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THE INFLUENCE OF LIGHTING CONDITIONS AND LIQUID DEPTH ON THE GROWTH ACTIVITY OF ENVIRONMENTALLY CONTROLLED ALGAL CULTURES

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A dissertation submitted in partial fulfillment
of the requirements for the degree of
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I. INTRODUCTION

Increased availability and utilization of our fresh water resources have made the public aware of aesthetic changes that are progressively degrading the general quality and limiting the usability of these facilities. These changes constitute a dynamic aging process that fresh waters experience. This aging process, which is known scientifically as eutrophication, is a self-induced phenomenon that, under natural influences, is regulated by the laws of nature. Evidence currently indicates that man himself is accelerating the process of fresh water eutrophication through his own activities. The activities of man which appear to be inducing eutrophication are: increased encroachment of the fresh water facilities, increased recreational use of the water, and discharge of untreated and treated wastes to the waters. Of these activities, the one that seems to be the real "trouble-maker" is the discharging of untreated and treated wastes. These wastes create problems through their contribution of organic and inorganic pollution to the receiving waters.

This fresh water aging process is of great concern to the Sanitary Engineering profession. In present day society this is the profession which is charged with the responsibility of providing the best possible waste treatment techniques, in order to insure that the water resources can be used to their fullest extent without

potentially endangering the health of the general public. In certain parts of this country and the world this concern is further emphasized when the thoughts of direct water reclamation, for the purpose of providing adequate water supplies, become closer to being realities.

Present day conventional, biological waste treatment processes are very effective in producing well oxidized effluents which contain little BOD. However, these effluents do contain nutrients that stimulate the growth of biological organisms, which generally obtain their metabolic requirements from sources other than organic matter. Algae are the major form of organisms in the aquatic community, whose growth activity is stimulated by the nutrients contained in sewage treatment plant effluents. The accumulation of these nutrients in the receiving waters will induce an increase in algal population to a level that greatly unbalances the biological community in the aquatic environment. This excess population of algae provides additional organic and inorganic materials that gradually fertilize the waters and thus accelerate the process of natural eutrophication. Another consideration currently of concern to water works personnel is what possible influence might the accumulation of extracellular metabolites from massive algal blooms have on the treatability of water sources.

It would seem only logical that if these fertilizing nutrients could be effectively removed from the waste effluents prior to discharging to receiving waters, this process of fresh water eutrophication might be reduced to its natural rate. Current knowledge

indicates that eutrophication is an irreversible process, so it is doubtful that much can be done about restoring prematurely aged waters to their original condition.

The use of algae as the active organism in a tertiary waste treatment process has been suggested (1) and also investigated to a certain extent (2) (3) (4) (5). This type of tertiary treatment is a bio-extraction process utilizing the algae's ability to assimilate the fertilizer nutrients in the waste effluent. Extraction of nutrients is currently taking place to some degree in sewage stabilization ponds, but its effectiveness is unpredictable. The use of environmentally controlled algal cultures offers a treatment technique which will provide effective and predictable nutrient extraction as long as an optimum environment is available to the algae. In order to provide the optimum algal environment on a continual basis, the limiting characteristics of algal growth in wastes must be adequately defined through research. With the growth limiting characteristics available, it might then be possible to design an optimum algal environment that is applicable as a waste treatment process.

PROPOSED STUDIES

As the result of a preliminary literature review and discussions with research colleagues, it was decided that a more critical investigation of the influence that culture liquid depth has on the growth characteristics of algae and their ability to extract

fertilizer nutrients from waste treatment plant effluents would be of value. The thought was that if lighting conditions throughout cultures of varying depths could be maintained relatively constant, a truer indication of the depth influence on the culture activity could be obtained. It was found in the literature concerning batch algal culturing, that when the culture depth was varied and other conditions maintained constant, a fairly stable numerical factor resulted from taking the maximum algal density value attained at a specific depth and multiplying it by the depth value. Therefore, it was assumed that if a constant depth-density factor was maintained for different culturing conditions, the self-shading effects that the algal cells have on one another would be stabilized and the lighting conditions throughout the various culture depths to be studied would be relatively the same.

Additional evaluation of light intensity effects on the algae were felt necessary since a new light source, which was developed specifically for the purpose of stimulating photosynthesis, was to be used. It was also decided to investigate the influence of a very intense light source on algal activity at different culture liquid depths.

With the information gained from these studies, it is hoped that a contribution will be made towards the further understanding of lighting conditions within algal cultures and the ultimate engineering design of an optimum algal environment applicable to tertiary waste treatment.

II. GENERAL CONSIDERATIONS

ALGAE -- WATER POLLUTION AND WASTE TREATMENT

Algae are classified in the plant kingdom under the phylum Tallophyta. This simple plant grouping includes a wide variety of organisms which differ greatly in cell organization, but all have the common characteristic of metabolic process initiation through photosynthesis (6). Algae differ from bacteria, fungi and protozoa in that the algal cell contains the light sensitive pigment chlorophyll and the biochemical mechanisms within the algal cell tend towards an accumulation of organic materials rather than a breakdown of the organics.

Massive, nuisance causing algal blooms in fresh water lakes, have been associated with sewage pollution for many years. Approximately 15 years ago Hasler (7) described 38 lakes in Europe and the United States that had experienced induced eutrophication through sewage pollution during the past century. Probably the most notorious algal problem associated with sewage pollution in this country is that involving the lakes around Madison, Wisconsin which has been thoroughly investigated by Sawyer and Lackey (8) (9) (10) and more recently described by Sarles (11). These studies revealed that non-agricultural drainage is the major contributor of inorganic nitrogen and phosphorus to the lakes. Edmondson et al (12), in a report on the

artificial eutrophication of Lake Washington, state that the most reasonable explanation of the increase in lake productivity is the increased discharge of treated wastes to the lake from the growing communities in the lake area. The incidence of the blue-green alga, Oscillatoria rubescens, in fresh water lakes has been reported (7) (13) (14), and this usually is an indication that inorganic and organic pollution is quite extensive.

Most of the literature agrees that there are critical levels of algal nutrient concentrations which must be available under the proper conditions in order for a fresh water to produce an algal bloom of nuisance variety. The most often quoted critical nutrient concentrations are those reported by Sawyer (10). Based on an extensive study of 17 Wisconsin lakes, Sawyer concluded that an algal bloom of nuisance level could be expected in any lake that contained concentrations in excess of 0. 01 milligrams per liter of inorganic phosphorus and 0. 3 milligrams per liter of inorganic nitrogen at the time of the spring overturn. Curry and Wilson (15), in a report on the effects of sewage-borne phosphorus on algae in Connecticut lakes, indicate that during the algal season Lake Candlewood had a phosphorus concentration of 8 parts per billion and no significant algal bloom was evident. They further indicated that algal blooms were found in lakes containing more than 10 parts per billion of phosphorus. In a report on stream pollution by algal nutrients, Stumm and Morgan (16) indicate

that besides nuisance algal blooms serious secondary pollutional problems can result from fertilization, through the oxygen demand associated with the ultimate degradation of the algal bloom. They further suggest that critical levels of nutrient concentrations be evaluated on the basis of the oxygen demand created by the metabolic up-take of the algal nutrients; with use of the relationship that as one milligram of phosphorus passes through the phosphorus cycle it has an ultimate oxygen demand of about 160 milligrams. It would be desirable to establish the critical concentrations of nutrients, such that the hypolimnetic waters do not turn anaerobic.(16).

The capabilities that algae have for surviving in sewage environments have been known for some time. The alga, Chlorella pyrenoidosa, was isolated from sewage in 1903 by Chick (17). Her research further revealed this organism's ability to metabolize ammonia and ammoniacal compounds. Based on these findings, Chick proposed that algae could possibly be effective as an active organism in the treatment of sewage polluted waters. Witt (2) presents a list of many algal forms that can be found in sewage polluted waters. This list shows that the Chlorophyta are the most prevelant group of algae, while the Chlorococcales appear to be the dominant order of algae found in polluted waters. The occurrence of algae in waste stabilization ponds and their contribution to waste treatment in these facilities has been well documented. Golueke (18) and Pipes (19) have reported on the biological aspects of the symbiotic relationship between algae and

bacteria in waste stabilization ponds. The operation of these ponds has been extensively studied under varying climatic conditions by the Public Health Service in the Dakotas (20) and at Fayette, Missouri (21). Oswald and associates (22) and Hermann and Gloyna (23) have both developed generalized equations for the engineering design of stabilization ponds. The development and use of stabilization ponds as waste treatment facilities has mainly been concerned with effective BOD removal. Along with the BOD removal, the extraction of significant quantities of algal nutrients has also been reported, but with a variable degree of reliability (21) (24) (25).

It appears that the most desirable approach to the control and elimination of aquatic growth nuisances is through the application of ecological control (26). This type of control can probably be most effectively applied by lowering the concentration of one or more of the critical nutrients in the waste waters to a level of concentration that will not stimulate aquatic blooms, before discharging the wastes into the receiving waters. The use of algae as the active organisms for the removal of algal nutrients from waste waters has been suggested by Imhoff (1) and others (2) (3) (4) (5). Studies by Gerloff et al (27) have shown that biologically treated domestic sewage is an ideal medium for the culturing of algae. The use of algal lagoons for the extraction of fertilizer nutrients from sewage plant effluents has been investigated by Bogan et al (3) and Bush et al (4), and they

have reported their efforts as being somewhat successful. Reid and Assenzo (28) have studied the culturing of attached algal forms for this same treatment purpose, but had little success on a continuous basis. Another application of stabilization ponds has been reported by Hermann (29). This author reports on the use of such ponds as nitrate reducing reactors for the treatment of high nitrate wastes. In most of these algal pond process investigations, the nutrient removal on a continual basis was somewhat unpredictable due to the uncontrolled environment that was offered to the algae.

The use of controlled algal cultures for the bio-extraction of fertilizer nutrients from sewage treatment plant effluents has been investigated by Witt (2), Gates (5) and recently reported on by Gates and Borchardt (30). This type of investigation has been performed and is being continued with the thought that through the engineering design and control of optimum algal environments, effective nutrient removal from sewage plant effluents can be realized on a continual basis.

FACTORS INFLUENCING THE GROWTH ACTIVITY OF ALGAE

Algal activity is governed by the basic life supporting process of photosynthesis. Photosynthesis is a photochemical process which involves the transformation of inorganic carbon into organic carbon materials, with the release of oxygen as a by-product.

Research has revealed that photosynthesis is actually composed of two reactions; one involving the photochemical splitting of water through the absorption of light energy and the other involving the use of the absorbed energy in the fixation of inorganic carbon into organic compounds (31). Factors which influence the growth of algae are of both a physical and chemical nature. Generally, in the evaluation of algal activity, the reaction rate of the algae is graphed against the influencing factor. Usually this type of an evaluation produces a pattern that reveals two distinct areas of interest. First, there is the rate limiting area where the magnitude of the influencing factor directly affects the reaction rate; and second, the rate non-limiting area where further increase in the magnitude of the influencing factor has no appreciable affect on the reaction rate. For engineering design purposes, the important information to obtain from evaluations of this nature is the magnitudes of the various influencing factors that produce maximum rates of reaction.

PHYSICAL FACTORS

Certain physical factors have been shown to be of significant influence on the algal activity under laboratory and field culturing conditions. Those factors to be considered here are; temperature, culture lighting conditions, and the liquid depth in the culture.

Temperature

Temperature is one of the external or environmental factors that affects the photosynthetic mechanism in algae. Witt (2) has reported the thermal classification of algae according to the different phyla. This classification indicates that the diatoms are usually associated with low temperatures, the green algae like somewhat warmer temperatures and the blue-green algae require the highest temperatures. The more common algal forms that have been utilized in various algal research studies have an optimum growth temperature in the range of between 20 and 30°C.

As stated before, the over-all photosynthetic mechanism is comprised of two processes; the photochemical process and the carbon fixation process. The photochemical process, which appears to involve the activation of the chlorophyll molecules contained in the algal cells, is generally not influenced by temperature changes. However, the carbon fixation process is sensitive to temperature changes, indicating that this process is chemical and does not require light stimulation (36). The carbon fixation process has been observed to have a Q_{10} of about 2 (37), which is equivalent to the Q_{10} reported for photosynthesis within the temperature range of 20 to 30°C (62). In general, under conditions of saturation light intensities and carbon concentrations which limit the fixation process, temperature changes exert greatest influence on the photosynthetic process (36).

Interest in the development of mass algal culturing techniques for the commercial production of algae and the production of algae for use as a food source has stimulated searches towards obtaining thermophilic species of algae with a rapid rate of growth. In particular, blue-green algae such as Anacystic nidulans and Oscillatoria subbrevis, with generation times of 2 and 3 hours respectively and growth temperature optima between 35 and 40°C, have been investigated (63). Dyer and Gafford (64) isolated a high temperature blue-green algal form from a mixed culture obtained at the hot springs in Yellowstone Park by holding the culture at 50 to 55°C. This algal form was tentatively identified as Synechococcus lividus. Another blue-green alga, Hapalosiphon luminosus, was isolated in pure culture from the flora of a hot water spring (65). In pure cultures this alga attained a maximum growth at the high temperature of 50°C. A thermophilic Chlorella form (strain 7-11-06) with a maximum temperature for growth of 42°C was isolated by Sorkin (66). Many of the physiological characteristics of this thermophilic Chlorella compared to those of Chlorella pyrenoidosa which does not grow at temperatures above 29°C.

As for the effect of temperature on algae grown in a waste environment, Oswald and Gotaas (25) have reported an optimum temperature of 20°C, with reduced growth characteristics occurring at both higher and lower temperatures. Gaur, et al (67), in their

study of the growth characteristics of Oscillatoria in organic wastes, satisfactorily employed a temperature of 27 ± 2°C. Based on controlled algal culture studies, Witt (2) indicated that the efficiency of nitrogen extraction by Chlorella and Scenedesmus as a function of the energy absorbed was higher at 20°C than at 25°C, and that the efficiency of phosphorus removal as a function of the same variable did not change in the temperature range of 20° to 25°C.

In the search for the mechanism by which high temperature alters the growth activity of algae, the action of this stress either on the external medium or directly on the algal cell has been considered (58). Considering the information already presented on the occurrence of algae in hot springs where the carbon dioxide and dissolved oxygen concentrations are undoubtedly quite low, it appears that the high temperature effect on the external medium would not alter the algal activity significantly. The consideration that the high temperature may alter metabolic activity through the unbalancing of biochemical reactions is also questionable, since evidence has been presented to indicate that algae are able to alter their metabolic pathways through substrate induced enzymatic changes (6). Thus, it appears that the mechanism by which high temperatures alter algal activity is through the often mentioned process of thermal denaturization of the proteins and other macromolecules contained in the algal cells (63). Fogg as reported by Marre (63) suggests that the molecular

structures of the proteins of blue-green algae may be more rigid than the proteins of other algal forms; thus, allowing the blue-green algae to sustain themselves in high temperature environments.

