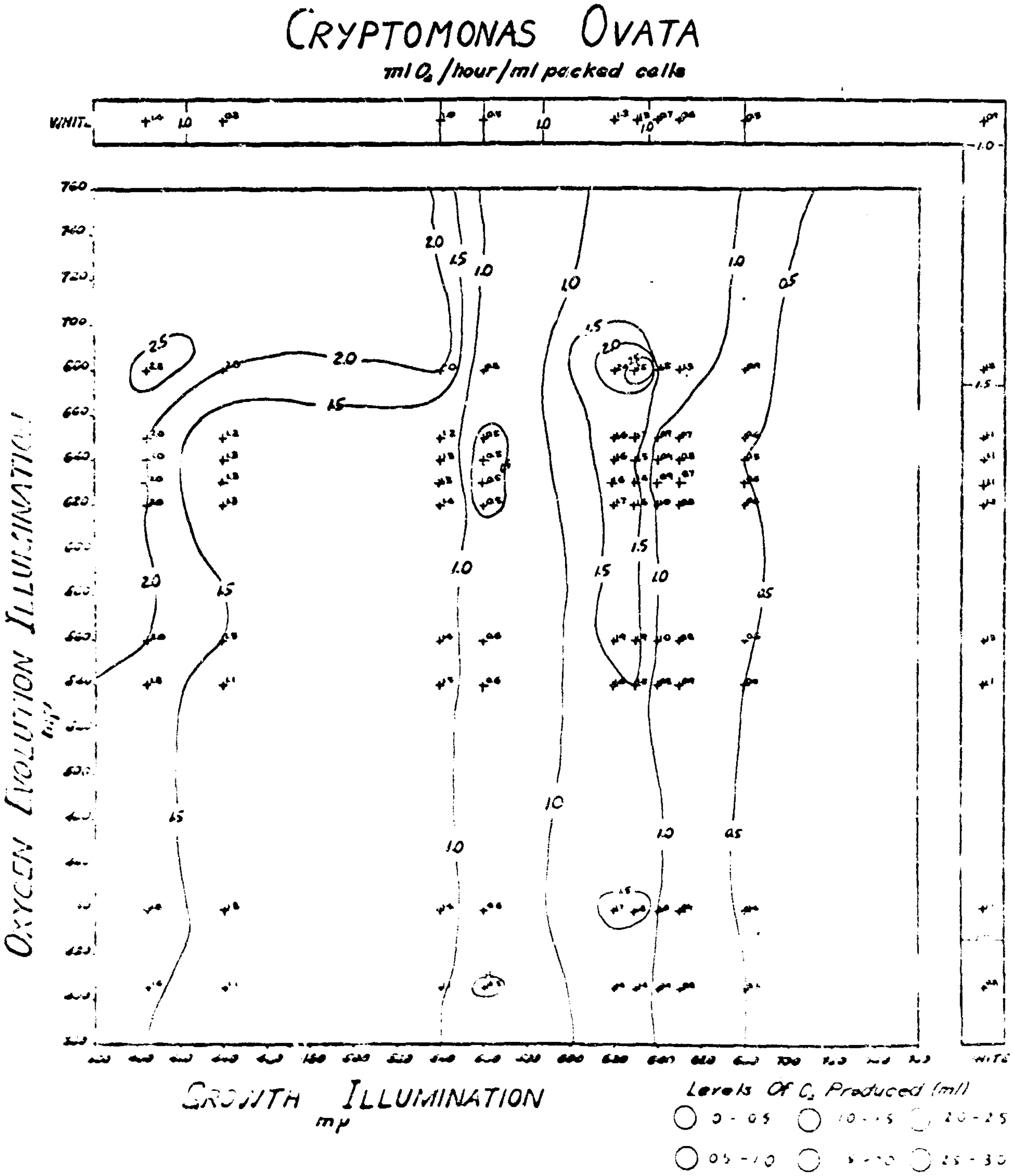
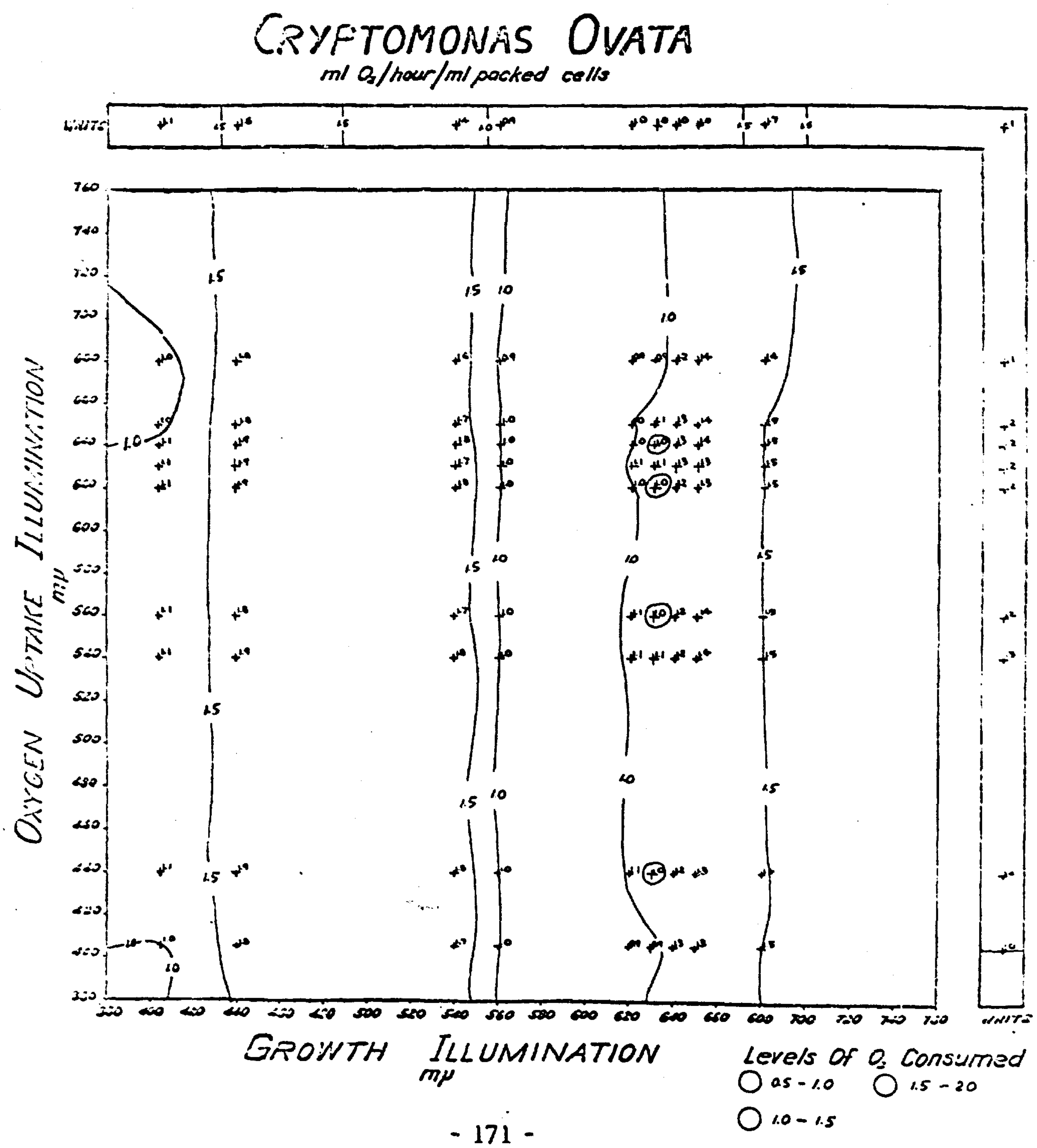
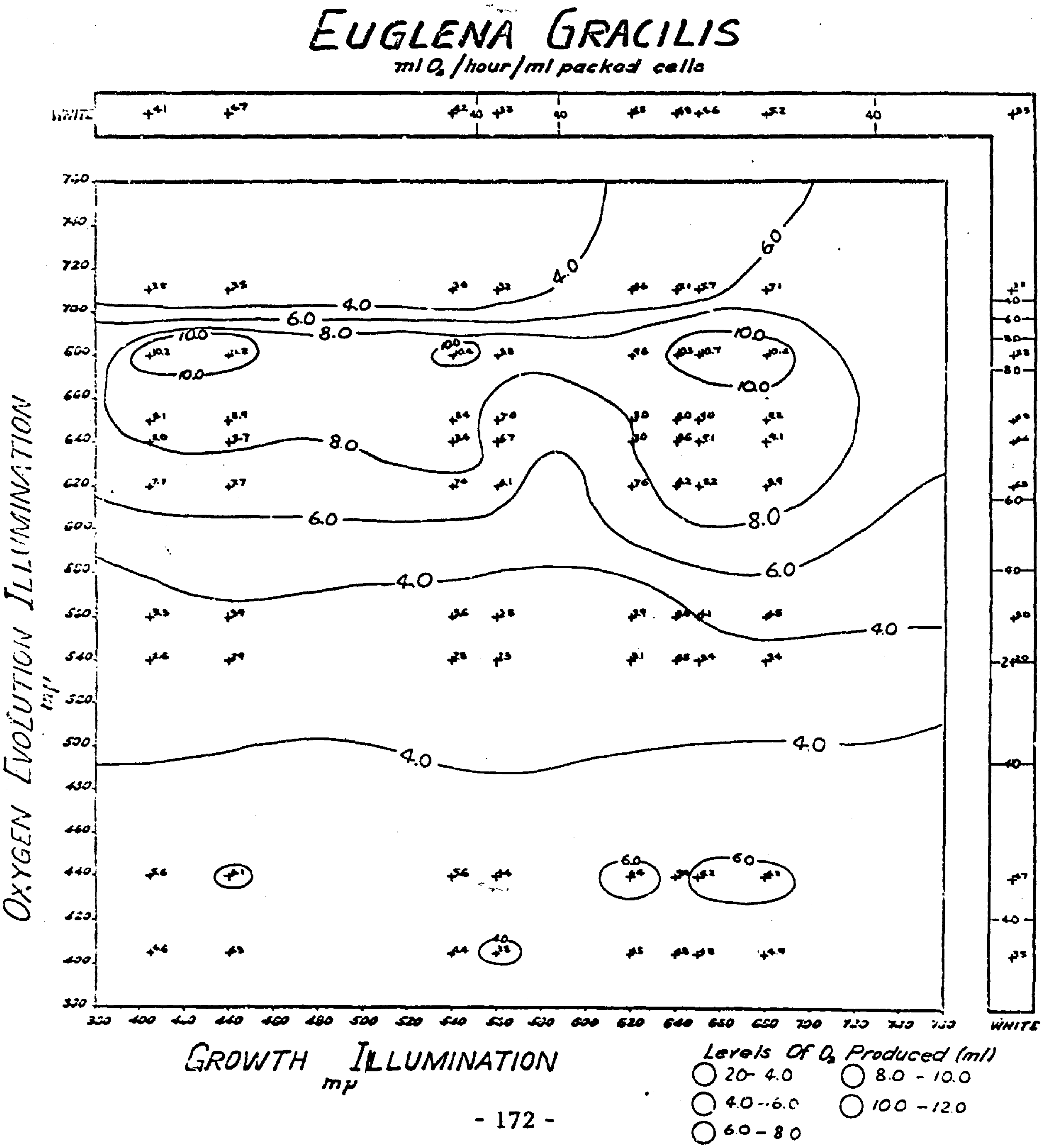
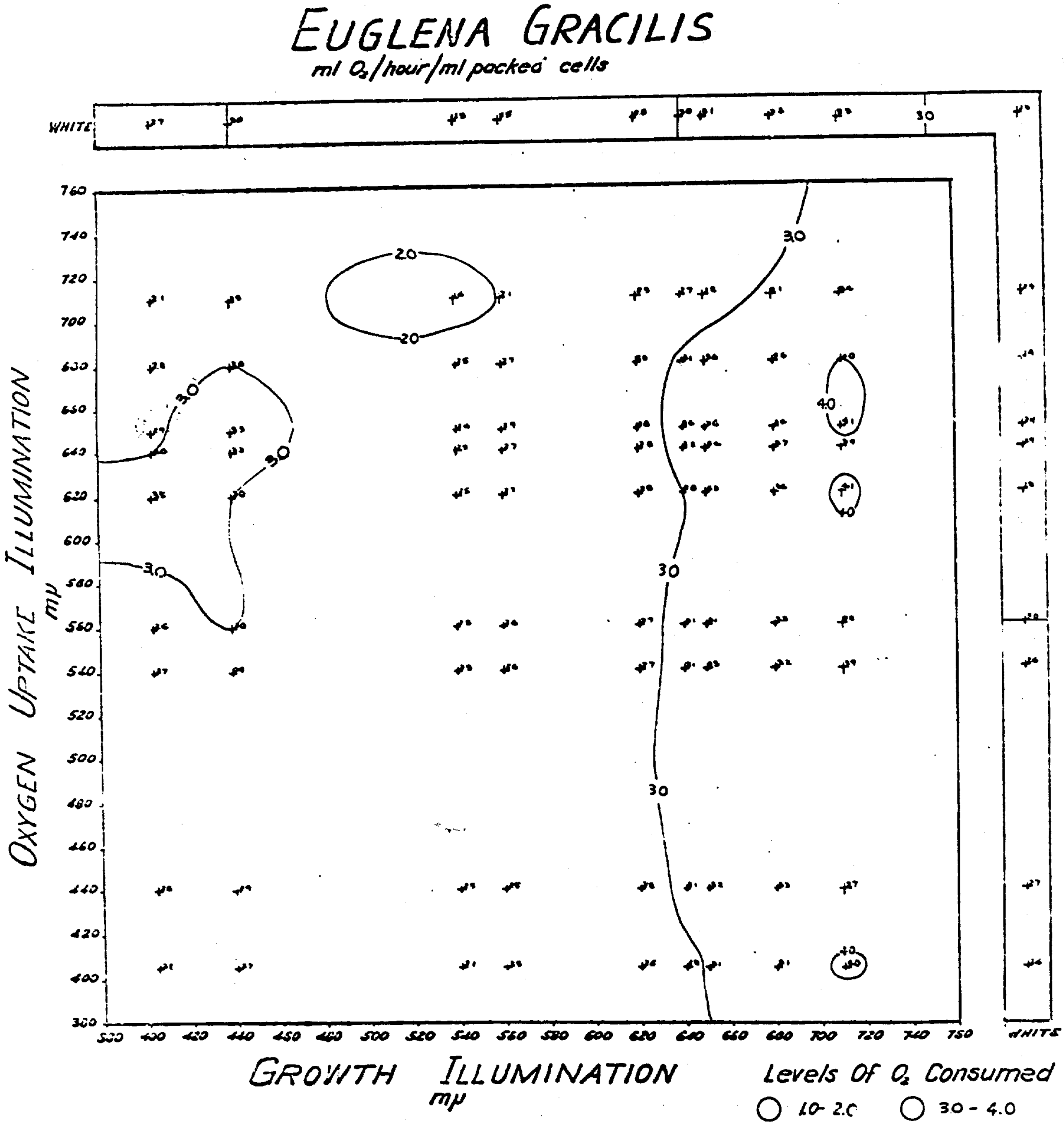
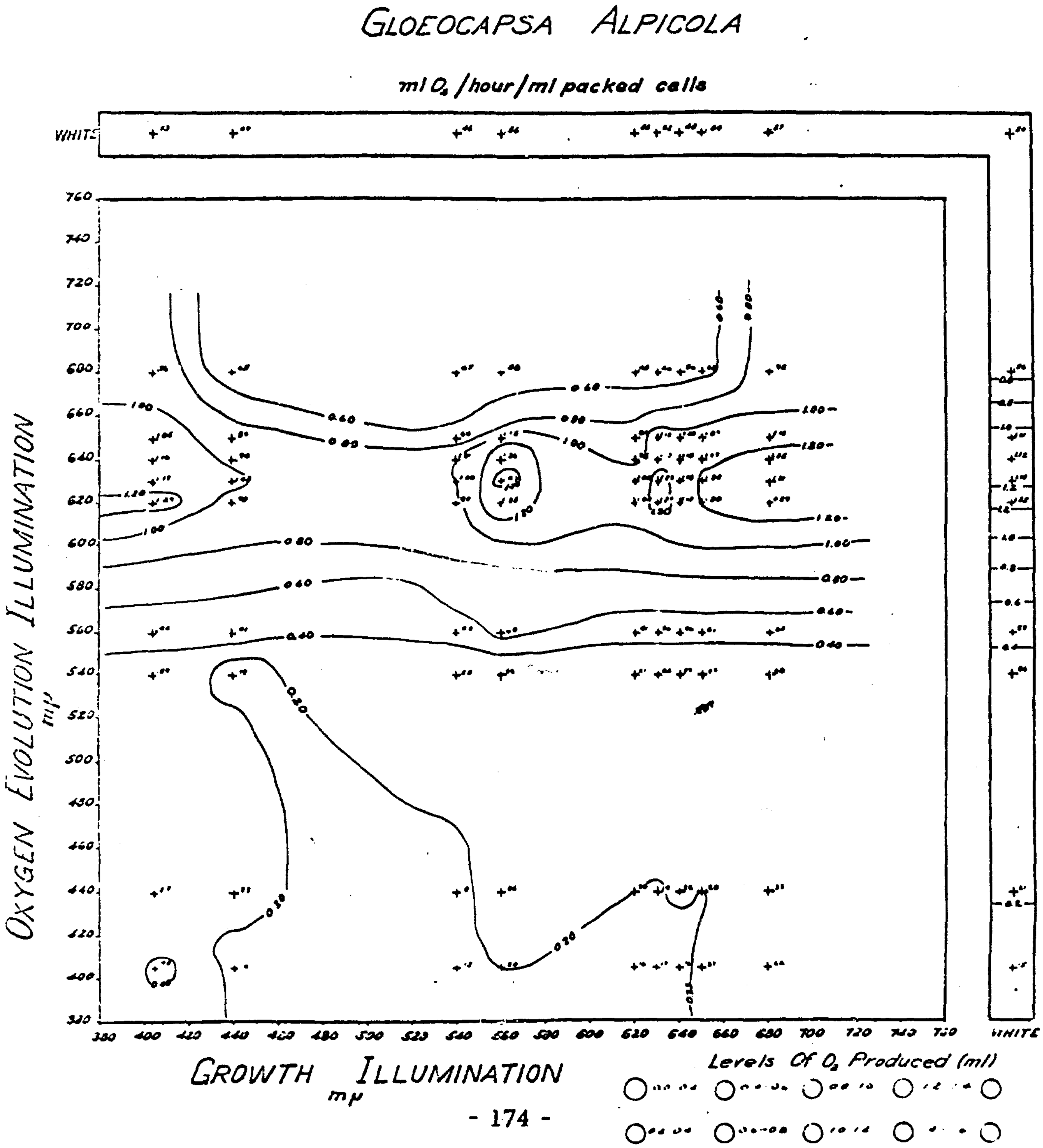


Fig. 73 Respiration




EUGLENA GRACILIS
ml O₂/hour/ml packed cells
OXYGEN EVOLUTION ILLUMINATION mμ
GROWTH ILLUMINATION mμ
WHITE
Levels Of O₂ Produced (ml)
2.0- 4.0
4.0--6.0
6.0 - 8.0
8.0 - 10.0
10.0 -12.0


EUGLENA GRACILIS
ml O2/hour/ml packed cells
WHITE
30
20
40
OXYGEN UPTAKE ILLUMINATION mμ
GROWTH ILLUMINATION mμ
Levels Of O2 Consumed
1.0-2.0
2.0-3.0
3.0-4.0
4.0-5.0


GLOEOCAPSA ALPICOLA
ml O₂ /hour/ml packed cells
WHITE
OXYGEN EVOLUTION ILLUMINATION mμ
GROWTH ILLUMINATION mμ
Levels Of O₂ Produced (ml)

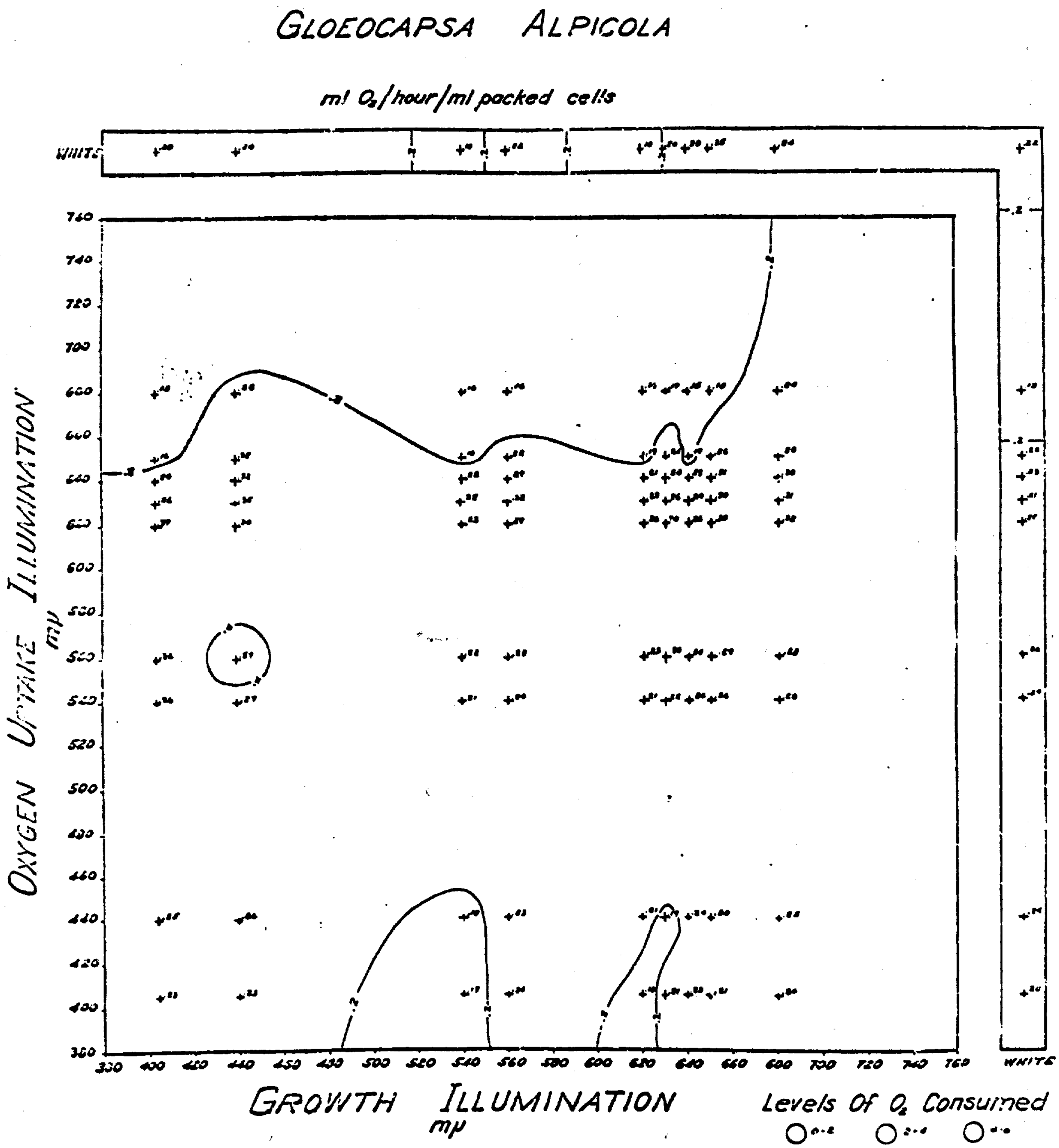

GLOEOCAPSA ALPICOLA
ml O2/hour/ml packed cells
WHITE
OXYGEN UPTAKE ILLUMINATION mμ
GROWTH ILLUMINATION mμ
Levels Of O2 Consumed
WHITE