Light

Light is the environmental factor that initiates the photosynthetic process Both the quality and the quantity of light have significant effects on photosynthesis. Investigations of the effects of light quality on photosynthesis have concluded that the wave lengths of light within the visible light spectrum, 400 to 700 millimicrons, are the photosynthetically effective portion of the total light spectrum. An action spectrum or efficiency curve of photosynthesis for Navicula minima prepared by Tanada and reported by Fogg (6) indicates that the quantum yield for this organism remains nearly constant from 520 to 680 millimicrons, drops rapidly at wave lengths greater than 680 millimicrons and shows a slight dip in the blue light region between 430 and 520 millimicrons. The light absorption spectrum for this alga shows the affect that light quality has on the absorption characteristics of the primary pigment (chlorophyll A) contained within the algal cell. Peak absorption occurs at the wave lengths of 430 and 670 millimicrons, with minimum absorption between 480 and 600 millimicrons. The comparison of these two relationships shows that other pigments contained within the Navicula minima cell (such as the

carotenoids) absorb the light passed by the chlorophyll A and contribute towards maintaining the quantum yield nearly constant between 520 and 680 millimicrons (6).

Light intensity as related to the growth characteristics of algae has three highly significant values. These values as described by Myers (32) are (1) the compensation point intensity which is the intensity that causes the algal organism's photosynthetic and respiratory processes to exactly equal each other; (2) the light saturation intensity which is the intensity that produces the maximum growth activity and (3) the growth inhibiting intensity which is the intensity that results in damage to the algal growth activity. Krauss (33) indicates that the influence of increasing light intensity on algal growth activity follows the typical growth curve pattern. Starting with the compensation point intensity there is an area where growth activity increase is directly proportional to the increasing light intensity, then followed by an area where growth activity increases at a decreasing rate as the light intensity increases to the point of light saturation. From the point of light saturation, growth activity remains relatively constant as light intensity is increased to the point where the intensity begins to inhibit the growth activity. Once the growth inhibiting light intensity is reached, the growth activity decreases at a rate equal to or greater than the above mentioned rate of growth activity increase with increasing light intensity. Figure 1 clearly illustrates this typical algal growth pattern.

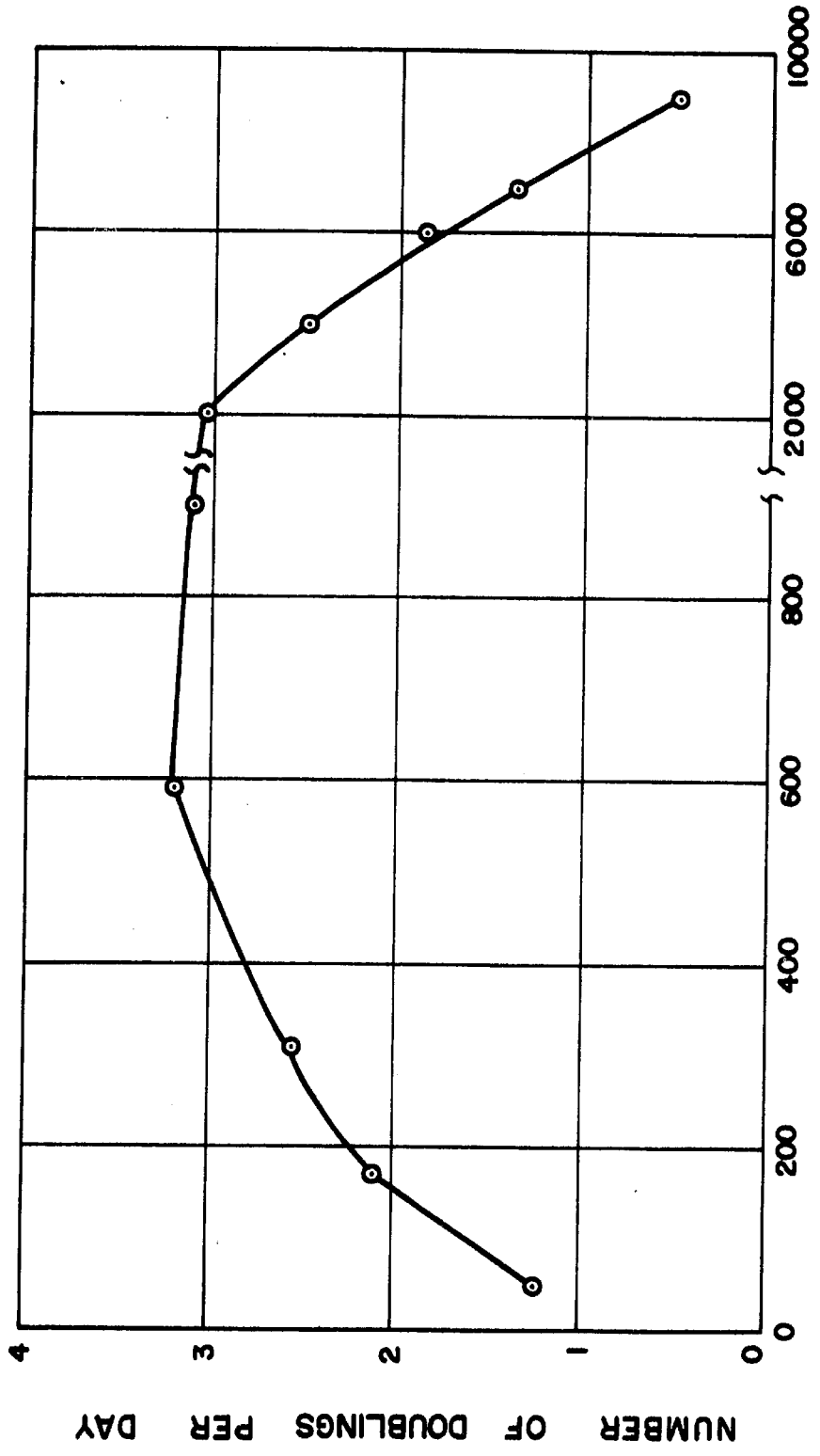


Figure 1. Typical Pattern of Algal Growth Activity as a Function of Limiting, Saturating and Inhibiting Light Intensities for Chlorella pyrenoidosa (33)

Evaluation of the above mentioned critical light intensity levels, through experimentation, has been performed by several investigators. Phillips and Myers (34) report a compensation point intensity for Chlorella growth of 24 foot candles. A light saturation intensity of around 600 foot candles has been reported for several green algae (33); and specifically for Chlorella, saturation intensities of from 400 to 600 foot candles have been indicated (33) (35). In the general consideration of the influence of light intensity on photosynthetic activity, Bonner and Galston (36) reveal that increased temperature raises the level of light saturation intensity. So it appears that in order to fully qualify the value of light saturation intensity, the temperature at which this value was determined must also be reported. As for the growth inhibiting light intensity, it is reported that some reduction in the photosynthetic activity can be expected to begin between 1000 and 4000 foot candles (35) (37). Certain algae could possibly withstand greater light intensities, provided they had been previously cultured under high light conditions (32). Information presented by Krauss (33) indicates that the level of growth inhibiting light intensity is somewhat dependent upon the algal genus and species under consideration. Also, the work of Tamyia (38) shows that, like light saturation intensity, the variation of the growth inhibiting light intensity with changing temperature is a direct proportionality. According to Nielsen (39) the damaging effects that high light intensities have on the photosynthetic system are through the photo-oxidation of enzymes

found within the chloroplasts in the algal cells.

The main purpose of light in its association with photosynthesis is the providing of absorbable energy for conversion into chemical energy. Experimental results reported by Arnon (40) (41) indicate that this process of energy conversion is more closely associated with phosphorous assimilation than with carbon assimilation. In green plants the conversion of light energy to chemical energy is accompanied by the formation of a carbon dioxide reducing power and the release of oxygen (31). The actual mechanism of photo-phosphorous assimilation is discussed in the section concerning the role of phosphorous in algal activity.

As for the effect that light intensity has on the growth activity of algae grown in a liquid waste environment, Witt (2) has shown that the typical light influencing patterns found in a synthetic medium are involved. For Chlorella grown in secondary sewage treatment plant effluent at 20°C, a light saturation intensity of about 900 foot candles was obtained. With a mixed culture of Scenedesmus and Chlorella grown in the same environment at temperatures of 20, 25 and 30°C, light saturation intensities of 800, 1000 and 1100 foot candles respectively were reported. Oswald, et al (42), in their waste oxidation pond studies where Euglena gracilis was the dominant organism, found that for a natural sewage (Warburg, 5-day-25°C BOD or 90 ppm) the optimum light intensity was from 400 to 1200 foot

candles. It was also reported that when the light intensity increased beyond the optimum level, the algae exhibited undesirable physiological changes. In the study that Bogan and associates (3) performed on the use of algae to remove phosphorus from sewage, they found that light intensity was the principal controlling factor. As for minimum light intensities, Oswald and Gotaas (25) indicate that, with a mixture of Scenedesmus and Chlorella at intensities below 30 foot candles, there was no conversion of light energy to cellular energy.

Intermittent Illumination

A given amount of illumination is more effectively used if a period of darkness follows the illumination period (43). This occurrence is based on the knowledge that the over-all photosynthetic process is comprised of two sub-processes; one photo-chemical and the other purely chemical. The algal cell mechanism is such that it can absorb adequate light in a very short time and then a much longer dark time is necessary for the complete utilization of the photo-chemical reaction products. Research by Kok (44) has shown that only a few milliseconds are needed for the critical light time, and that for Chlorella pyrenoidosa the dark time should be at least 10 times longer than the light time. Since the dark reaction is chemical in nature, the length of time necessary to insure that the reaction goes to completion is a function of temperature. Therefore, as with

the reporting of light intensity saturation values, the temperature at which the dark time was determined should probably be reported so as to fully qualify the critical dark time value. Optimum conditions would result when each algal cell, upon receiving its maximum quota of light energy, was immediately plunged into darkness for the time necessary to complete the dark reaction. Under these optimum conditions the illuminated algal cell, when placed in darkness, would immediately be replaced by an algal cell demanding illumination, thus allowing none of the incident light energy to be wasted. Such optimum conditions would effect an environment in which perfect integration of light energy times exposure time occurs (43).

Intermittent illumination also occurs in dense algal cultures through the shading effect that the individual algal cells have on one another. In a dense algal culture with a depth adequate to absorb the incident light almost completely, the algal cells at increasing depths will be exposed to decreased light intensity through self-shading of the cells (43). It has been shown by several investigators that in an algal culture, light intensity decreases with increasing depth and cell concentration according to the Beer-Lambert Law of light absorption (5) (45). Witt (2) and Tamiya, et al (38) have evaluated the self-shading of algal cells with the use of probability theory. The shading curves prepared by Witt indicate that as the algal density increases the probable per cent of shaded cells increases to a constant shading level. These curves also show that with increased algal cell

diameters, the maximum shading level is attained at lower algal densities. Davis, et al (46) indicate that self-shading of algal cells is probably a limiting factor in Chlorella cultures, since the number of cells per unit area of illuminated culture reaches a maximum value that is probably dictated by the shading factor.

Intermittent illumination is accomplished in dense algal cultures through induced turbulent mixing within the culture. The operation sought, through the application of turbulent mixing in dense algal cultures, is that of rapidly moving the algal cells into and out of a narrow layer at the culture surface. The algal cells would receive illumination at their saturation light intensity level only while they are in the narrow surface layer. This type of culturing operation would probably offer an environment in which the algae could accomplish light integration. Based on the results of induced turbulence studies in algal cultures, Gates (5) concluded that total algal growth increased with increasing mixer speeds; but that with the experimental apparatus used, true light integration by the algae was not obtained. These turbulence studies also indicated that with adequate mixing the exponential growth rate of an algal culture can be maintained for longer time periods. A culturing modification that would probably work towards the offering of an enviroment conducive to algal light integration, is that of providing the rolling flow pattern found in activated sludge aeration tanks to rapidly move the algae into and out of a narrow surface layer.

Other investigators have also experienced improved algal activity when culture mixing was applied. It has been stated that turbulent mixing serves the purposes of keeping the algal cells in suspension and at uniform density; maintaining a condition of equilibrium between the individual cells and the growth substrate; and moving the cells from the high light intensity area into the low light intensity area so as to prevent cell damage and to maintain maximum cell activity (47). Davis (48) in his experiments on Chlorella cultures measured increased algal growth, which he stated was probably due to the intermittent illumination effect caused by induced turbulent mixing. Ippen (49), studied the relation of turbulent flow to intermittent illumination of Chlorella cells and concluded that actual intermittent illumination was a function of depth, velocity and depth of light penetration. In culture studies on green algae, Brannon and Bartsch (50) determined that culture mixing for 5 minutes of every hour increased the culture density and the efficiency of substrate utilization by the algae. They concluded that this improved algal activity, resulting from the periodic culture mixing, was due to easier release of auto-spores, improved gas diffusion and removal of possibly toxic materials from around the individual algal cells. Studies conducted by Zehnder and Gorham (51) with Microcystis aeruginosa revealed that continuous culture shaking greatly increased the algal yield over that of an unshaken culture; and they concluded that the increased yield was due to improved gas exchange brought about by the continuous shaking. In conjunction with the latter

two discussed research studies, the effect of intermittent illumination resulting from culture mixing, although not mentioned by the researchers, probably played a major role in developing the increased algal activity that was observed. As for the effect that vertical mixing has on algae grown in a sewage environment, Gotaas, et al (45) indicate that in pilot lagoon studies the algal growth activity was greatly influenced by the mixing.

Efficiency of Light Energy Utilization

Energy utilization efficiency in algal systems has most often been evaluated on the basis of the quantity of absorbed light energy needed for the photosynthetic transformation of one carbon dioxide molecule into sugar. Light energy travels as an electromagnetic radiation having the wave properties of length and frequency. This light is emitted and absorbed in the form of individual packets of energy known as quanta (36). The energy concentration per quantum of light is a function of the wave length of the light, with shorter wave length light containing more energy per quantum than longer wave length light (31). According to Moore and Duggar (52) the quantum efficiency of photosynthesis in Chlorella is essentially the same for red, green, blue or violet light. Therefore, with the use of the long wave length red light, which contains less energy per quantum than the other light colors, a greater efficiency of energy utilization is obtained in

photosynthetic activity, since the quantum efficiency does not appear to vary with light quality. Most generally it is reported that from 8 to 12 quanta of light are necessary for the reduction of one molecule of carbon dioxide to sugar; however, Warburg has reported a very controversial quantum efficiency value of 4 (53). It also appears that the same quantum requirements exist regardless of the type of photosynthetic cells that are involved (31).

When considering the efficiency of energy utilization in conjunction with the unit process of mass algal culturing, the yield or productivity of algal material is used as an expression of this efficiency. The evaluation of algal culture yield or productivity involves a relationship between the culture density and rate of culture growth. Gates (5) has shown the confusion that the methods and terminology used to indicate culture density, culture yield or productivity and culture growth rate provide for the literature reader. Methods used to express culture density include milligrams or grams per liter, number of organisms per unit volume, per cent light transmission through algal suspensions for different light wave lengths and light paths, and packed volume of cells per milliliter or liter of culture determined at a non-standarized centrifuge speed. Some of the terminology for expressing the rate of culture growth is $\log_e$ or $\log_{10}$ units per day or hour, generation time of the organism, volume of overflow per unit of time from constant density cultures, grams of algal material per hour or day and number of organisms per hour or day.