Fig. 78 Photosynthesis

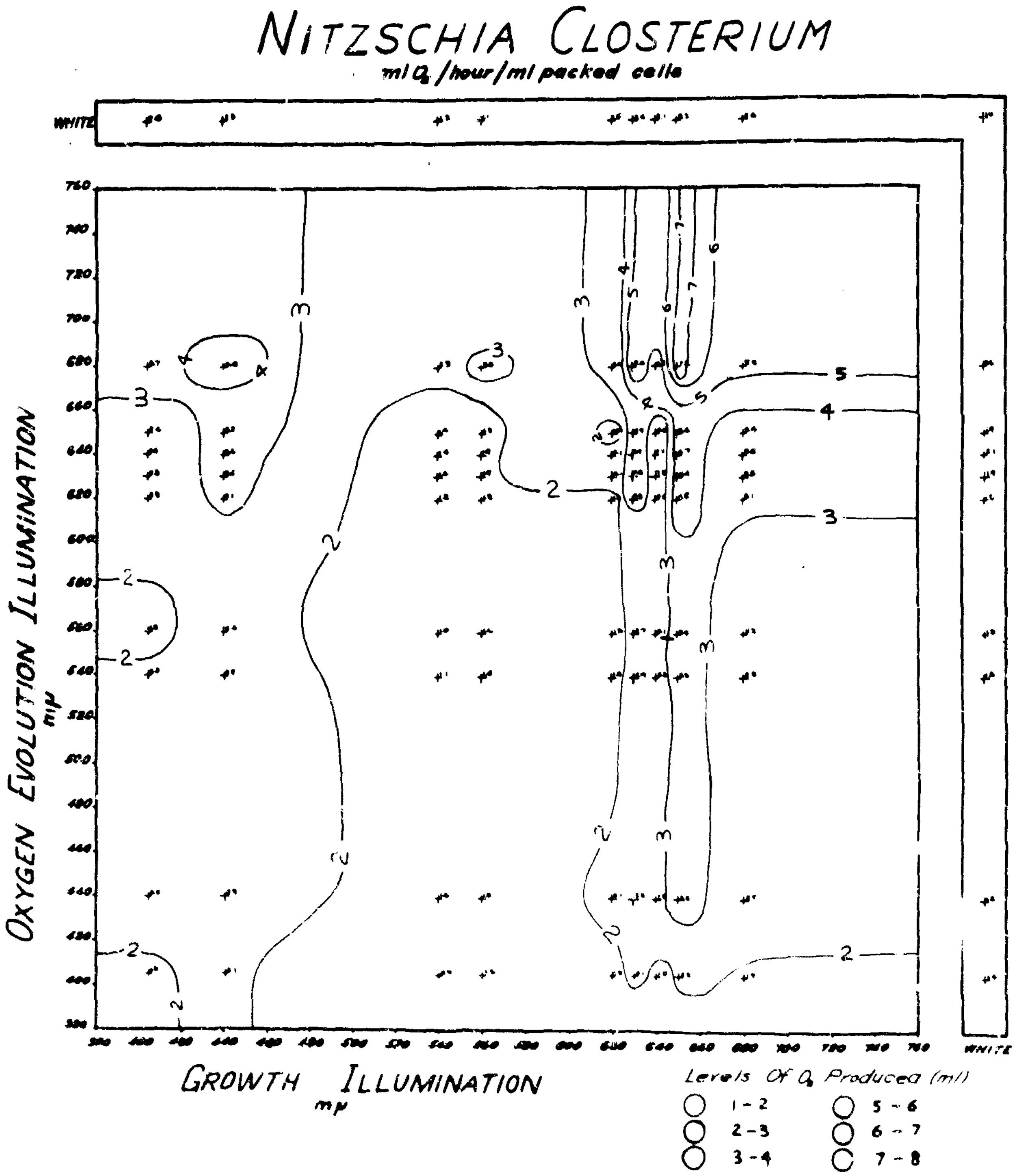
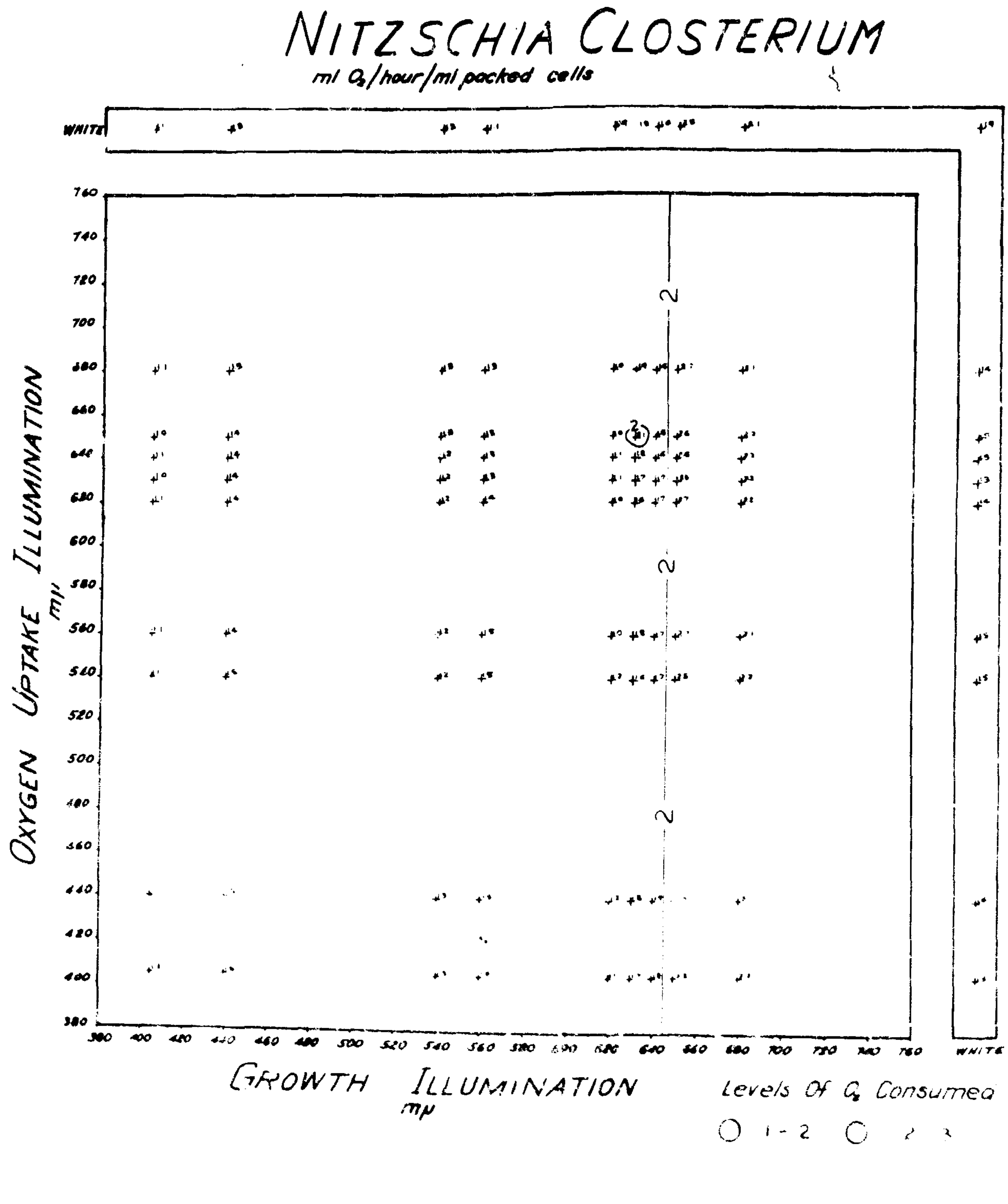
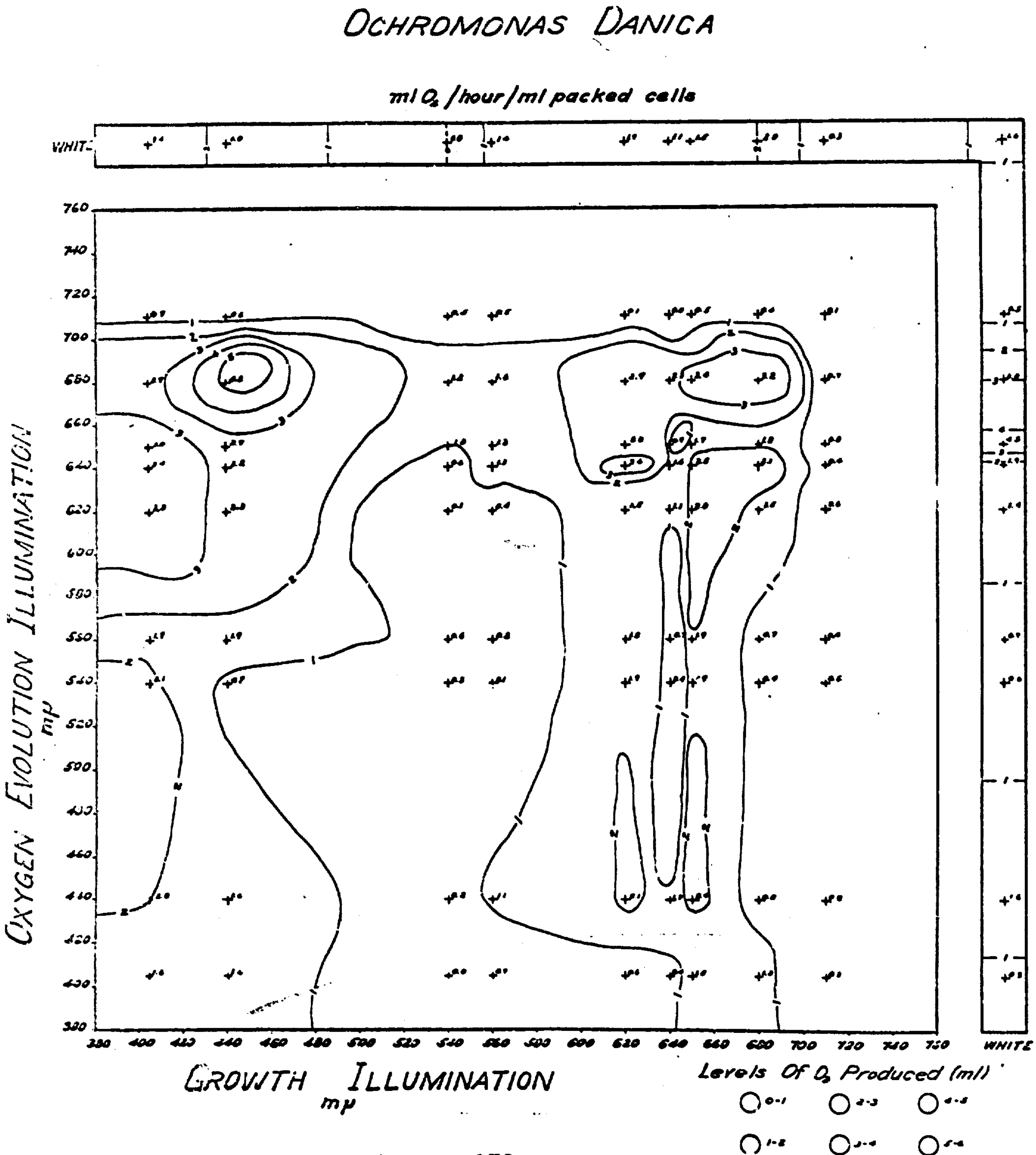