The rate of culture growth can be evaluated through the methods used to express culture density. Culture yield or productivity indicates the rate at which the culture produces cellular material. The evaluation of culture yield or productivity is usually in terms of grams per liter of culture per day or grams per square meter of illuminated surface per day. In certain sewage pond studies (45) and pilot-plant studies in Chlorella production (54), algal productivity has been expressed in terms of tons per acre per year.

Burlew (43) indicates that the yield or productivity and cost per unit of illuminated area are the fundamental considerations in the planning of a mass algal culturing process. This is because the yield per unit area is a direct measure of the efficiency of light energy utilization by the algal culture. Gates and Borchardt (30) also state the importance of surface productivity considerations in the development of controlled algal cultures for the bio-extraction of fertilizer nutrients from sewage treatment plant effluents. They continue by indicating that the yield per unit of culture volume or volume productivity is also of importance, since this factor reveals the efficiency of nutrient extraction from the sewage medium by the algae. Therefore, to provide an algal process for effective nutrient extraction, an enviroment must be designed, in which maximum surface and volume productivity occur together (30). Optimal environment designs must include techniques to provide maximum light energy distribution and utilization within the culture. Myers and Graham (55) attempted to

overcome the light saturation effect on algae by providing improved light distribution within algal cultures through the use of inverted light diffusing cones. This technique increased the maximum culture yield by a factor of 2 and also increased the culture density that produced the maximum yield by more than a factor of 2. Increased culture yield has been experienced by creating intermittent lighting in cultures through induced turbulence (43). It has been stated that in order to create a true light integration effect by turbulence, culture densities in the range of 2 to 5 grams per liter would be necessary (46). Gates (5) in his induced turbulence studies with batch Chlorella cultures, failed to experience light integration. Possibly through investigation with different mixing patterns, higher density algal cultures and with light intensities in the range of 5,000 to 10,000 foot candles, light integration effects may be experienced with controlled algal cultures.

Factors which affect the utilization of the light energy are the same as the factors that influence algal activity, since yield or productivity is a function of the algal growth rate. One interesting influencing factor is that of culture density. Ketchum (56) has presented a "theory of optimum yield" which states that for every population there is an optimum density which produces a maximum yield. This theory has been supported in the results from the controlled algal culture studies reported by Gates and Borchardt (30). As for maximum culture yields, it has been stated that with complete use of the visible light spectrum, it might be possible to produce 110 grams of dry algae

per square meter of illuminated surface per day (54). It is doubtful that this maximum surface yield has ever been or ever will be realized, since the algae effectively use only the blue and red portion of the visible light spectrum.

Myers, et al (57), using a small annulus growth chamber, concentrated synthetic media, and full sunlight were able to obtain maximum Chlorella yields of 55 grams per liter. Maximum surface yields of 16 grams per square meter per day have been reported for cultures in 5 gallon bottles (58). Studies performed by Mayer, et al (59) in Israel using concentrated nutrient media in outdoor tanks with a capacity of 2,100 liters produced Chlorella yields of 14 to 21.2 grams per square meter per day. Gates (5), in his induced turbulence study with Chlorella pyrenoidosa in synthetic media, realized a maximum surface productivity of 52.3 grams per square meter per day with a light intensity of about 1300 foot candles. As for algal productivity in sewage environments, Oswald and Gotaas (60) reported average yields of 30 to 35 tons of dry algae per acre per year from sewage ponds at Richmond, California, with maximum July and August yields of 60 to 75 tons per acre per year. Meffert (61) obtained surface yields of 7 to 10 grams per square meter per day during warm weather and 4 grams per square meter per day during cool cloudy weather. In his studies on the use of controlled algal cultures for the bio-extraction of nutrients from sewage plant effluents, Gates (5) experienced a maximum surface yield of Oscillatoria of about 9 grams per square meter per day

at a light intensity of approximately 1000 foot candles. Bush, et al (4) reported, from their study on the dissolved solids removal from waste water by algae, an efficiency of solar energy utilization of 4 per cent, which more than doubles the efficiency for agricultural crops.

Culture Liquid Depth

The culture depth is usually considered as that depth of liquid measured parallel to incident illumination. The influence of culture depth on the growth characteristics of algae has mainly been considered in conjunction with studies concerning the mass culturing and harvesting of algae for the purpose of developing a potential food source. Liquid depth has also been considered to some extent in sewage stabilization pond studies, which for the most part have been concerned with BOD removal from sewage rather than the bio-extraction of algal nutrients from the waste.

Illuminating conditions within an algal culture are altered with each incremental increase in culture depth. The quantity of light is decreased with increasing depth due to the light absorbing characteristics of the algae. For each culturing condition there is a particular depth at which the penetrating light intensity just equals the saturation light intensity for the algae (43). The light penetrating below this saturation depth will be utilized with maximum efficiency by the

algae; while above this critical depth, only the saturation intensity fraction of the penetrating light is completely used by the algae. It is probable that light quality is also affected throughout the depth of an algal culture. Beeton (68) reports on the quality of light penetration in the Great Lakes and states that in the more productive lakes, the spectral distribution of light at depth shifts towards the red end of the light spectrum. In the less productive lakes of Superior and Huron, the spectral distribution at depth is in the blue-green range. Beeton's findings indicate that in productive bodies of water, which contain significant quantities of phytoplankton, the shorter wave length light is absorbed in the upper portions of the body of water and that the longer wave length light penetrates to deeper depths. This report concerning the quality of light at depth in the Great Lakes is in full agreement with the Wisconsin Lakes report by Birge and Juday (69). Based on the evidence available in connection with the variation of penetrating light quality in productive lakes, it is probable that the same pattern of light quality variations exists within algal cultures.

The significance of liquid depth has been considered to some extent in connection with sewage stabilization pond studies. Mackenthun and McNabb (70) evaluated the rate of light absorption with liquid depth in several Wisconsin sewage ponds and found that when the reduction in light was related graphically to the liquid depth on semi-logarithmic graph paper, a straight line relationship results when the

suspended material is uniformally distributed. Variations from this straight line relationship did occur when there was a vertical stratification of phytoplankton. Oswald and Gotaas (25), in their development of a general equation for the design of waste stabilization ponds, have evaluated liquid depth as a function of algal cell concentration. As stated in a previous section, an algal suspension absorbs light in agreement with the Beer-Lambert Law, which is expressed as follows:

$$\frac{I}{I_o} = e^{-C\alpha d}$$

I = measured light intensity at depth (d)

I_o = incident light intensity

C = algal cell concentration

α = specific light extinction (or absorption) coefficient.

This development then states that for a practical pond design all of the available light should be absorbed, thus, leaving the transmitted light at the pond bottom approximately equal to zero. Then by taking the natural logarithm of both sides of the above equation and equating I to zero, the following expression for the liquid depth is obtained:

$$d = \frac{\log_e I_o}{C\alpha} \quad .$$

From this equation it can be seen that the cell concentration and specific extinction coefficient have an inverse effect on the depth value.

It has been shown that the light absorbing characteristics of an algal culture are a function of the inorganic nitrogen concentration in the culture feed (5); therefore, as the nitrogen concentration is altered, so is the specific extinction coefficient of the algal suspension. This indicates then that for every change of extinction coefficient, a different depth should be provided so as to maintain the treatment process conditions optimum.

In pilot plant studies of sewage stabilization ponds, Gotaas, et al (45) found that maximum oxygen production and BOD removal resulted at the shallower pond depths. In another study Gotaas and Oswald (71) found that the maximum photosynthetic efficiency occurred at greatest depth and the maximum energy yield occurred at minimum depth. From this they concluded that the maximum efficiency in the utilization of light is obtained at the sacrifice of efficiency in the utilization of algal nutrients. A recent article by Varma and Wilcomb (72) further emphasizes the earlier findings of Gotaas and Oswald by indicating that there appears to be an optimum pond depth for maximum BOD removal and maximum algal concentration. Varma and Wilcomb suggest that this optimum pond depth occurs at the point where the algal concentration curve intersects the BOD removal curve when a graphical representation of these two variables with pond depth is prepared. As for the influence of culture depth on controlled algal cultures grown in sewage treatment plant effluent, Gates and Borchardt (30) indicate that the shallower depths make better use

of the available light energy. They also state that for a series of culture depths, there will be a single combination of culture growth rate, culture density and depth that will utilize the available light energy to a maximum.

CHEMICAL FACTORS

The influence of the chemical concentrations within growth media has been extensively investigated under various algal culturing conditions. Algal nutrition has been studied for the prime purpose of providing adequate growth media to be used in the development of mass algal culturing techniques, which could be used if and when algae may be necessary as a supplementary food source. It has been shown that the concentrations of the various algal nutrients in the growth medium influences the carbohydrate, protein and lipid contents within the algal cells (73). Another chemical factor which appears to influence algal activity is the extracellular metabolites produced by the algae themselves. Certain of these metabolites may benefit the algae while others may inhibit the algal activity (86). Investigations concerning the use of algal cultures for the extraction of nutrients from waste waters have shown that the concentrations of the nutrients in the wastes influence the algal growth activity and the ability of the algae to remove the nutrients (5). Recent thoughts on the use of controlled algal cultures as the active biological unit in closed ecological

systems to provide gas exchange, waste treatment and supplementary food during space travel, have stimulated additional research concerning the chemical and bio-chemical factors that influence algal activity (74) (118) (119).

The chemical factors to be considered here are: culture pH; the influence and utilization of the macronutrients of carbon, nitrogen and phosphorus; the influence of the known micronutrients and other growth factors; and the influence of extracellular metabolites on the growth activity of algae.

pH of Culture Medium

Variation of culture pH does not appear to have any direct affect on the physiological mechanism of the algal cell, but it does influence the chemical quality of the growth medium which in turn alters the growth characteristics of the algae. It has been reported that algae of the types commonly present in sewage environments have been cultured in media with pH values in the range of 3.0 to 11.0 with no apparent inhibition of photosynthesis or respiration (75). Emerson and Green (76) studied the effects of hydrogen ion concentration on Chlorella photosynthesis and concluded that under conditions of carbon dioxide saturation neither hydrogen nor bicarbonate ion concentration influenced the rate of photosynthesis for a range of pH values from 4.9 to 8.9. An investigation by Witt (2) reveals that Scenedesmus

growth stopped when the pH was lowered to 5. 5 and as growth started again the pH increased to a maximum of 11 which again stopped algal growth. Within this wide pH range, steady growth was observed from pH 6. 0 to 9. 5. Witt made no attempt to explain why the algal growth stopped at these lower and upper pH values. Further studies by Witt indicate that the maximum growth rate for a mixed culture of Chlorella and Scenedesmus occurred around a pH value of 8. 3 and that lower growth rates resulted at higher pH values. These findings are in agreement with results that have been reported by Osterlind (77).

Increasing the pH of an algal culture above a level of 8. 0 will produce precipitates which will probably reduce the availability of necessary nutrients. Pipes (19) suggests that the concentration of phosphate may become limiting through precipitate formation if the pH is allowed to exceed values of about 9. 0. Alkaline conditions within a culture may cause necessary trace nutrients such as iron to be precipitated in an unavailable form that results in curtailed algal activity (78). Culture pH values around 9. 5 or above may cause the ammonium present to leave the culture in the gas phase which would be beneficial when considering nutrient removal from wastes but not when considering the mass production of algae. Another adverse effect of culture pH levels above 8. 3 is the production of calcium carbonate precipitate which would interfere with light penetration throughout the culture and thus reduce the rate of photosynthesis. One beneficial consideration is that a high pH in an open culture or stabilization pond

would favor more rapid absorption of carbon dioxide from the atmosphere (25).

In the symbiotic relationships between algae and bacteria in sewage environments, high pH levels also appear to be of concern. Pipes (75) indicates that Oswald found pH values greater than 8.0 limiting the light conversion efficiency of the algae. It was concluded that this problem was not due to any direct action on the algae, but was due to the limitation of the amount of available carbon dioxide since bacterial oxidation was inhibited by the high pH levels.

Carbon Utilization

Carbon is the most fundamental nutrient element associated with algal activity. The photosynthetic fixation of inorganic carbon into sugar compounds is the initial step in a chain of biochemical reactions, which are stimulated through the metabolism of the photosynthetically produced sugar. The final result of this series of metabolic reactions is the propagation of new cellular material. Calvin (79) found that the first stable product of photosynthetic carbon fixation in Chlorella is the 3-carbon phosphorylated compound of 3-phosphoglyceric acid (PGA). This material is then reduced to the level of a carbohydrate or aldehyde level by means of the reducing power developed through the photolysis of water. This reducing power is a reduced pyridine nucleotide that aids in the electron transport associated

with the oxidation-reduction reactions of metabolism within the cell (31). Six carbon sugars are probably produced through the metabolic cycle proposed by Calvin and Bassham (79) which is pictured in Figure 2. The sugars are then metabolized through the well known pathways of carbohydrate breakdown, which also stimulates the uptake of other necessary nutrients from the growth medium by the algae and their incorporation into new cellular products.

Under natural conditions the carbon dioxide present in the atmosphere and the natural alkalinity of the water provide limitless sources of inorganic carbon for photosynthetic fixation. Inorganic carbon exists in the forms of carbon dioxide, bicarbonate and carbonate, which are a part of one system that maintains itself in equilibrium. When the concentration of one inorganic carbon form is altered, the amounts of the other ionic carbon forms shift so as to reestablish the equilibrium of the system and these changes usually result in a change of pH. On the other hand, a pH change in water will shift the concentrations of the inorganic carbon forms present (80).