Fig. 79 Respiration




OCHROMONAS DANICA
ml O₂ /hour/ml packed cells
OXYGEN EVOLUTION ILLUMINATION mμ
GROWTH ILLUMINATION mμ
WHITE
Levels Of O₂ Produced (ml)
0-1
1-2
2-3
3-4
4-5
5-6

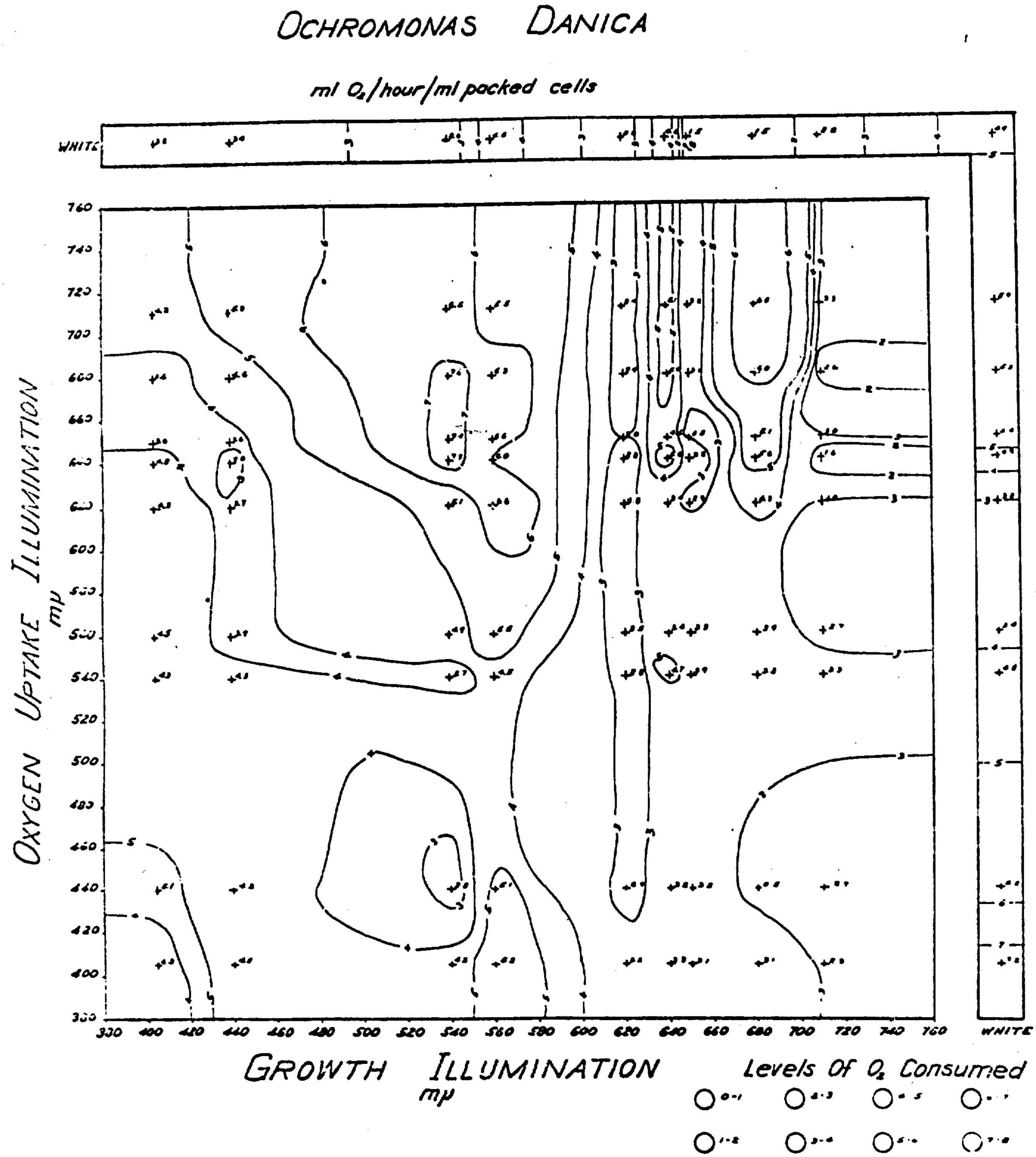

OCHROMONAS DANICA
ml O2/hour/ml packed cells
WHITE
760
740
720
700
680
660
640
620
600
580
560
540
520
500
480
460
440
420
400
380
OXYGEN UPTAKE ILLUMINATION mμ
380 400 420 440 460 480 500 520 540 560 580 600 620 640 660 680 700 720 740 760 WHITE
GROWTH ILLUMINATION mμ
Levels Of O2 Consumed