Depending upon the inorganic carbon species present, the environment will be more conducive to the growth of one algal form over another. Osterlind (81) states that algal types can be classified as to their ability or inability to assimilate the bicarbonate form of inorganic carbon. One type is that of Chlorella pyrenoidosa which according to Osterlind has no possibility of utilizing bicarbonate. The other extreme is represented by Scenedesmus quadricauda which

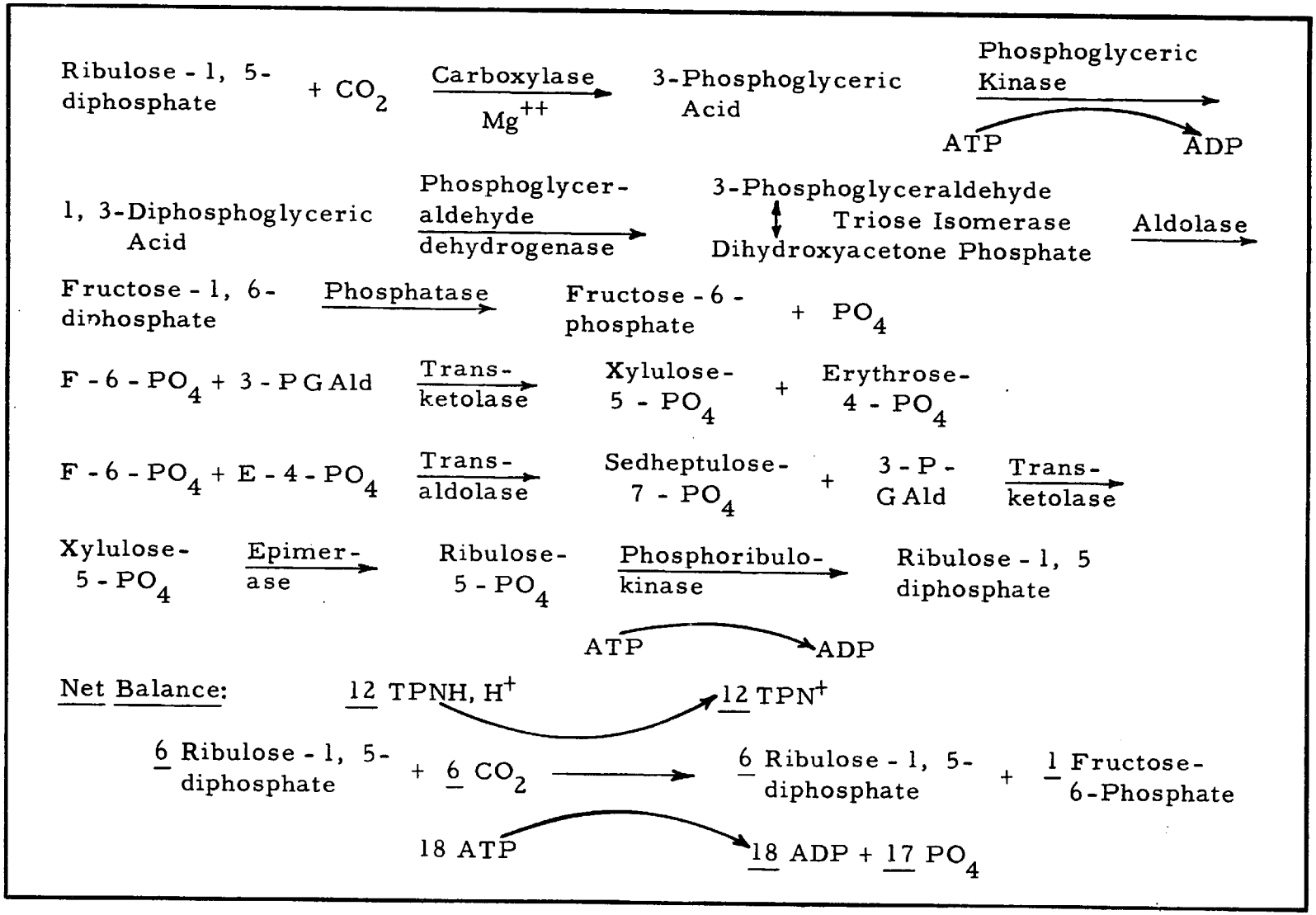


Figure 2. Carbon Reduction in Photosynthesis (79).

assimilates bicarbonate very well. This classification does not appear to hold true under all conditions, since in the Sanitary Engineering Laboratories at The University of Michigan very healthy cultures of Chlorella pyrenoidosa have been grown with bicarbonate as the inorganic carbon source. Both of these algal species appear to utilize carbon dioxide as a carbon source equally well (81). As a result of further studies with Scenedesmus quadricauda, Osterlind (82) found that carbon dioxide assimilation begins as soon as the culture is illuminated, whereas the bicarbonate assimilation usually follows a lag period of some tens of minutes. It is suggested that the factor which must be photoactivated in order to instigate bicarbonate uptake is either connected to the absorption of bicarbonate or is the emzyme carbonic anhydrase. Conditions which favor the utilization of bicarbonate-carbon are low pH values and a low carbon dioxide concentration (82).

As to the provision of adequate carbon to insure effective laboratory culturing of algae, Myers (78) indicates that the production of 1 milligram of algal dry weight requires an uptake of about 0.5 milligram of carbon, which is equivalent to 1.8 milligrams or about 1.0 milliliter of carbon dioxide under laboratory conditions. Aeration of cultures with 5% carbon dioxide in air has been a universal algal culturing practive for some time. When 5% carbon dioxide is used, it is usually assumed that the characteristics of the algal cells are independent of the carbon dioxide concentration in the medium (78). Convincing evidence to contradict this assumption is lacking, although

according to Myers (78), Nielsen has questioned the advisability of using higher carbon dioxide concentrations. Contrary to this, the continual addition and intermittently controlled addition of 100% carbon dioxide to ensure adequate carbon and to control the pH in algal cultures has been effectively accomplished, without noticeable changes in algal characteristics or toxicity to the algae (5) (30).

It has been stated that the superiority of a particular algal medium is often a function of pH or carbon dioxide concentration rather than the superiority of certain salts in specified ratios (78). Osterlind (83) found that optimum growth of Scenedesmus quadricauda occurred when a carbon dioxide concentration of 80 micromoles per liter was provided. Nielsen (84) in his studies with very dilute concentrations of Chlorella pyrenoidosa, showed that the algal activity was independent of the carbon dioxide down to concentrations of 0.01 to 0.03%. Both Scenedesmus quadricauda and Chlorella pyrenoidosa assimilate carbon dioxide, but the rate of assimilation is directly proportional to the carbon dioxide concentration in the range from 10 to 100 micromoles per liter (85).

In considering bicarbonate as the carbon source, Osterlind (83) found that a bicarbonate concentration of 10 to 20 micromoles per liter produced the same optimum growth of Scenedesmus as did the above mentioned optimum carbon dioxide concentration. One factor that appears to influence the utilization of bicarbonate is the age of the culture. One study indicated that a 5 day culture of Scenedesmus

quadricauda assimilated bicarbonate independently of the concentration from 10 to 100 micromoles per liter, while a 10 day culture had lost its ability to assimilate bicarbonate (85). In additional Scenedesmus studies, Osterlind (77) found that the best algal activity occurred at pH 8. 1, at which level almost all of the available inorganic carbon is in the bicarbonate form.

Reports have indicated that certain concentrations and forms of inorganic carbon may be inhibitory to algal activity. It is generally thought that algae are unable to utilize the carbonate species of inorganic carbon (37) (77). In fact, Krauss (37) states that carbonate ions may be inhibitory to algal activity. Osterlind (77) studied the retarding effect of high concentrations of carbon dioxide and carbonate on Scenedesmus quadricauda and found that at pH levels above and below the optimum pH of 8. 1, algal growth decreased. This indicates that at low pH levels the increased concentration of carbon dioxide limited growth and at high pH levels the increased carbonate concentrations inhibited growth. It was suggested that carbonate will start to inhibit growth at a concentration of about 4 micromoles per liter. From this study it was concluded that the medium with lesser amounts of carbon dioxide and carbonate at any pH level will offer a better environment for the growth of the Scenedesmus form studied. As for carbon dioxide toxicity, Krauss (37) indicates that the concentration level at which toxicity becomes apparent is somewhat dependent upon pH but will generally show up when the carbon dioxide concentration is

between 5 and 10% in air. Nielsen (84) has reported that a carbon dioxide concentration above 1% has a narcotic effect on photosynthesis under high pH conditions. No reports of limited algal activity due to excessive bicarbonate concentrations were found.

For many algae, carbon may be provided as pre-formed organic substances in the medium, rather than as carbon dioxide in the gas phase (78). Hexose sugars and acetic acid are the organic substances most generally utilized by algae as carbon sources, but certain algal species may need very specific organic substances to support their growth (6). Saunders (86) has prepared very extensive lists of algae capable of utilizing organic substrates and the organic substrates that algae use for energy or growth. According to Myers (78), the simplest method for producing Chlorella in large quantities requires only large cotton-stoppered flasks, glucose as a carbon source, illumination and aeration or mechanical agitation.

Reports on studies of algal activity in sewage environments indicate that carbon is usually the limiting nutritional element (25) (71). Ludwig, et al (87) state that when considering the reclamation of nutrients from sewage, carbon dioxide fortification is necessary to insure that the algae will make maximum use of the available nitrogen. In studies on the ecology of a biotic community of algae and bacteria, Golueke (18) found that optimum concentrations of carbon dioxide were within the range of 1 to 3% and that inhibitory effects were noted at concentrations of 4%. It was also reported that the algal cell density

was highest at carbon dioxide concentrations of 1 to 3%, and that the effect of carbon dioxide concentrations was greatly influenced by light energy input and less so by detention time. Gotaas, et al (46) made a study of the influence of various concentrations of carbon dioxide on the growth of Euglena gracilis and found that 0.03% carbon dioxide gave fair growth, 0.5 and 2.0% gave good growth, 4% gave fair growth and 8% gave poor growth. From controlled algal culture studies, Witt (2) found that the concentration of carbon dioxide is probably incidental as long as it is sufficient, and also states that best results might be obtained if the supply of and demand for carbon dioxide were balanced. It is generally felt that secondary sewage treatment plant effluents will support about 1,000,000 Scenedesmus per milliliter without the use of a supplemental carbon source (5).

Algae will also utilize organic carbon in waste environments. Pipes (88) reports that under carbon dioxide limiting conditions organic acids, some organic nitrogen compounds and some vitamins will increase the growth rate of Chlorella. Consequently, it was concluded that in sewage stabilization ponds with low carbon dioxide concentrations, the algae will probably use organic materials from the sewage in addition to photosynthetically produced carbohydrates for the building of new algal cellular material.

Nitrogen Utilization and Extraction

Nitrogen is of importance since a major portion of the

cellular components of algae have a nitrogenous character. In natural environments and laboratory growth media, nitrogen can appear in the inorganic forms of ammonia, nitrate or nitrite. Organic nitrogen materials such as urea, proteins and products of protein degradation may also be present and available for utilization by the algae. Another nitrogen source is the atmosphere and certain blue-green algal forms appear to have the ability to assimilate or fix elemental nitrogen from the atmosphere (6). Through their metabolic activities, algae absorb nitrogen and incorporate it into amino acids which are used to synthesize the proteins that make-up new algal cellular material. According to Myers (78) most unicellular algae contain about 8 per cent nitrogen; therefore, every gram (dry weight) of algal cells produced per liter of culture requires 80 milligrams per liter of nitrogen.

Most studies concerned with the nitrogen assimilating capabilities of algae have been performed with the use of nitrogen starved algal cultures. Syrett (89) states that ammonia is rapidly assimilated by nitrogen starved cells and that this assimilation is at the expense of endogenous carbohydrate reserves. Nitrate and nitrite are assimilated slower than ammonia and at about the same rate as with normal cells. It appears that the absence of illumination does not influence the nitrogen assimilation, since in darkness nitrogen deficient cells of Chlorella vulgaris continue to assimilate ammonia until their carbohydrate reserves are exhausted (89). Ammonia may be

either absorbed by the algal cell or oxidized to nitrate before absorption. Within the cell the nitrate is reduced back to ammonia which is then coupled into cellular nitrogen compounds (31).

Biochemical evidence indicates that the major reaction whereby ammonia is incorporated into amino acids is the reductive amination of ketoglutaric acid to glutamic acid (89). Other amino acids can then probably be formed through transamination reactions (6). Ketoglutaric acid is produced within the algal cell through the conventional pathways of carbohydrate metabolism. Ammonia nitrogen can serve as the sole nitrogen source for most algae and is usually preferred over other nitrogen sources. If ammonia and nitrate are offered together as nitrogen sources, the ammonia is preferentially utilized with the nitrate being consumed only after the ammonia has been exhausted. The reason for the ammonia preference is probably because the ammonia nitrogen is at the same reductive level as the organic compounds within the cell and thus can be immediately coupled with these compounds without utilization of additional cellular energy (6).

In spite of the fact that nitrate nitrogen is less available to algae than ammonia nitrogen, nitrates are commonly used as the nitrogen source in synthetic algal media. Certain side effects associated with ammonia assimilation appear to make this nitrogen source less desirable than nitrate nitrogen. Ammonium nitrogen is absorbed into the algal cells most readily as undissociated ammonium hydroxide, leaving the medium surrounding the algal cells in a more acid

condition (89). The absorption of nitrate nitrogen creates a more alkaline environment but the changes are less extreme than with ammonia absorption. Therefore, in order to successfully use ammonia as a nitrogen source, a well buffered environment must be provided so as to prevent the acid situation from developing to a level that would inhibit algal growth (90). McLachlan and Gorham (91) studied the effects of pH and nitrogen sources on the growth of Microcystis aeruginosa and found that ammonium bicarbonate prevented the pH from decreasing when ammonia was used as a nitrogen source.

A great deal of research in the area of algal nutrition has been directed toward the determination of satisfactory nitrogen concentrations that will sustain optimum growth activity for various forms of algae. As a result of studies concerning the influence of mineral nutrition on the growth of a representative group of algae, Chu (92) indicates that the upper limit of the optimum range of nitrogen concentration varies from about 5. 3 to 13 parts per million (ppm) when ammonium salt is the nitrogen source and from 3. 5 to 17 ppm when nitrate nitrogen is provided. The lower limit of the optimum nitrogen concentration range varies from 0. 3 to 5. 3 ppm when the nitrogen source is an ammonium salt and from 0. 3 to 0. 9 ppm when nitrate is the source of nitrogen. When the nitrogen concentration was increased beyond the upper limit range, growth inhibition became more evident; and as the nitrogen concentration was reduced below the lower limit range, the growth rate decreased with additional decreases in the

nitrogen concentration. If the growth of algae is continued for some-time in an environment where the nitrogen concentration is limiting to the growth rate, algal cells deficient in nitrogen will develop. Along with decreased growth activity, one of the first indications of nitrogen deficiency is a fading of the characteristic green pigmentation that healthy algae possess (89). What appears to be happening is that in order to satisfy the algal cell's nitrogen needs, the nitrogen incorporated in the chlorophyll molecules is being metabolized and since the growth medium is deficient in nitrogen, the chlorophyll storage is not being replenished. Krauss (93) reports that during 14 days of growth in a nitrogen deficient medium, the nitrogen content in the Scenedesmus species under study decreased from 7. 5% of the dry weight to 2. 07%, and that at any time the major portion of the nitrogen was in the form of protein.

From studies on the growth conditions of Scenedesmus quadricauda, Osterlind (83) indicates that a nitrogen concentration in the growth medium of from 0. 1 to 0. 5 milligram per liter in the form of ammonium is the best nitrogen source, but that a concentration of 10 to 50 milligrams per liter in the form of nitrate is a more desirable nitrogen source, due to the lack of the afore mentioned pH problem that is associated with ammonium nitrogen utilization. Rodhe (94) found under his algal culturing conditions that the maximum growth of Scenedesmus quadricauda requires at least a nitrogen concentration of 5 ppm in the growth environment. In a report on the factors influencing

the growth of Microcystis aeruginosa, Zehnder and Gorham (51) state that the maximum algal yield was obtained with 10 millimoles of sodium nitrate in the growth medium and that the yields were increased from 685 ppm dry weight in 20 days to 1500 ppm dry weight in 7 days by increasing the sodium nitrate concentration. Gerloff, et al (95) found that the nutrient solution known as "Chu #10", which contains approximately 40 milligrams per liter of calcium nitrate, gave the most promising results in the isolation, purification and culturing of blue-green algae. In a review on mineral nutrition of phytoplankton, Ketchum (96) indicates that the optimum nitrogen concentration for several algae has proven to be from 5 to 17 ppm, that 31.5 ppm of ammonium has been reported to be toxic to Chlorella pyrenoidosa, and that nitrite has been found to be both toxic and nontoxic to algae. It is evident that one is not able to prescribe an exact nitrogen concentration which will be optimum for algal growth media in general. The problem of developing a desirable nitrogen concentration is unique to each algal study, and is dependent upon the algal species and culturing conditions that are to be used.

Organic nitrogen compounds are also available to algae as nitrogen sources. In a summary on how organic materials influence phytoplankton, Saunders (86) reports that several species of autotrophic algae are able to utilize alanine, aspartic acid, asparagine, glutamic acid, glycine and succinamide as sole sources of nitrogen. Saunders also presents an extensive list of organic nitrogen compounds that are

utilized by algae for energy or growth. Fogg (6) states that any substance which is an intermediate in nitrogen metabolism appears to be available as a nitrogen source for algae provided it can be absorbed into the algal cell. According to Syrett (89) urea can be used as the total nitrogen source for Chlamydomonas, Chlorella and other unicellular green algae. Syrett further states that urea has been proposed as the best nitrogen source for the mass culturing of Chlorella because it does not stimulate bacterial contamination, it produces faster algal growth than nitrate-nitrogen does, the culture pH remains fairly constant, and a large concentration can be added to the medium without inhibiting the algal growth. Urea is possibly assimilated without any prior breakdown to ammonia or perhaps during assimilation, the urea carbon is released as carbon dioxide while the amide groups are coupled to some material so that no free ammonia is formed (89). The first step in amino acid assimilation by algae appears to be deamination so that the amino nitrogen enters into assimilation as ammonia (6). It has been reported that Scenedesmus obliquus deaminates glycine so rapidly that ammonia-nitrogen accumulates in the growth medium (89). The amide nitrogen contained in acetamide, succinamide, asparagine and glutamine appears to be more available for algal assimilation than the amino acid nitrogen. It is understood that the amide nitrogen is first liberated as ammonia before being assimilated by the algae (6). Another consideration is that organic nitrogen compounds may act as chelating agents and thus influence algal growth by maintaining essential elements available

to the algae by chelation, which is quite different from their usefulness as a nitrogen source (86).

Another source of nitrogen for algal utilization, which is worthy of consideration, is that provided through the fixation of elemental nitrogen from the atmosphere. According to Dugdale and Neess (97), the ability of blue-green algae to fix nitrogen from the atmosphere into their growth medium has been definitely established. Fogg (98) indicates that there is no satisfactory evidence to establish nitrogen fixation by algae belonging to groups other than the Cyanophyta, and that most of the known nitrogen fixing species belong to the three genera of Anabaena, Cylindrospermum and Nostoc. Williams and Burris (99) found with tracer studies using radioactive nitrogen that, of 10 blue-green algal species, only Nostoc muscorum, Calothrix parietina and another tentatively identified Nostoc species had the ability to fix nitrogen. It appears that the mechanism of nitrogen fixation in blue-green algae is generally similar to that in the nitrogen fixing bacteria (98). The nitrogen fixing mechanism in blue-green algae is functional only in growing cells, consequently any environmental change that influences algal growth will in turn affect the nitrogen fixing ability of the algae. Nitrogen sources such as ammonia and urea, which are more readily available to the algae, completely inhibit nitrogen fixation, as does nitrate, provided the algae are adapted to the assimilation of nitrate-nitrogen (6). A molybdenum concentration in the range of 0. 1 milligram per liter is a nutritional requirement of blue-green algae (98).

Ammonia appears to be the first recognizable product of nitrogen fixation and in Nostoc muscorum the nitrogen primarily accumulates in aspartic acid and glutamic acid, from which it enters into further nitrogen metabolism (6). Based on laboratory culture studies with blue-green algae, Allen (100) concluded that fixation of nitrogen, under optimum conditions, could amount to about 480 pounds per acre per month.

It has been conclusively shown that algae growing in a waste environment have the ability to extract significant quantities of inorganic nitrogen from the waste and that the nitrogen concentration of the waste influences the growth activity of the algae (24) (2) (5). Oswald and Gotaas (25) suggest the relationship between sewage nitrogen and growing algae of

$$C_c = 10 \times N$$

where N is the nitrogen required to produce an algal concentration of C_c with N and C_c being expressed in the same concentration terms. The constant 10 arrives from the assumptions that 80% of the nitrogen in the sewage is recovered and that the composition of the algal cells is 8% nitrogen. In an evaluation of stabilization pond literature, Fitzgerald and Rohlich (24) report that the main nutrient changes recorded are the reduction of ammonia-nitrogen from 15 to 40 ppm to less than 2 ppm in the pond effluent. Also up to 60% of the organic nitrogen may be removed and that nitrate and nitrite nitrogen may

increase, but their amounts are insignificant when compared to the concentration of ammonia-nitrogen. Neel, et al (21) indicate that through the treatment of raw sewage in experimental lagoons from 94 to 98% of the nitrogen was removed. It was also found that with waste loadings below 40 pounds of BOD per acre per day, the photosynthetic activity in the lagoons was influenced by the changes in available algal nutrients.

Algal processes for the purpose of extracting nutrients from wastes have been studied in pond treatment methods and with environmentally controlled algal cultures. Reid and Assenzo (28), in their studies with attached algae grown in sewage, related the removal of total nitrogen to a first order reaction and developed a k-rate for removal of total nitrogen from sewage plant effluent of 0. 128 unit per hour. One main conclusion was that attached algae are relatively inefficient nutrient removers. Bush, et al (4) reported on the use of algae for the removal of dissolved solids from waste water and indicated that efficiencies for removal of ammonia ranged from 63 to 90%, for nitrate 27 to 60% and for organic nitrogen 32 to 74%. The use of stabilization ponds as nitrate reducing reactors has been discussed by Hermann (29). His studies revealed that at loadings greater than 15 milligrams of nitrate-nitrogen per liter of reactor per day, the efficiency of nitrate reduction declined to less than 50%. Hermann also stated that in aerobic-anaerobic stabilization ponds, the bacterial biota of the algal-bacterial commensal system are mainly responsible for the oxidation and reduction reactions that take place.

In a discussion of Hermann's paper, Oswald (101) states that in order to have an efficient nitrate reducing process, the environment must be such that atmospheric reaeration, photosynthetic oxygenation and methane fermentation do not contribute greatly toward maintaining the oxygen resources or satisfying the BOD.

Based on environmentally controlled algal culture studies using sewage treatment plant effluent as a growth medium, Witt (2) concluded that Scenedesmus and Chlorella absorb ammonia in preference to other inorganic and organic forms of nitrogen present in the sewage. It was also stated that a tertiary sewage treatment process utilizing algae would require a process time of approximately 20 hours under optimum condition in order to accomplish almost complete removal of the ammonia from the sewage substrate. Gates (5) found that the growth rates of controlled Oscillatoria cultures, in a biologically treated sewage environment, were a function of the total inorganic nitrogen concentration in the culture feed; and that there was a critical range where the nitrogen concentration directly influenced the culture growth rate. It also was found that the quantity of inorganic nitrogen extracted from the waste was a function of the inorganic nitrogen in the culture influent. From these studies Gates predicted that with multiple exposure of the waste to algal cultures of varying densities, it might be possible to attain 98% extraction of the inorganic nitrogen initially present in the waste treatment plant effluent.

Phosphorus Utilization and Extraction

Algal growth in natural environments as well as in laboratory cultures is influenced by the amount of available phosphorus. In the algal cell phosphorus is present mainly as a structural component of nucleic acids and as part of certain phospholipids which are thought to be important components of the cell membrane structure. A phosphorus deficiency is therefore quite critical since it would affect the formation of new genetic material in the cell and also new membrane material around the surface of the cell. Phosphorus is also critically involved in all energy transfer steps associated with cell metabolism, since energy storing compounds such as adenosine triphosphate contain three phosphate groups coupled through high energy bonds to an extensive ring structure (31).

Phosphorus occurs in solution in the forms of orthophosphate, polyphosphates and organic phosphorus complexes. The various polyphosphate species gradually hydrolyze in aqueous solutions to regenerate the orthophosphate forms from which they were derived. This rate of orthophosphate regeneration is increased by increasing the temperatures and lowering the pH. The rate of hydrolysis is also influenced by bacterial activity, as indicated by the fact that the hydrolysis is very slow in pure water and much faster in sewage (80). The release of phosphorus compounds through the degradation of organic phosphorus materials is also of importance in the cycling of phosphorus in biological systems, but information on

this consideration is lacking. When considering the possibilities of whether an environment will support the bloom and growth of an algal population, the total concentration of phosphorus present in its various forms is of extreme importance. Experience indicates that algal bloom problems do not develop when phosphorus concentrations are very limiting. The critical phosphorus concentration level has been reported to be 10 micrograms per liter (10) (14).

Phosphorylated metabolites within the algal cell are involved in both the light and dark reactions of photosynthesis. According to Kuhl (102), Emerson, et al proposed that the prime role of the light energy absorbed by the chlorophyll is in the formation of high energy phosphate bonds, which are then able to sustain all succeeding dark reactions. With the discovery of photosynthetic phosphorylation by Arnon (40), the proposal by Emerson was further substantiated. Arnon demonstrated with isolated chloroplasts from higher plants that light energy is used for esterification of inorganic phosphate, resulting in the production of the adenosine phosphate compounds.

As a result of studies on the assimilation of phosphorus by Chlorella pyrenoidosa, Alkholy (103) found two limits of phosphorus concentration within the individual cells, a maximum of 1.5×10^{-6} micrograms per cell and a minimum of 0.10×10^{-6} micrograms per cell. From this, it was concluded that the minimum value must be considered as more important, since growth cannot continue once this

value is reached. Kuhl (102) states that in nutrient solutions phosphorus is exclusively present as phosphate, and that the phosphorus content of Chlorella pyrenoidosa and Scenedesmus cells depends strictly on the concentration of phosphate in the medium. This cell phosphorus content consideration is the same for the diatoms Asterionella japonica and Asterionella formosa. Also stated was the fact that when phosphate is supplied in excess, the phosphorus content per cell remains constant. Osterlind (83) found from his studies on the growth conditions for Scenedesmus quadricauda that a phosphorus concentration in the growth medium of from 0. 10 to 10 ppm produced little change in growth activity. In the consideration of organic phosphorus utilization, Chu (104) indicates that dissolved organic phosphorus in the sea may also be absorbed and utilized by phytoplankton. On the other hand, Alkholy (103) states Chlorella pyrenoidosa does not consume organic phosphorus in the culture medium. In the case of phosphorus deficient cells, phosphate is absorbed before growth starts and there is always a lag phase associated with this activity (103). Other symptoms of phosphorus deficiency appear to be chlorophyll reduction and accumulation of fat, starch and cell wall materials, which indicate some interference with nitrogen metabolism (102).

It has been shown by many investigators that sewage, either untreated or treated, has a phosphorus content which is adequate to support the growth of algae and that algae are effective in reducing the phosphorus concentration in sewage (27) (2) (3) (5). Fitzgerald

(105) states that phosphorus levels were decreased by 96% through stabilization ponds, but concluded that most of the reduction was probably through precipitation resulting from the increased pH of the ponds rather than by algal absorption. From a study concerning stabilization ponds following a trickling filter sewage treatment plant, Nemerow (106) found that a phosphate reduction of 42% resulted from the actively growing algae in the ponds. Bogan, et al (3) describe phosphorus removals from secondary sewage plant effluent varying between 10 and 90% or more. The phosphorus was removed through the processes of bio-absorption and bio-precipitation which were governed by the activity of the algae. Pilot plant studies on the removal of dissolved solids from waste water by algae have been reported on by Bush, et al (4) and reveal a phosphate removal ranging from 10 to 68%. It is further indicated that the phosphate removal appeared to be influenced by the rate of waste flow. At low rates the algal activity appeared to be limited by phosphate availability and at the high flow rates the algal activity was increased through the greater availability of phosphate, which resulted in a greater removal of phosphate and other ions. Reid and Assenzo (28) described the removal of total phosphate from domestic wastes by attached algae through the use of a first order equation. They report a reaction rate (k) for total phosphate removal from sewage treatment plant effluent of 0.103 per hour. Witt (2) concludes that an almost complete removal of the phosphate in secondary sewage treatment plant effluent through

tertiary treatment with algae would require about 20 hours under optimum conditions. Studies of controlled algal cultures by Gates (5) show significant removals of orthophosphates from sewage treatment plant effluents. These studies also indicate that under nutrient limiting conditions the growth characteristics of the algae do not appear to be influenced by the orthophosphate concentration in the culture feed.

Micro-Nutrient and Auxiliary Growth Factors

Elements that are necessary in small or even trace quantities in order to insure the growth of algae are known as micronutrients. These elements include iron (Fe^{+2} or Fe^{+3}), manganese (Mn^{+2}), zinc (Zn^{+2}), copper (Cu^{+} or Cu^{+2}), molybdenum (MoO_4^{-3}), boron (BO_3^{-3}) and chloride (Cl^{-}), which are usually necessary. Very minute quantities of cobalt (Co^{+2}) and vanadium (VO_4^{-2}) also may be necessary for certain algal forms (31). Some forms of algae have very specific micronutrient requirements, such as the diatoms who need silica so as to build their silicious cell walls.

Iron is probably the most important of the micronutrient elements since it is part of the respiratory electron carriers called cytochromes and the oxidative enzymes peroxidase and catalase. In these enzymes the iron is located in the coenzyme group where it occupies the central atom position and is connected to four pyrrole rings which are joined by a large cyclic structure (31). The iron functions due to its reversible oxidative and reductive properties. The optimum

amount of iron necessary for growth appears to depend upon the algal species in concern. Iron concentrations of from 2.6×10^{-8} to 1.8×10^{-7} molar have been found adequate for the growth of Chlorella (107). Goldberg's (108) studies on iron assimilation by marine diatoms revealed that they utilized only particulate and/or colloidal iron as a growth nutrient, and the minimal content of iron per cell necessary for further cell division was 10×10^{-8} micromoles per liter. In nutrient requirement studies with Chlorella, Myers (109) used a normal iron concentration of 13.3×10^{-5} moles per liter, but found that the iron concentration could be varied 270 fold without noticeable changes in the growth and photosynthetic behavior of the organism. Zehnder and Gorham (51) obtained a maximum yield of the blue-green alga Microcystis aeruginosa in a medium with an iron content of 0.75 ppm. Due to the importance of iron within the metabolic pathways of algae, the most immediate symptom of iron deficiency is a retardation of growth and photosynthetic activity.