Fig. 82 Photosynthesis

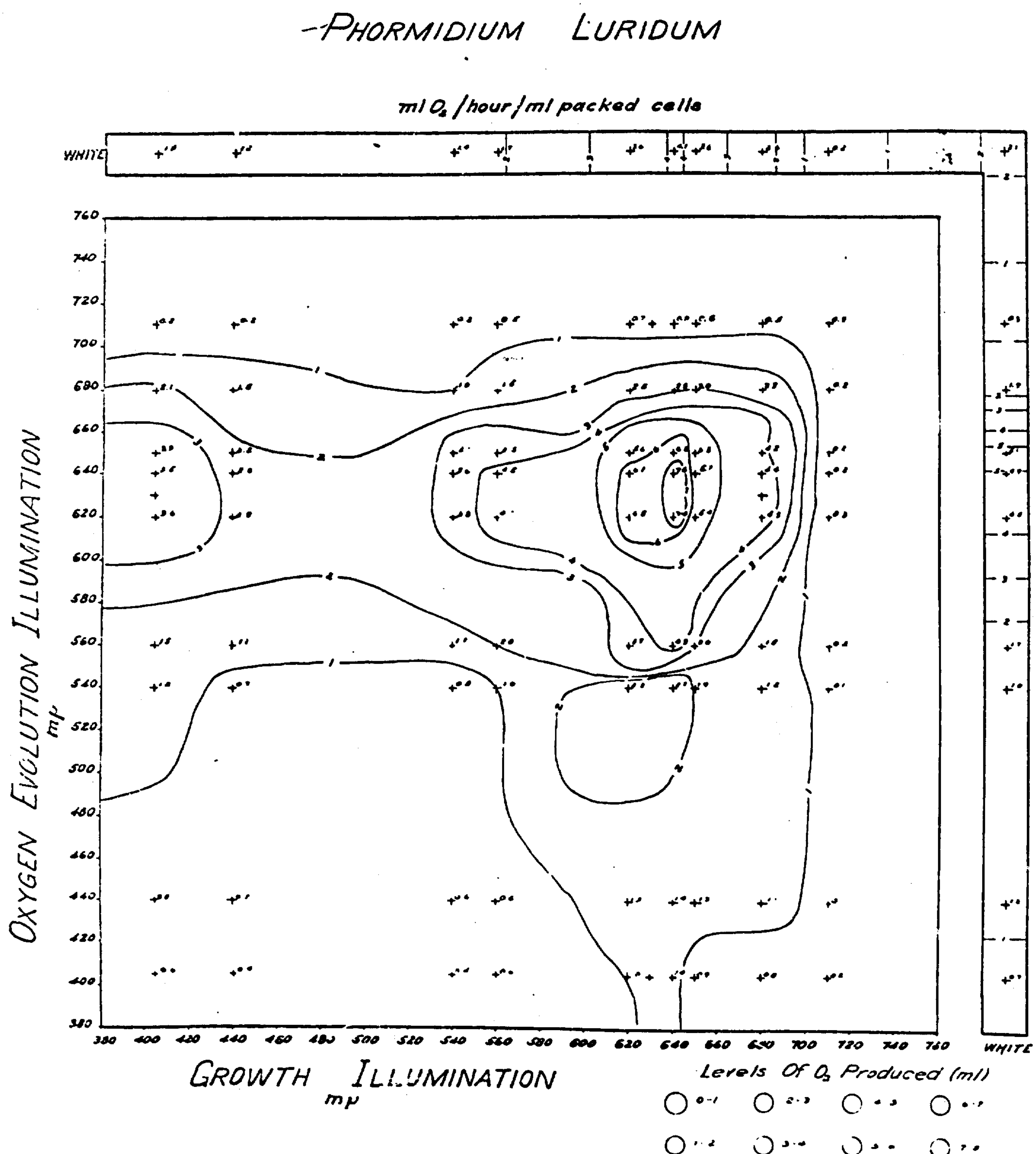
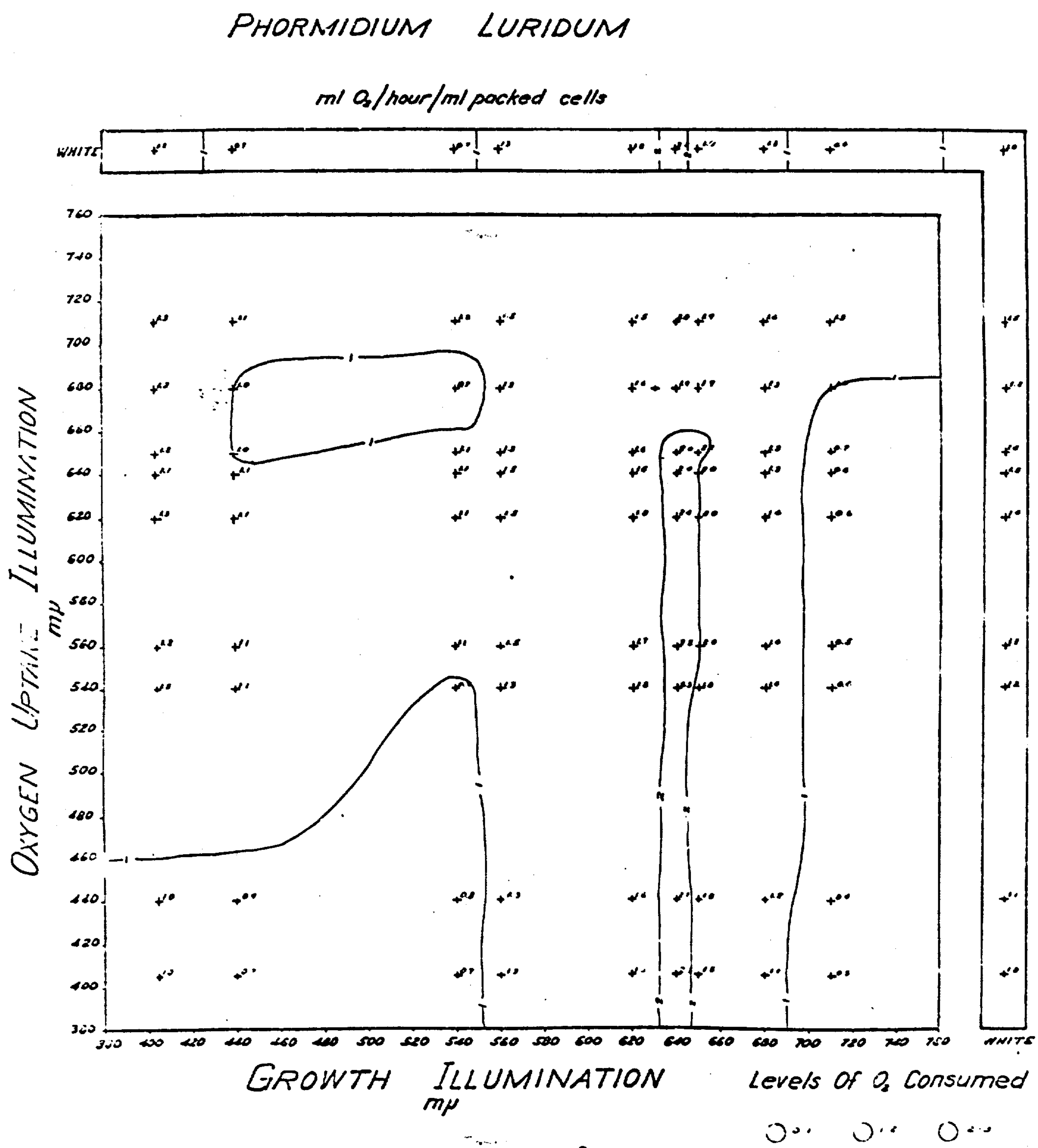
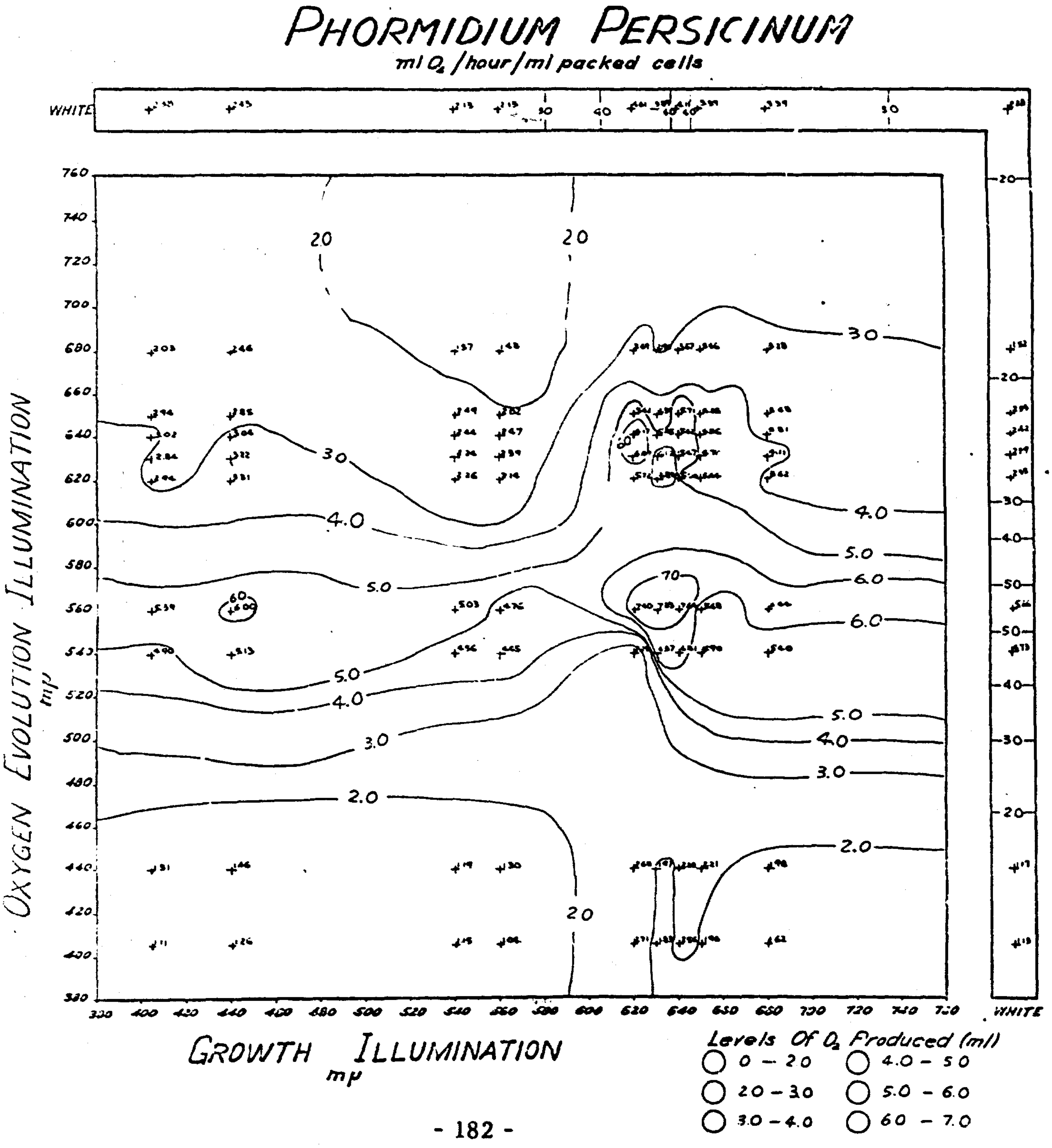
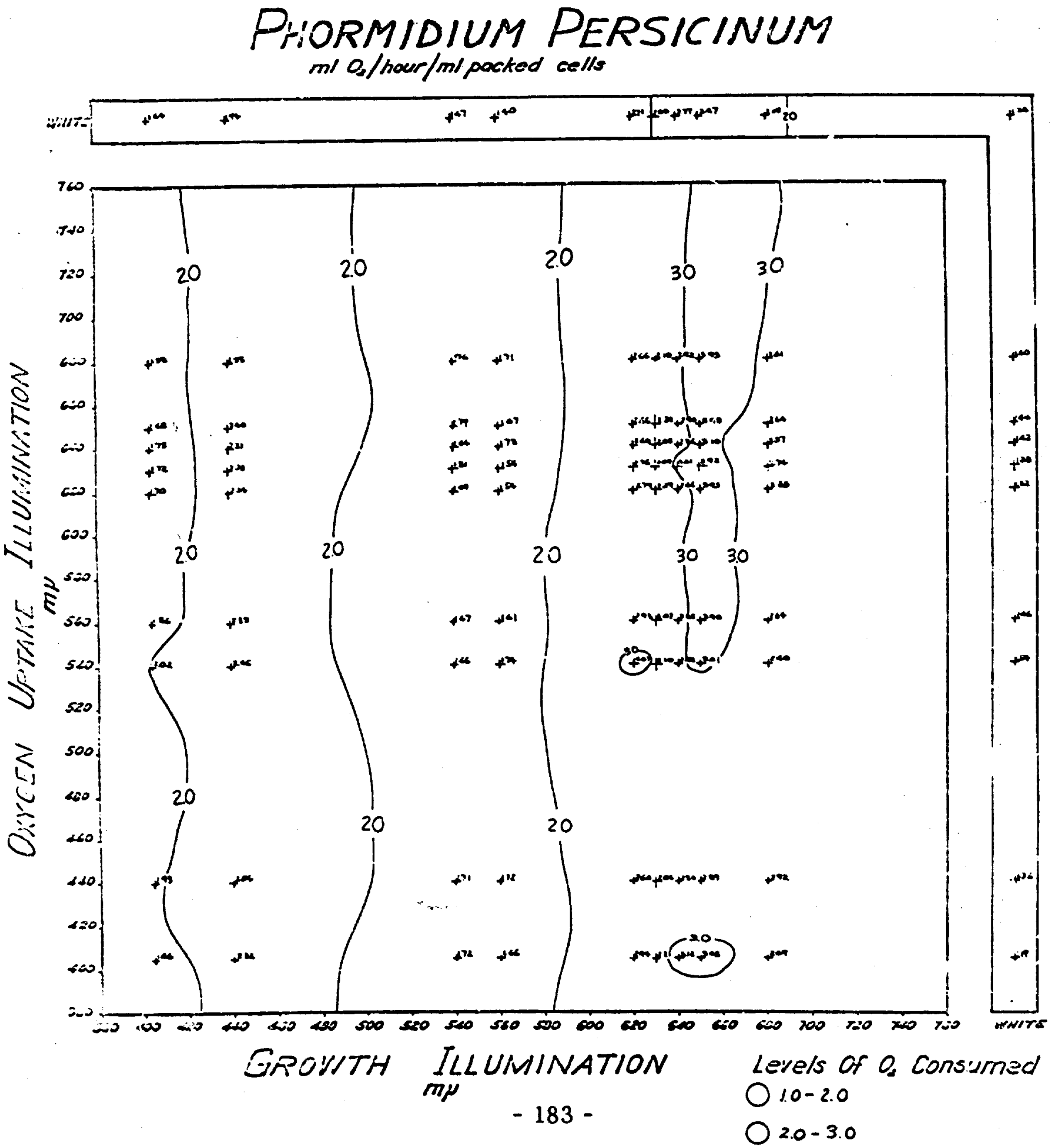


Fig. 83 Respiration




PHORMIDIUM PERSICINUM
ml O2 /hour/ml packed cells
WHITE
OXYGEN EVOLUTION ILLUMINATION
mμ
GROWTH ILLUMINATION
mμ
Levels Of O2 Produced (ml)
0 – 2.0
2.0 – 3.0
3.0 – 4.0
4.0 – 5.0
5.0 – 6.0
6.0 – 7.0


PHORMIDIUM PERSICINUM
ml O2/hour/ml packed cells
OXYGEN UPTAKE ILLUMINATION
mμ
GROWTH ILLUMINATION
mμ
WHITE
2.0
3.0
Levels Of O2 Consumed
1.0 - 2.0
2.0 - 3.0