According to Galston (31) the other metallic micronutrients previously mentioned perform metabolic duties similar to that of iron by consisting as entire or as part of prosthetic groups or coenzymes. Copper, manganese and zinc probably function as iron does, since they all possess reversible oxidative and reductive properties. Copper is a part of certain oxidative enzymes such as tyrosinase and ascorbic oxidase. A copper concentration below 10^{-7} molar has been reported as being inadequate for cultures of

Chlorella (107). Manganese has been shown to be essential for various species of Chlorella, Ankistrodesmus, several diatoms and some algal flagellates (107). Also the ability to carry out the Hill reaction is completely suppressed by a manganese deficiency, but the ability can be immediately restored upon the addition of manganese to the growth medium. The role that manganese plays in algal metabolism appears to be very specific in the process of nitrate reduction (89). The occurrence of zinc in the enzyme carbonic anhydrase suggests that this metal may assist the photosynthetic mechanism at the level of carbon dioxide fixation (107). Symptoms of zinc deficiency, which appear to be decreases in dry weight, chlorophyll formation and photosynthetic activity, in Chlorella pyrenoidosa become apparent at concentrations below 10^{-7} molar (107).

Some of the other micronutrients which are necessary in lesser amounts and about which less is known are molybdenum, chloride, cobalt, vanadium and boron. Molybdenum appears to be involved in the functioning of the enzyme, nitrate reductase (89). According to Wiessner (107), a molybdenum requirement for a number of nitrogen fixing blue-green algae (Nostoc, Anabaena and Cylindrospermum) has been demonstrated. For Nostoc muscorum the minimum amount of molybdenum for nitrate reduction is 10^{-10} molar which is much less than the 10^{-7} molar concentration required for nitrogen fixation. In the case of green algae, the molybdenum deficiency symptoms of inhibited cell division and chlorosis appear

when the molybdenum concentration falls below 10^{-9} molar. The lack of molybdenum affects not only the reduction of nitrate by Anabaena cylindrica, but also the synthesis of proteins (89). Chloride was found to be necessary in the process of photosynthetic phosphorylation (41), but its exact role in this process has not been fully defined (31). The element cobalt has been shown to be necessary for the healthy growth of several blue-green algae and to stimulate the growth of the green alga, Scenedesmus obliquus (107). The exact metabolic role of cobalt is not fully understood, but it is an integral part of vitamin B_{12}, and its replaceability in growth media by vitamin B_{12} has been demonstrated. The necessity of vanadium in algal culturing is somewhat obscure. Wiessner (107) reports that in strong light the evolution of oxygen from a vanadium deficient culture of Scenedesmus obliquus was inhibited, but that the oxygen evolution was reactivated after a lag period by the addition of vanadate. From this it was suggested that vanadium may be involved in one of the dark reactions of photosynthesis. Galston (31) states that the metabolic role of boron in photosynthesis is completely unknown. It has been reported that the minimum amount of boron necessary for maximum growth of Nostoc muscorum is 0.9×10^{-5} molar and that the optimum concentration for Chlorella vulgaris is 0.5 milligram per liter (107).

One unfortunate problem that must be considered in connection with micronutrients is that their presence in a culture medium does not always insure their availability for use by the algae.

Many of the heavy metal micronutrients have a tendency to shift from soluble molecules to insoluble or colloidal materials and complexes; thus, making themselves unavailable for algal metabolism. Iron is of main concern since its ferric form tends to precipitate as a hydroxide or phosphate in alkaline environments. The organic chelating agents of citrate and EDTA (ethylenediamine tetracetic acid) have been shown to be quite useful in maintaining iron and other trace metals in solution (37). EDTA seems to be the more desirable chelating agent as it does not appear to stimulate bacterial contamination of algal cultures as citrate does. The effectiveness of the chelating agent is somewhat dependent upon the pH of the solution and whether or not competing complexing systems are present (86). In the University of Michigan Sanitary Engineering Laboratories, citrate has been used very successfully as an iron complexing agent in algal culturing studies using synthetic media, with the only problem being that bacterial contamination usually occurred in the cultures. The use of EDTA eliminated the problem of bacterial contamination, but unfortunately the algae did not grow either. The mechanism by which the complexed micro-element is released to the algal cell is somewhat obscure. Krauss (33) suggests that the chelates may release the elements by progressive dissociation; the chelates may be destroyed by such things as light energy and release the element or the whole complex structure might be absorbed and metabolized by the algal cell. He concludes by stating that further research in this area is needed.

Various organic growth factors have been shown to be necessary for algal growth, and Saunders (86) lists the organic growth factor requirements for a number of algae. Droop (110) states that of all the known vitamins and growth factors, only vitamin B_{12}, thiamine and biotin have been found to be of any general importance in algal metabolism. It is further indicated by Droop that there is no general correlation between vitamin requirements and heterotrophy in respect to major carbon or nitrogen sources. The majority of the algal species known to require vitamins are autotrophic in all other considerations, except that many are facultative heterotrophs in regard to their nitrogen source (110).

Consideration as to the requirements of micronutrients, chelating agents and other growth factors is necessary only when synthetic media are used for the mass culturing of algae or for the study of how various environmental changes affect algal activity. When considering the development of algal processes for the treatment of domestic wastes, it is not necessary to consider micronutrient additives, since according to current knowledge, untreated and treated domestic wastes contain all of the essential micronutrients and auxiliary growth factors necessary to support active algal growth.

Extracellular Metabolites

In the development of mass algal culturing processes in synthetic media, consideration is given to the reuse of the substrate

after harvesting the algae. As suggested by Gates (5), a working process for the bioextraction of algal nutrients from sewage treatment plant effluents might involve multiple exposure of the effluent to algal cultures with the algae being harvested between exposures. With the multiple use of the growth substrate in these two algal culturing processes, it is possible that the extracellular metabolites from the algae might reach a concentration where they will exert a harmful or beneficial influence on the algal activity.

Saunders (86) states that certain algae excrete organic materials that appear to act as toxins towards themselves and also other algal forms. It has been reported by Pratt (111) that under usual culturing conditions with Chlorella vulgaris, a growth inhibiting substance, which has been given the name chlorellin, accumulates to a concentration level that limits the maximum attainable algal density in the culture. It was also shown that when a culture, in which growth had stopped, was treated with Norite A, growth resumed again and extended the maximum attainable algal density towards a higher level. Based upon a review of Pratt's work, Myers (32) suggests that this algal secretion of a growth inhibiting material may be limited only to Chlorella vulgaris. Krauss (112) indicates that the discovery of the growth inhibitor chlorellin stimulated algal studies with other species of Chlorella, but no similar substances were detected. Auto-inhibition of growth, apparently due to an accumulation of specific extracellular metabolites in the growth medium, has been

reported for Nostoc punctiforme and Nitzschia palea (113). Chemical investigations into the characterization of chlorellin indicate that the active portion of the material is an organic base; that it is heat liable but probably not affected by oxidation and that it limits growth more effectively at high pH levels, indicating that the base form of the material is more active than the salt form (114).

It is known that when actively growing algal cultures are exposed to contamination, few bacteria ever develop, which may be due to the accumulation in the growth medium of extracellular products that exhibit anti-bacterial properties (113). Jorgensen and Nielsen (115) investigated the influence that filtrates from cultures of Chlorella vulgaris had on the growth of Staphylococcus aureus and found that both growth inhibiting and growth accelerating substances were present simultaneously in the culture solutions. It was also found that the growth inhibiting effects were inactivated by heating the filtrate at 70°C for 20 minutes, by illumination of the culture filtrate and by treatment of the filtrate with activated carbon. After inactivating the growth inhibitors, bacterial growth in the filtrates exceeded that in the controls which indicated the presence of bacterial growth accelerating materials. Fogg (113) states that pathogenic and coliform bacteria die out rather quickly in sewage stabilization ponds that have abundant algal growths, and suggests that this could possibly be due to the production of anti-bacterial substances by the algae. It is felt that the disappearance of these bacterial organisms through their

natural die-away is of more significance than the removal methods suggested by Fogg.

During normal growth, the blue-green alga, Anabaena cylindrica, secretes a polypeptide material which has the ability to form complexes with such ions as copper, zinc, ferric irons and phosphate (116). Fogg and Westlake (116) indicate that the chelating ability of the Anabaena polypeptide is of significant biological importance, since the complex formation between the polypeptide and cupric ion greatly reduces the toxicity of the copper towards the Anabaena cylindrica and also other algal forms present. They further state that the production of this polypeptide is greatest in young cultures but that the concentration level continues to increase as the culture ages. From this it can be seen that the chelating ability of extracellular metabolites from algae might be of additional ecological importance by maintaining necessary micro-elements in a form which is available to the algae.

III. EXPERIMENTAL FACILITIES AND PROCEDURES

ALGAL CULTURING FACILITIES

DEPTH STUDY CULTURING UNIT

For the depth study, the constant density culture unit designed by Witt (2) and described by Gates (5), was used. This unit consisted of two identical chambers in which the depth could be varied from 2.5 to 45 centimeters. The sides, bottom and division wall of this unit were constructed with black opaque plexiglas. Clear plexiglas was used to construct the front of the culturing unit and the movable back partition of each chamber.

Previous research indicated that a separate photometric control device, instead of a control chamber was satisfactory (5). As a result of this finding, both chambers were operated simultaneously under the same culturing conditions.

Culture depth was varied by moving the back partition of each chamber to the desired location, as illustrated in Figure 3. In order to have a water tight chamber, the movable partition was sealed in place with EC 612, a black adhesive compound produced by Minnesota Mining and Manufacturing Company.

The liquid volume in the culture chambers at each depth was established and maintained by a constant head overflow device.

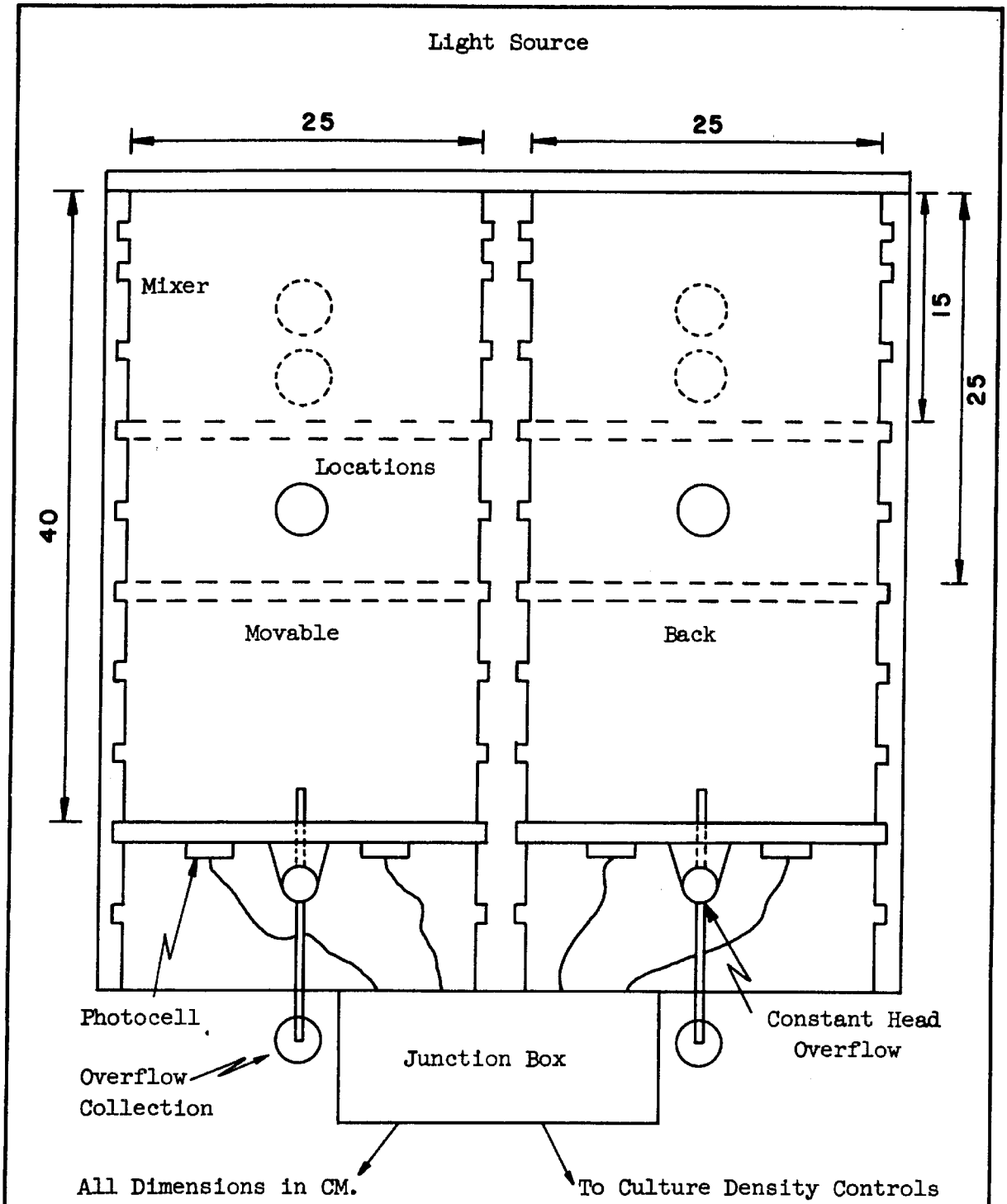


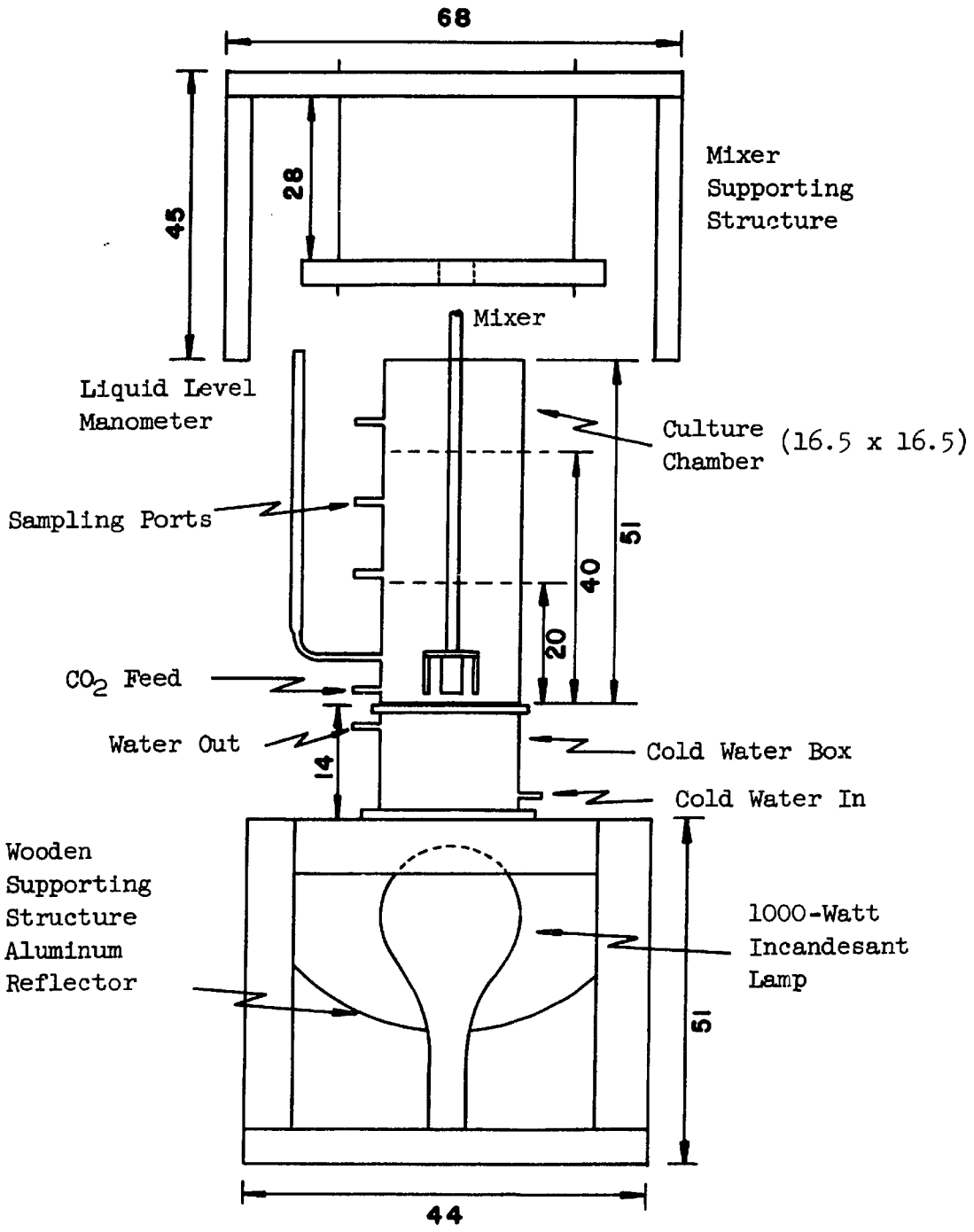
Figure 3. Liquid Depth Study, Algal Culturing Unit

This overflow facility was attached to and extended through the middle-third portion of the backs of the culture chambers.

BATCH CULTURING UNIT

The batch culture study was carried out in the culturing unit shown in Figure 4. The culturing chamber, which has the outside dimensions of 6-1/2 x 6-1/2 x 20 inches, was mounted on top of a water box with dimensions of 6-1/2 x 6-1/2 x 4-3/4 inches. Both the culturing unit and the sides of the water box were constructed out of 1/4 inch thick clear plexiglas, with top and bottom of the water box being 1/2 inch thick clear plexiglas. The bottom of the water box, being 8 inches square, rested on two metal angles which were part of the wood frame structure that supported the complete culturing unit above the light source. The light source was centrally located below the culturing unit, with the bottom of the water box approximately 4 inches above the top of the incandescent bulb.

Heavy black paper covered the outside of the culture chamber, so that the only culture surface illuminated was the 21.2 square centimeter bottom surface of the chamber. The sides of the water box were covered with aluminum foil and an aluminum foil reflector was placed around the base of the light source in order to concentrate and reflect all the light possible upwards towards the culture chamber.



All Dimensions in CM.

Figure 4. Batch Algal Culturing Facility

Distilled water was pumped from a constant head reservoir through a refrigeration unit into the bottom of the water box and then discharged at the top of the water box back into the reservoir. There was a tendency for air bubbles to accumulate at the top of the water box and these were periodically expelled to the reservoir. Culture liquid depths were established and maintained with an adjustable liquid manometer connected to the culture chamber.

LIGHT SOURCES

In general, the design of the light source used in the culture depth study was patterned after that of Witt (2). It consisted of 12, 110 watt "Gro-Lux" fluorescent lamps spaced 2 inches center to center. Light intensity at the face of the culture chambers was altered by increasing or decreasing the number of lamps in operation, and by changing the distance between the face of the culturing unit and the light source. Each of the six switches on the light source controlled the operation of two lamps. The back board and interior of the supporting structure for the light source were lined with aluminum foil, so as to reflect all possible light from the lamps towards the culturing unit. Figure 5 pictures the location of the light source with respect to the culturing unit.

The "Gro-Lux" fluorescent lamp was developed by the Sylvania Corporation as an artificial source of illumination for the stimulation of plant growth. According to the manufacturer, these

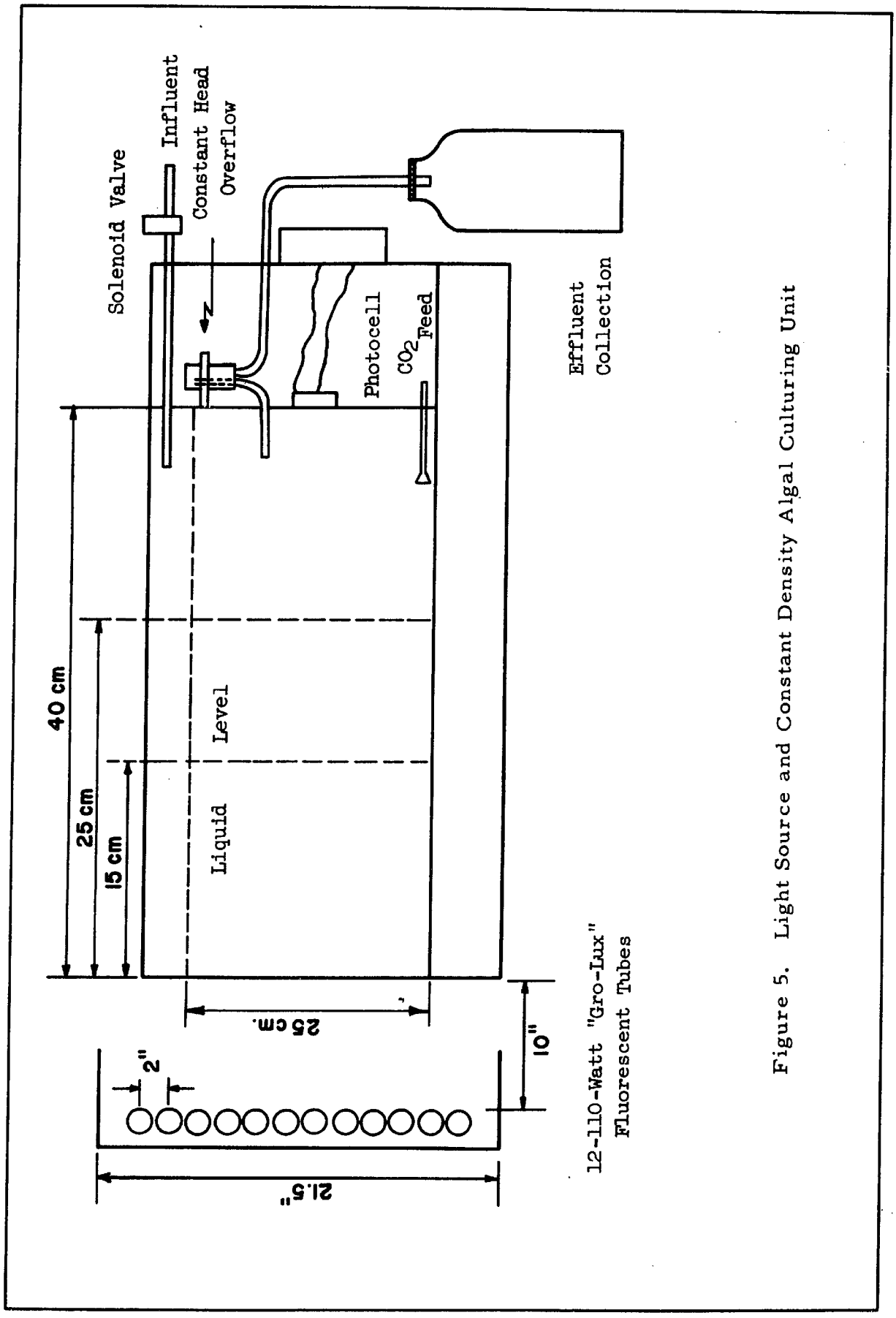


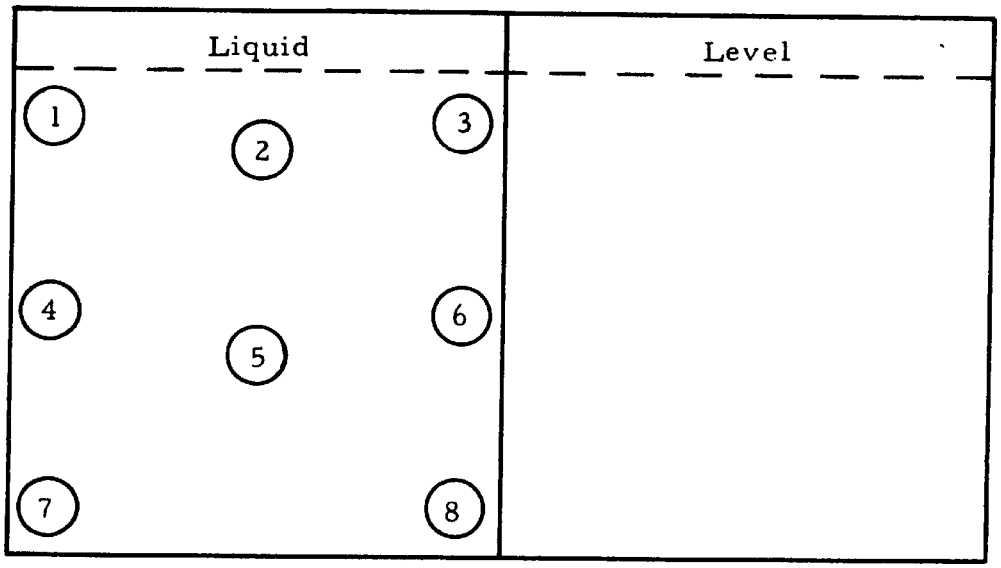
Figure 5. Light Source and Constant Density Algal Culturing Unit

lamps contain a blend of two high energy phosphors which produce the proper proportions of blue and red light for effective photosynthetic stimulation. As shown in Appendix I, the spectral energy distribution for the "Gro-Lux" lamps falls between the wavelengths of 400 and 700 millimicrons, with peaks at 450 and 660 millimicrons. This spectral distribution corresponds very close to the light absorption characteristics of chlorophyll. Also, as part of Appendix I is a table of the relative amounts of photosynthetically effective light energy produced by various fluorescent lamps. With the warm white lamp as a base, it can be seen that the "Gro-Lux" lamp produces well over twice the total effective light energy as does the warm white lamp.

Light intensities obtained at the middle of the chamber faces ranged from 800 to 2500 foot candles, depending on the number of lamps in operation. Table I summarizes the light intensity distribution over the face of the constant density culturing unit for the "Gro-Lux" fluorescent light source. As the lamps began to age, the desired intensities were maintained by moving the culturing unit closer to the light source.

The light source utilized in the short duration batch culture study was a 1000 watt incandescent lamp. This source was located approximately 8 inches from the illuminated culture surface, as shown in Figure 4. After the light passed through a cooled water box for the purpose of attenuating the unnecessary infrared light energy, an intensity of 5000 foot candles was obtained at the culture surface.

FACE OF CONSTANT DENSITY CULTURING UNIT



LIGHT INTENSITY DISTRIBUTION

(Intensity in Foot-Candles)

No. of Lights	Light Reading Position							
	1	2	3	4	5	6	7	8
4	650	700	780	710	800	820	650	700
6	1200	1200	1400	1350	1350	1300	900	1000
8	1500	1600	1600	1600	1750	1700	1500	1500
10	2200	2300	2500	2050	2200	2250	1750	1850
12	2400	2600	2600	2400	2600	2600	2350	2400

Table 1. Light Intensity Distribution Over Face of Constant Density Culturing Unit

CULTURE DENSITY CONTROLS

For the culture depth study, the culture density controls used were the same as those used and described by Witt (2) and Gates (5). After a culture depth had been established and the proper dry weight concentration determined so as to give the desired depth-density factor, the culture was allowed to concentrate to the desired density. When this density was attained, the percent transmission at 425 millimicrons was determined, and this value was then used as the standard upon which to base the control adjustments. At the start of each specific depth study, the photometric controls were adjusted, using neutral filters of varying densities to approximate the density of the algal culture in front of the control photocell. The fine adjustments of the controls were made with the micro-potentiometers incorporated in the electrical control circuits. Extremely complete diagrams of the constant density, electrical control circuitry can be found elsewhere (2) (5).

When the balanced control circuits became unbalanced, due to increased algal density, fresh substrate was introduced into the cultures by gravity flow through actuated solenoid valves to reestablish the desired culture density. The fresh substrate was introduced above the liquid level and fairly close to the mixer shaft, so as to provide an instantaneously complete diffusion of the added substrate in the culture liquid, in order to prevent over dilution of the culture. The mixing conditions in the 15 and 25 centimeter

cultures were sufficient to provide relatively immediate control system response to the addition of substrate. With the 40 centimeter culture, the mixing conditions were such that there was enough delay in the control system's response to added substrate, so that over dilution of the culture was common.

CONTINUOUS pH CONTROLS

The pH of the constant density cultures was continuously monitored by the same control devices as described and illustrated by Gates (5). The controls were set so as to maintain the pH of the culture liquid within the range of 7.5 to 8.0. Whenever the pH increased above this range, the control systems were actuated so as to allow 100% carbon dioxide to diffuse into the culture liquid through finely fritted glass diffusers and reestablish the pH within the desirable range. The carbon dioxide cylinder was equipped with a pressure regulating gage that controlled the outlet pressure at 15 pounds per square inch during the first part of the study and then at 20 pounds per square inch for the remainder of the study.

Soon after the culture depth study started, it was noticed that as carbon dioxide entered the culture liquid, the bubbles flowed up in front of the photocells mounted at the back of the culture chambers and caused the density controls to be activated. This actuation of the controls usually resulted in unnecessary dilution of the cultures. The problem was satisfactorily corrected by extending

the fritted glass diffuser, which came through the lower back of the culture chamber, out closer to the mixer and also increasing the outlet pressure at the carbon dioxide cylinder to 20 pounds per square inch. With this new arrangement, the incoming carbon dioxide was more quickly diffused throughout the culture liquid resulting in little to no unnecessary culture dilution.

During the batch culture studies, the 100% carbon dioxide was continually added to the culture liquid in order to maintain the pH at approximately 8. 0. Daily pH readings were taken with a pH meter and the necessary carbon dioxide feed adjustments were made with a fine needle valve in the feed line. The carbon dioxide entered the culture just above the culture face and close to the stirrer, so that quick diffusion of the carbon dioxide was accomplished.

TEMPERATURE REGULATION

The culturing units were housed in a constant temperature room. Temperature controls were adjusted every time the culture density or the light intensity were changed. Throughout the studies the culture temperatures were maintained within a range of plus or minus 2°C around the desired temperature of 20°C. During the periods of higher light intensity, it was necessary to strategically locate and operate electrical fans to move cooled room air around the culturing units, in order to maintain the desired temperature. For the depth studies three fans were positioned behind the culturing

unit and one fan was located to move air across the light source.