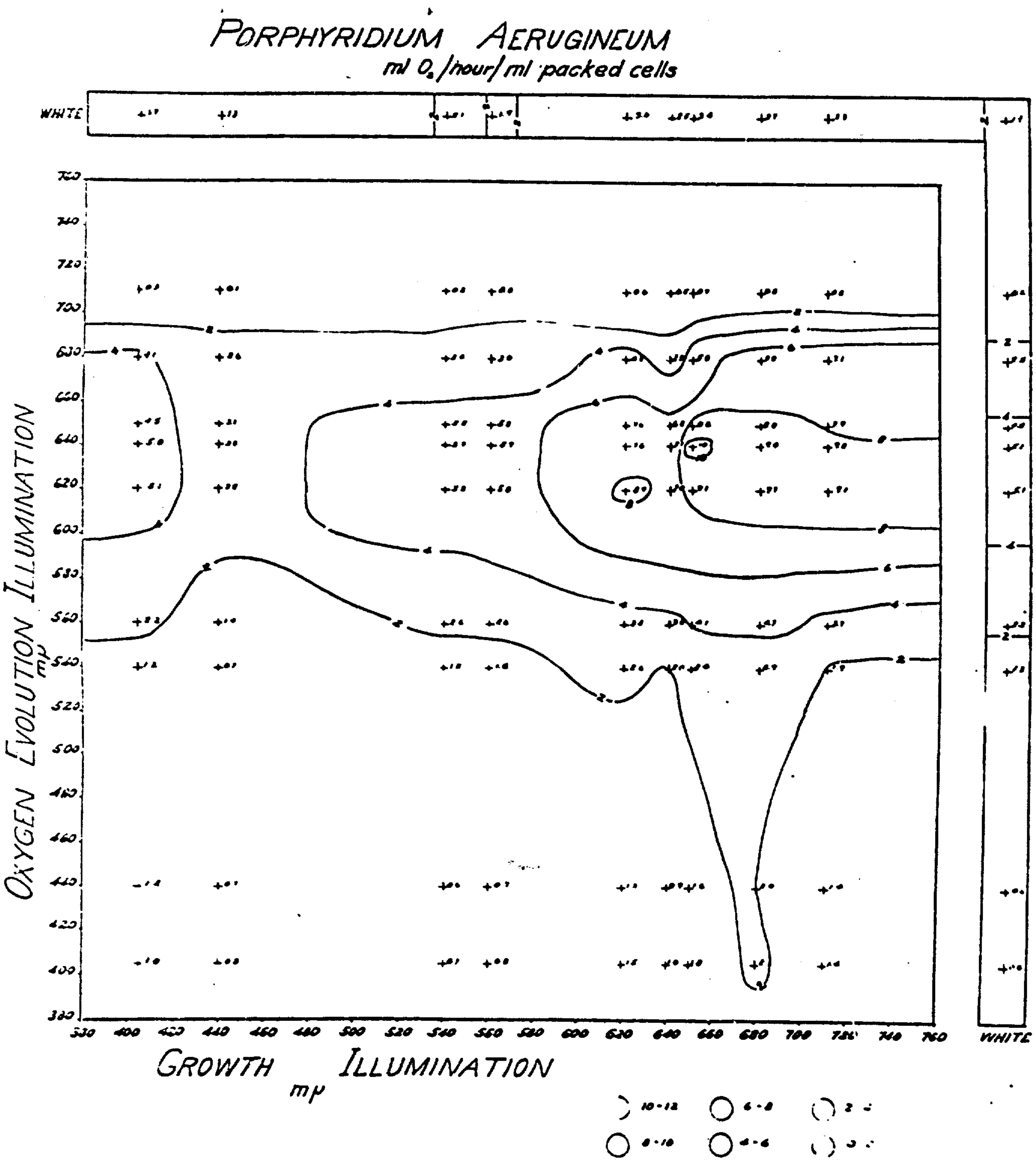

PORPHYRIDIUM AERUGINEUM
ml O_2/hour/ml packed cells
WHITE
OXYGEN EVOLUTION ILLUMINATION mμ
GROWTH ILLUMINATION mμ
WHITE

Fig. 87 Respiration

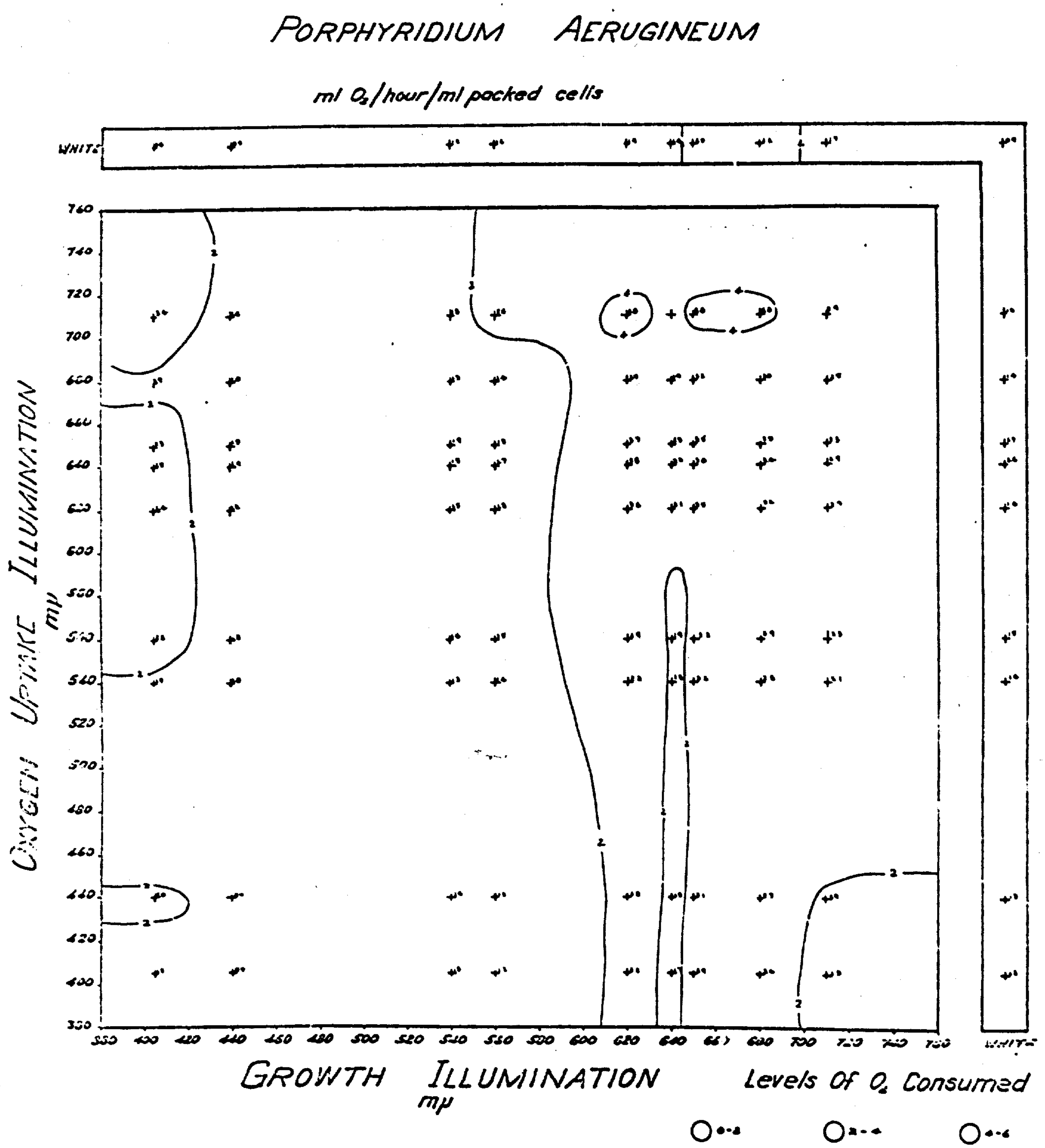


Fig. 88 Photosynthesis

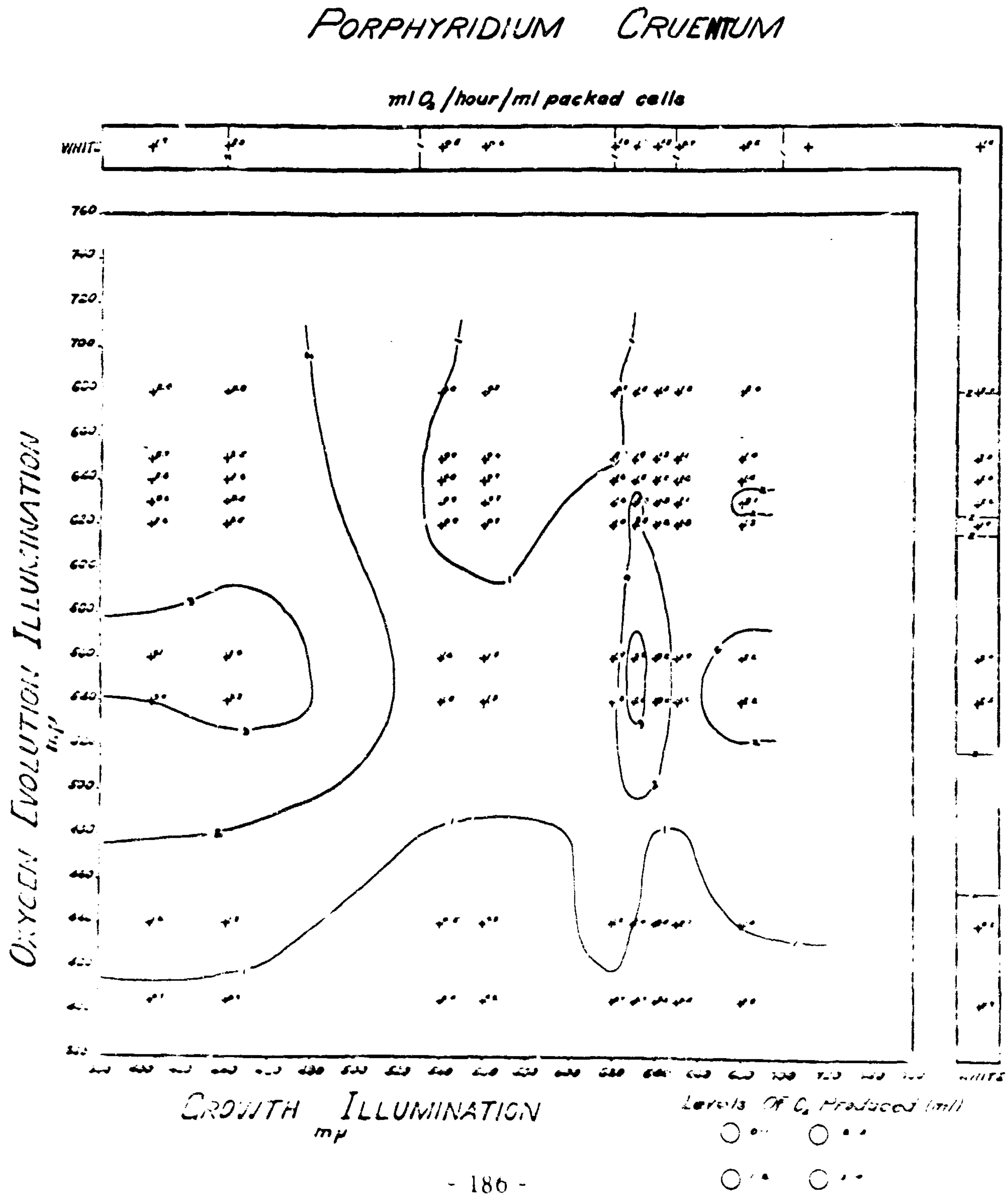
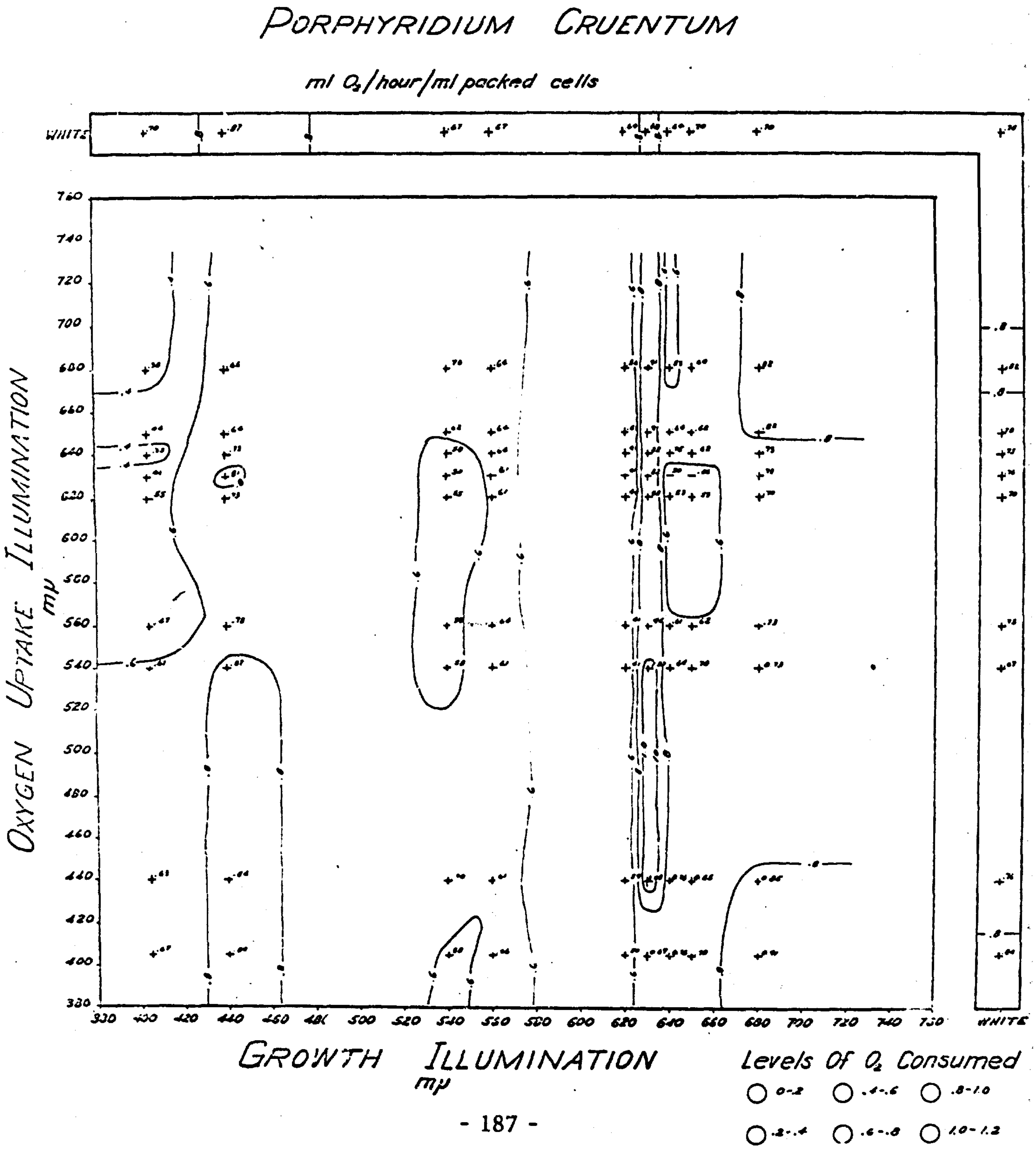
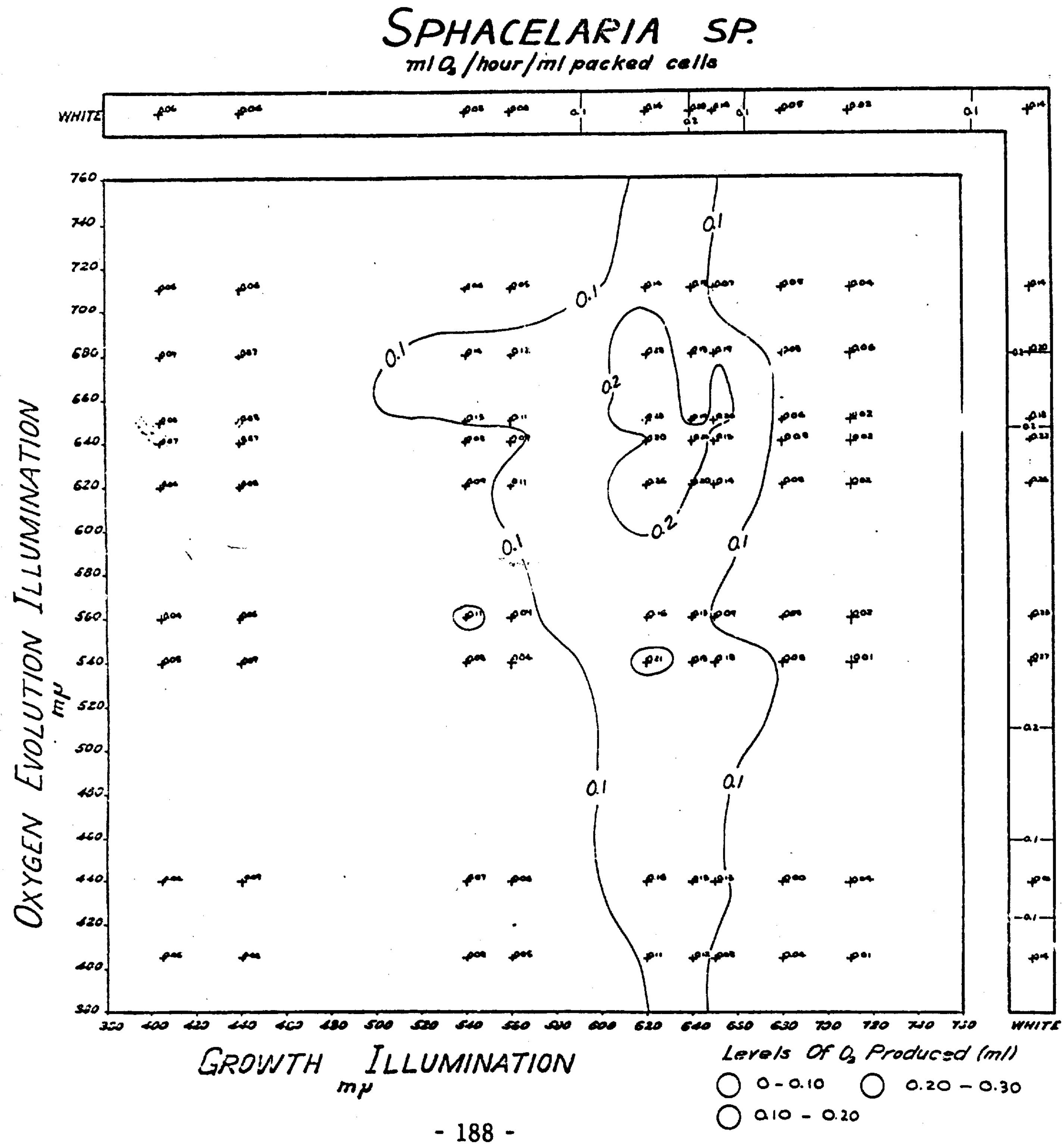


Fig. 89 Respiration




SPHACELARIA SP.
ml O2/hour/ml packed cells
WHITE
OXYGEN EVOLUTION ILLUMINATION mμ
GROWTH ILLUMINATION mμ
0.1
0.2
Levels Of O2 Produced (ml)
0 - 0.10
0.20 - 0.30
0.10 - 0.20

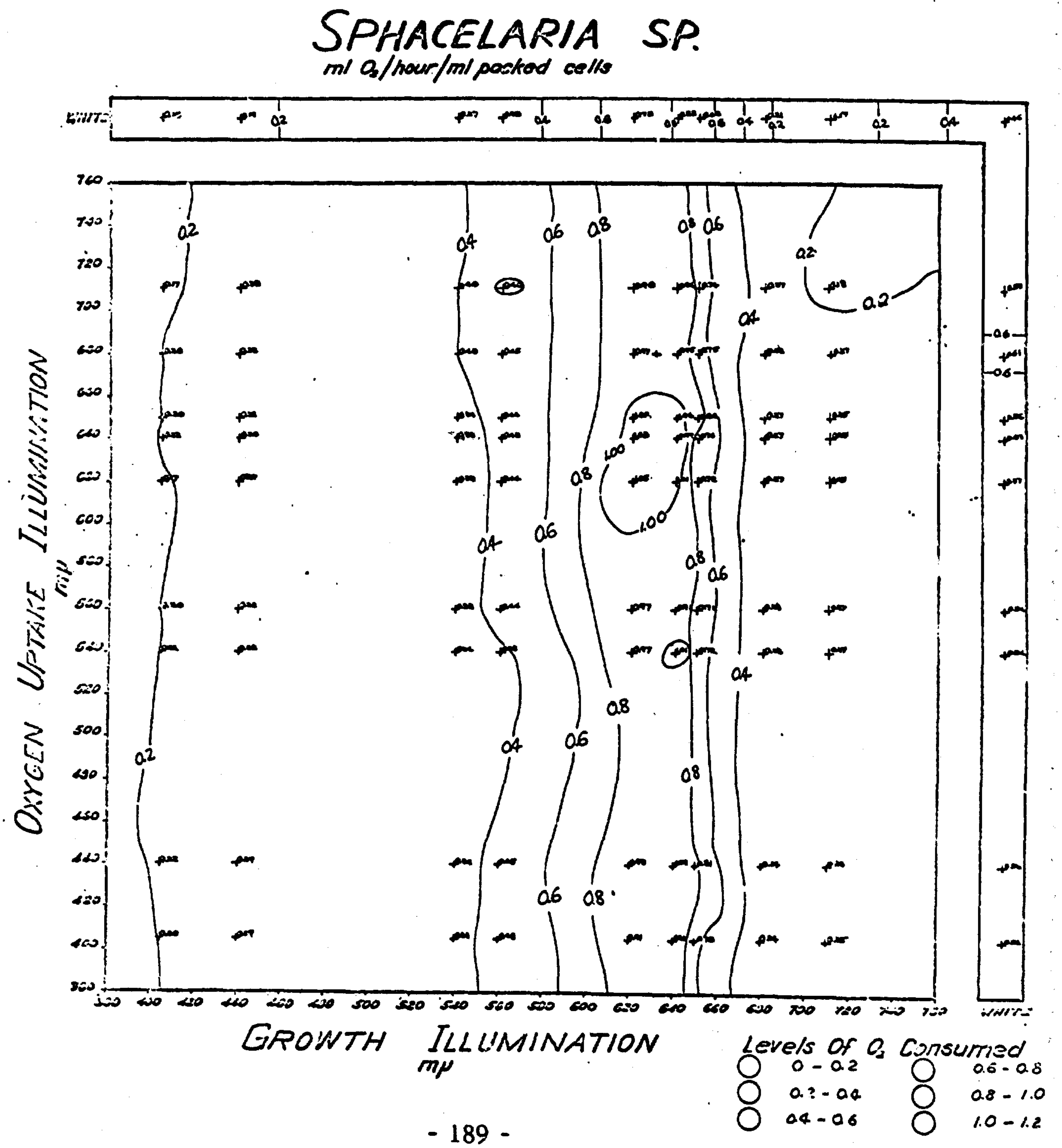

SPHACELARIA SP.
ml O2/hour/ml packed cells
OXYGEN UPTAKE ILLUMINATION mμ
GROWTH ILLUMINATION mμ
WHITE
Levels Of O2 Consumed
0 - 0.2
0.2 - 0.4
0.4 - 0.6
0.6 - 0.8
0.8 - 1.0
1.0 - 1.2

Fig. 92 Photosynthesis

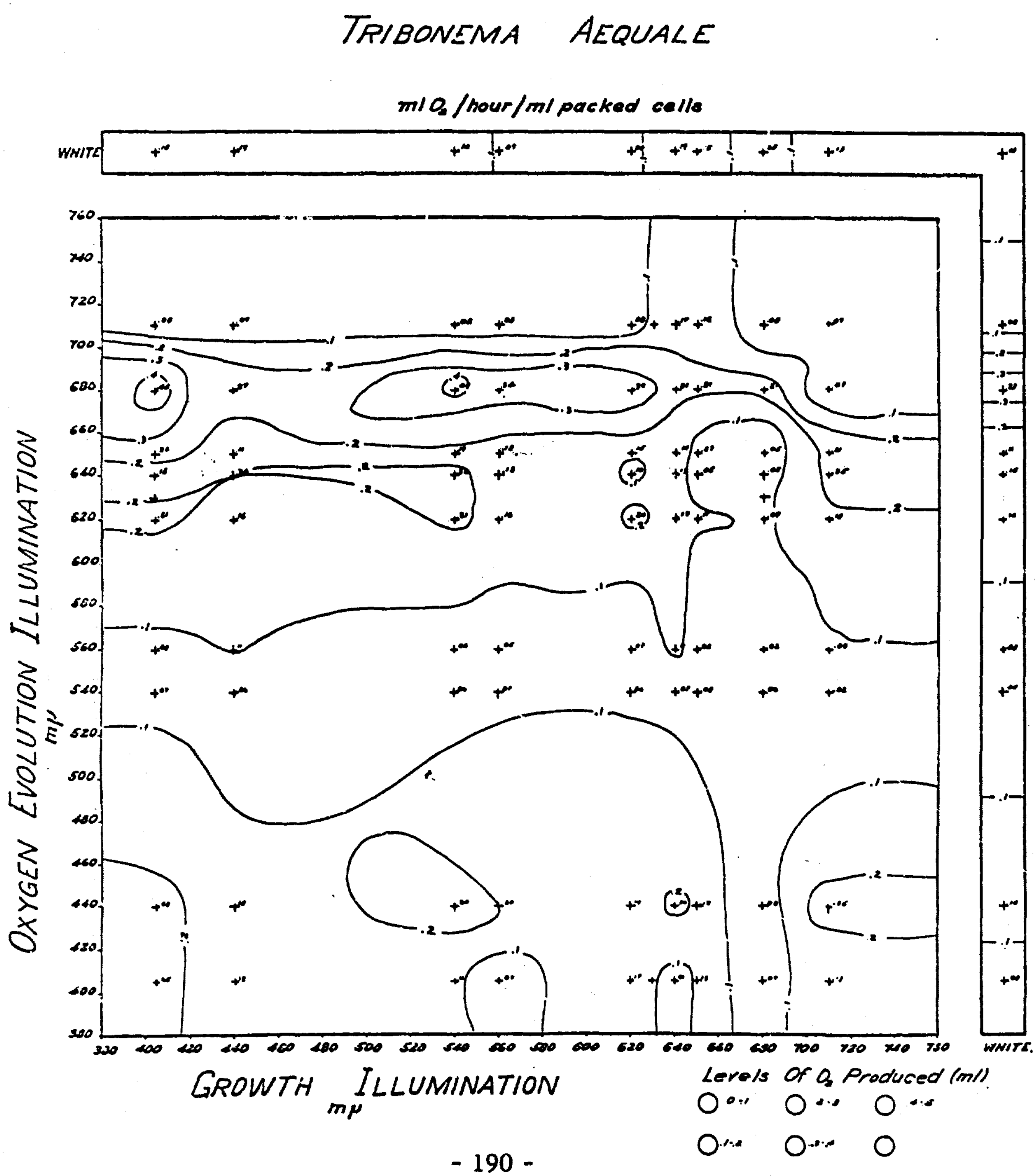
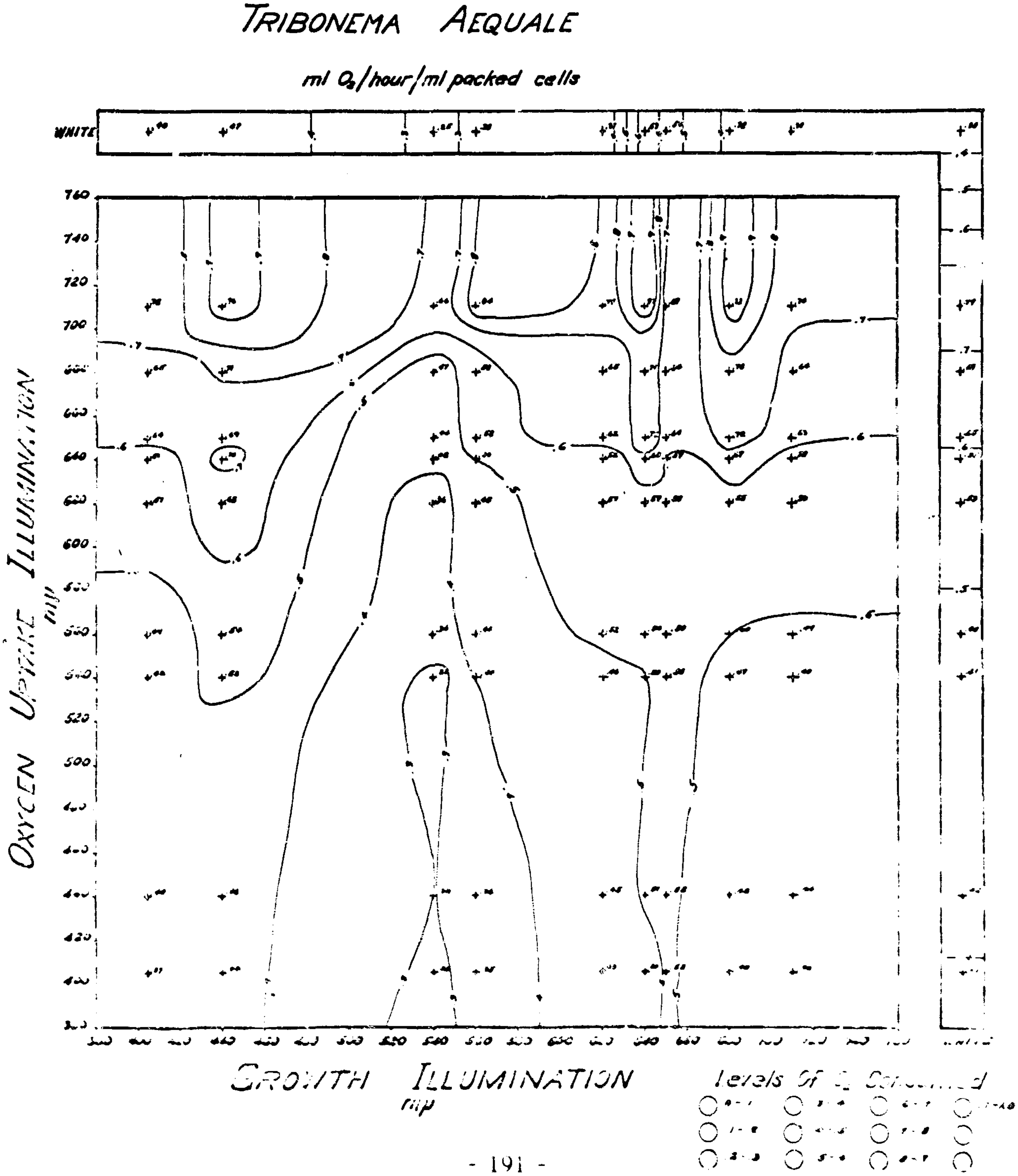


Fig. 93 Respiratic n



Unclassified
Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author) Charles F. Kettering Research Laboratory Yellow Springs, Ohio	2a. REPORT SECURITY CLASSIFICATION Unclassified 2b. GROUP

3. REPORT TITLE

SPECTRAL LIGHT REQUIREMENTS OF ALGAE

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)

Final 1 July 1965 - 1 July 1967

5. AUTHOR(S) (First name, middle initial, last name)

Thomas E. Brown

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
October 1968	191	37

8a. CONTRACT OR GRANT NO. DA 19-129-AMC-565(N) b. PROJECT NO. 1C014501A71C c. d.	9a. ORIGINATOR'S REPORT NUMBER(S) 9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report) 69-45-FL FL-82

10. DISTRIBUTION STATEMENT

This document has been approved for public release and sale; its distribution is unlimited

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY U.S. Army Natick Laboratories Natick, Massachusetts 01760

13. ABSTRACT

Seventeen species of algae representing ten taxonomic divisions were individually grown in white light and light of nine separate 10 nm bandwidths corresponding to the major absorption peaks of known photoactive pigments. Energy levels of the incident light were equalized through the entire series, approximating 15,000 ergs cm^{-2} sec^{-1}. Measurements of growth, pigmentation, photosynthesis, respiration, and where possible, morphology and structure were made following seven to ten days continuous exposure to the light regimes. The rates of photosynthesis and subsequent respiration were determined using the same full light regime as for growth. Light enhancement characteristics and wavelength requirements are shown for these parameters and compositions of specific illumination sources are suggested.

DD FORM 1 NOV 65 1473 REPLACES DD FORM 1473, 1 JAN 64, WHICH IS OBSOLETE FOR ARMY USE.

Unclassified
Security Classification

Unclassified
Security Classification

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Light	6		9			
Spectra	6					
Intensity	6					
Wavelengths	6					
Color	6					
Algae	7		9			
Physiology	7					
Growth	7					
Pigments	7					
Photosynthesis	7					
Respiration	7					
Morphology	7					
Requirements			8			

Unclassified
Security Classification