Auxiliary temperature control for the batch culture study was provided by recirculating water through a water cooler and a water chamber located between the light source and the culture chamber. Also a fan was positioned to move cool room air across the top of the light source.

MIXING FACILITIES

Individual mixing facilities were provided in each culture chamber. Turbulent mixing was maintained in the culture chambers during the depth study by a four blade clear plexiglas impeller mounted at the end of a stainless steel shaft and impelled by a high pressure air driven hand drill. The mixing shafts were located in the approximate center of the chambers as shown in Figure 3, and were slightly inclined towards the front of the chamber so as to maintain vortexing around the shaft at a minimum. The bottom of the impellers was situated about 3 inches above the bottom of the chambers. The air mixers, which were located above the culture chambers, were operated at a speed of approximately 1000 revolutions per minute throughout the culture depth study.

For the batch culture study, a similar mixing facility was used. A smaller six blade, clear plexiglas impeller mounted at the end of a brass shaft maintained turbulent mixing in the chamber. Again the impeller was located in the center of the chamber with its

bottom approximately 2-1/2 inches above the illuminated surface of the chamber, as shown in Figure 4. The air mixer speed was maintained around 1500 revolutions per minute throughout the batch culture study.

CULTURING SUBSTRATE

The substrate used for the culture depth study was Ann Arbor Sewage Treatment Plant effluent. Ann Arbor's sewage treatment plant provides complete treatment utilizing the activated sludge process for secondary treatment. The effectiveness of sewage and sewage treatment plant effluent as algal growth media has been well established (2) (5) (27).

Approximately 50 gallons of effluent were collected from the treatment plant at a time and returned to the laboratory in 5 gallon containers for processing. Effluent processing consisted of filtering through glass wool to remove undesirable particulate matter and steam sterilization. Sterilization was performed in stainless steel pails for 1 hour at a temperature of 121^{o}C. The effluent sterilization was necessary so as to minimize chemical quality changes that would have resulted due to bacterial activity in unsterilized effluent. The processed effluent was stored in a 50 gallon plastic container located in the algal study constant temperature room.

Based upon chemical analyses before and after sterilization, the chemical quality of the effluent was reestablished by

adding appropriate amounts of reagent grade chemicals. The chemicals used were ammonium chloride, sodium bicarbonate, potassium phosphate and sulfuric acid. Throughout the culture depth study, the total inorganic nitrogen (TIN) concentration was in the concentration range which, according to Gates (5), does not influence algal growth. A representative chemical analysis of the Ann Arbor Sewage Treatment Plant effluent before and after sterilization appears in Appendix II.

An artificial substrate was used in the batch culture study. The contents along with their concentrations in this artificial substrate are listed in Appendix III. The desired quantity of substrate was prepared with fresh distilled water and sterilized in the aforementioned stainless steel pails for one hour at a temperature of 121°C. Following sterilization the medium was immediately cooled to redissolve the flocculant material that tended to form during sterilization. Then the medium was placed in the batch culturing chamber and inoculated with the algal culture that was being investigated.

ALGAL ORGANISMS

During the culture depth study a dominant algal culture was maintained. The dominant alga was a blue-green, filamentous form that originally had been identified, on a tentative basis, as Oscillatoria rubescens by Dr. A. M. Beeton of the Civil Engineering Department at the University of Michigan (5). Just recently, this filamentous algal form has been positively identified as Schizothrix

calcicola (Ag.) Gom. by Dr. Francis Drouet, who is associated with the Academy of Natural Sciences in Philadelphia. This Schizothrix form was originally isolated from the Ann Arbor Sewage Treatment Plant effluent. The major contaminating alga during this study was a Scenedesmus form, and during brief intervals amounted to as much as 30% of the total algal count. Other minor contaminants were a Chlorella form and some diatoms. For most of the 25 centimeter depth study, the diatoms contributed quite significantly to the total algal count, indicating that the lighting conditions at this depth were favorable for diatom growth, since lighting in the culture was essentially the only environmental factor that changed with depth.

The alga used in the batch culture study was Chlorella pyrenoidosa which was originally obtained from the algal culture collection maintained at the University of Indiana by Dr. Starr. This organism was maintained on proteose agar slants in the laboratory. An inoculum for the study was obtained by aseptically transferring a significant quantity of algae from the slant culture into a liter of sterilized inorganic media with a microbiological transfer loop. The liquid inoculum culture, contained in a 1-liter Florence flask, was allowed to grow in a constant temperature water bath provided with diffused air agitation and continuous fluorescent lighting. When the inoculum culture density reached a level that would provide an initial concentration of approximately 1×10^6 organisms per milliliter in the batch culturing unit, the inoculum was transferred

to the culturing unit and the study commenced. About 50 milliliters of the inoculum were retained and added to a fresh flask of media. The inoculum culture was maintained in pure culture form and in an active growing state by periodically transferring a small volume of the culture into fresh media.

ANALYTICAL TESTS AND PROCEDURES

PHYSICAL

Mixer Speed

The speed of the high pressure air driven mixers was evaluated with a stroboscope, capable of measurements within a range of 250 to 18, 000 revolutions per minute. Line air pressure fluctuated quite significantly at the beginning and end of the working day and remained relatively constant on week-ends. Due to this pressure fluctuation, mixer speed adjustments were necessary at the times of air pressure variation. Also the desired mixer speed was verified on the average of twice daily at times other than those already mentioned.

Incident Light Intensity

Light intensities incident to the face of the culturing units were determined with a Weston Model 756 light meter and a Weston

Illumination Target with a viscor filter. The target with viscor filter being sensitive to incident light within the wavelength range of 420 to 710 millimicrons. As the light passed through the plexiglas fronts of the culturing units, the intensity at the liquid face was reduced to approximately 0. 90 of the intensity incident to the outside face of the culturing units.

Line voltage fluctuations, that significantly altered the incident light intensity, occurred at the beginning and end of the working day. Between these critical times and throughout the week-ends the light intensity remained relatively constant. Light intensity readings were taken at the center of the chamber surface during the morning and evening of each day. The incident light intensity associated with each phase of the culture depth study is the average of all intensity readings taken throughout that specific phase of the study.

Algal Density

The density of the algal cultures was determined daily by four different techniques. The four techniques were; (1) dry weight concentration, (2) per cent transmission, (3) microscopic algal particle counts with the hemacytometer counting chamber and (4) electronic particle counts with the Coulter Counter.

Dry weight concentrations were evaluated with the use of Millipore Filter Type RA and the standard Millipore vacuum

filtration apparatus. The Type RA filter membrane, with a pore size of 1.2 ± 0.3 microns, assured complete removal of algae from the algal suspensions. Prior to use, the filters were dried at 103°C for 24 hours and then stored in a desicator. The analytical procedure used consisted of removing a filter from the desicator, weighing the filter on an analytical balance, filtering a known volume of suspension through the filter and then drying the filter plus algae at 103°C for 24 hours. At the end of the drying period, the filter plus algae were removed from the oven, cooled in a desicator and again weighed. The increase in filter weight was the dry weight concentration of the algal suspension, expressed as milligrams of algae per liter of culture (mg-algae/liter-culture). A control filter was not used with each determination, since laboratory experience indicated that a control was not necessary. The dry weight concentration associated with each culture depth is the average of all dry weight determinations made throughout each culture depth study. This average value was then used to determine the depth-density factor for each culturing condition. During the culture depth study, samples for the dry weight determination were collected from the culture overflow and the filtrate produced was used for the chemical determinations. For the batch culture study, samples were collected from the culture chamber and the filtrate was returned to the chamber.

Per cent transmission (%T) of monochromatic light through the various algal suspensions was determined with a

Coleman Model 14 spectrophotometer. For the culture depth study a 20 millimeter light path was used and for the batch cultures a 13 millimeter light path. All per cent transmission readings were made at a wavelength of 425 millimicrons. The selection of this wavelength was based on laboratory experience, and the successful use of this wavelength during previous research (5). Once the desired dry weight concentration of the algal suspension was attained, the approximate per cent transmission was determined and then used as the basis for the adjustment of the constant density controls throughout each specific phase of the culture depth study. Transmission readings of the culture liquid and culture overflow were recorded daily. Also, at various other times transmission readings were made for the purpose of monitoring the effectiveness of the constant density controls. The procedure used was that of first pouring the algal suspension back and forth between a beaker and the spectrophotometer cuvette two or three times, and then immediately placing the cuvette in the spectrophotometer and reading the per cent transmission. It is felt that with this procedure, the condition of the algal suspension in the cuvette very closely approximated that in the culture chambers.

Microscopic counts of algal particles were made daily, utilizing the hemacytometer counting chamber and a magnification of 645. Five fields were counted, with a field constituting the central 1 square millimeter area of the counting chamber. A fresh drop of algal suspension was used to fill each

counting chamber of the hemacytometer. In the recording of the micro-counts, the total number of dominant and secondly dominant algal particles were recorded with the total number of algal particles remaining being listed as other algal particles. The sum of the three micro-counts was termed the total micro-count. The ruled surface of the counting chamber is 0.10 millimeter below the cover glass, so the volume of algal suspension over the counting field is 0.10 cubic millimeter. Based on this volume characteristic of the hemacytometer, the total micro-counts are expressed as algal particles per milliliter. .

Total particle counts were made daily with the Coulter Counter. Basically this counter is an electronic instrument that is able to evaluate the number and size distribution of microscopic particles that are suspended in an electrolytic solution. The theory of the Coulter Counter and its application to the counting of algal particles have been amply described by Gates (5). Samples for the Coulter counts were taken from the culturing chambers. The operational procedure used was similar to that described elsewhere (5). One milliliter of the sample or one milliliter of an appropriate dilution of the sample was diluated to 50 milliliters with electrolyte solution using a 24 cubic centimeter automatic pipetter and 1 milliliter of distilled water. A blank solution, which lacked the milliliter of sample or sample dilution, was counted each time before a sample count was made. By counting the blank first, this count was not influenced by the much larger sample count. Counts were made at various pre-determined

threshold settings and at the end of each phase of the depth study an average series of counts was calculated from the gathered data. The average sample counts were corrected for coincidence using the Coulter correction table, and the average blank counts were subtracted from their corresponding corrected sample counts. The blank corrected counts multiplied by the dilution factor furnished the total particles per milliliter of original sample, at the various threshold settings. The total counts were then graphed as a function of lower threshold setting as shown in Figures 6 and 7. The total count at the point of curve direction change in Figure 6 and the total count at the point where a projected tangent to the curve plateau intersects the vertical axis in Figure 7 were taken as the total particle counts per milliliter of original sample. The Coulter counts were made for the purpose of evaluating whether or not the changing environmental conditions had any affect on the size distribution of the algae in the depth study cultures. Based upon experience and prior research, the counter settings for counting the batch Chlorella cultures were: amplification control, 1/8; aperature current, 1/4; and lower threshold, 40. The same corrections were made in evaluating the total particles per milliliter of Chlorella culture. Throughout both studies, there was little to no agreement between the Coulter Counter counts and the micro-counts, which is in agreement with previously reported results (5).

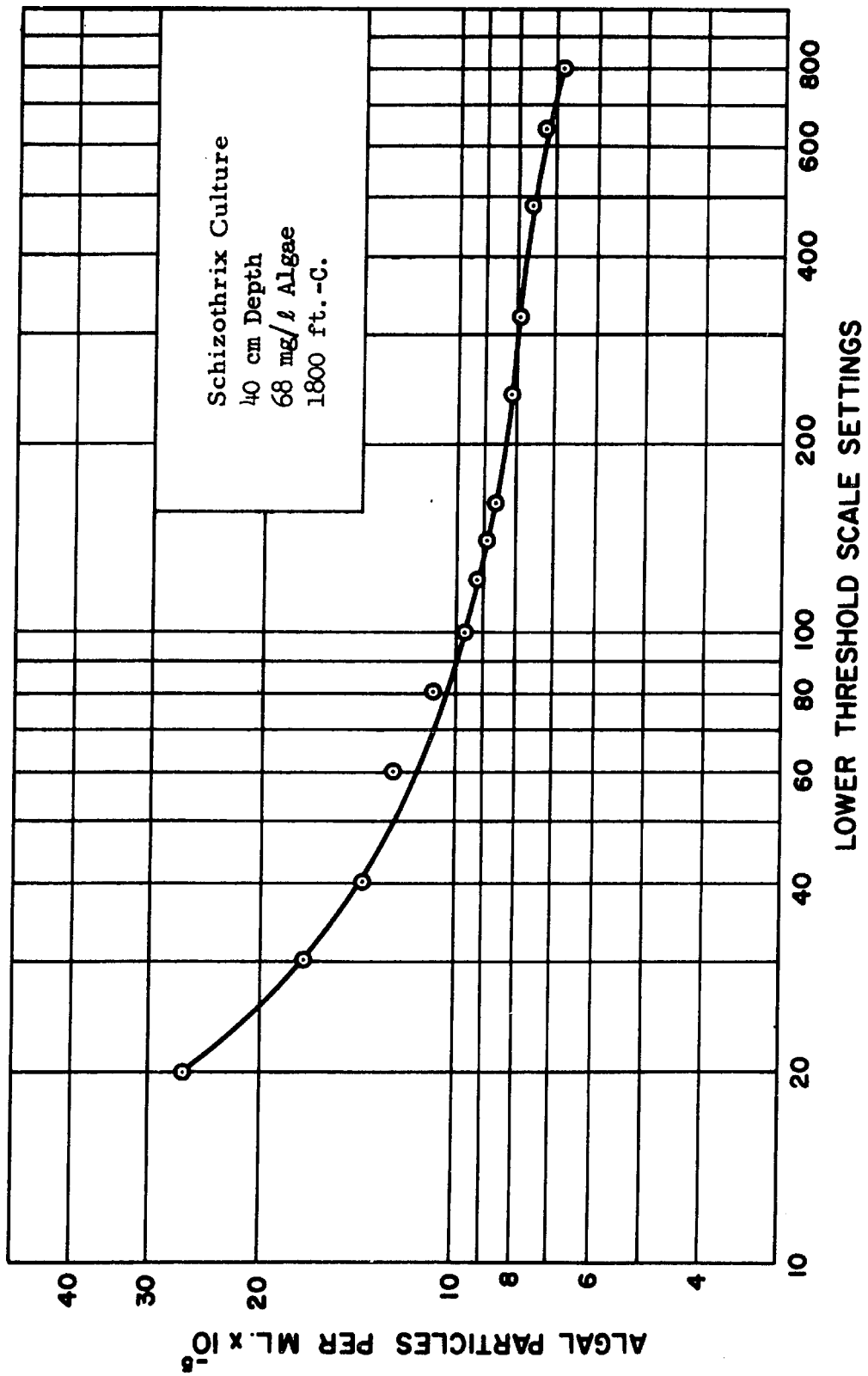


Figure 6. Algal Particle Counts as a Function of Coulter Counter Lower Threshold Settings at 1/4 Amplification and 1/8 Aperature Current

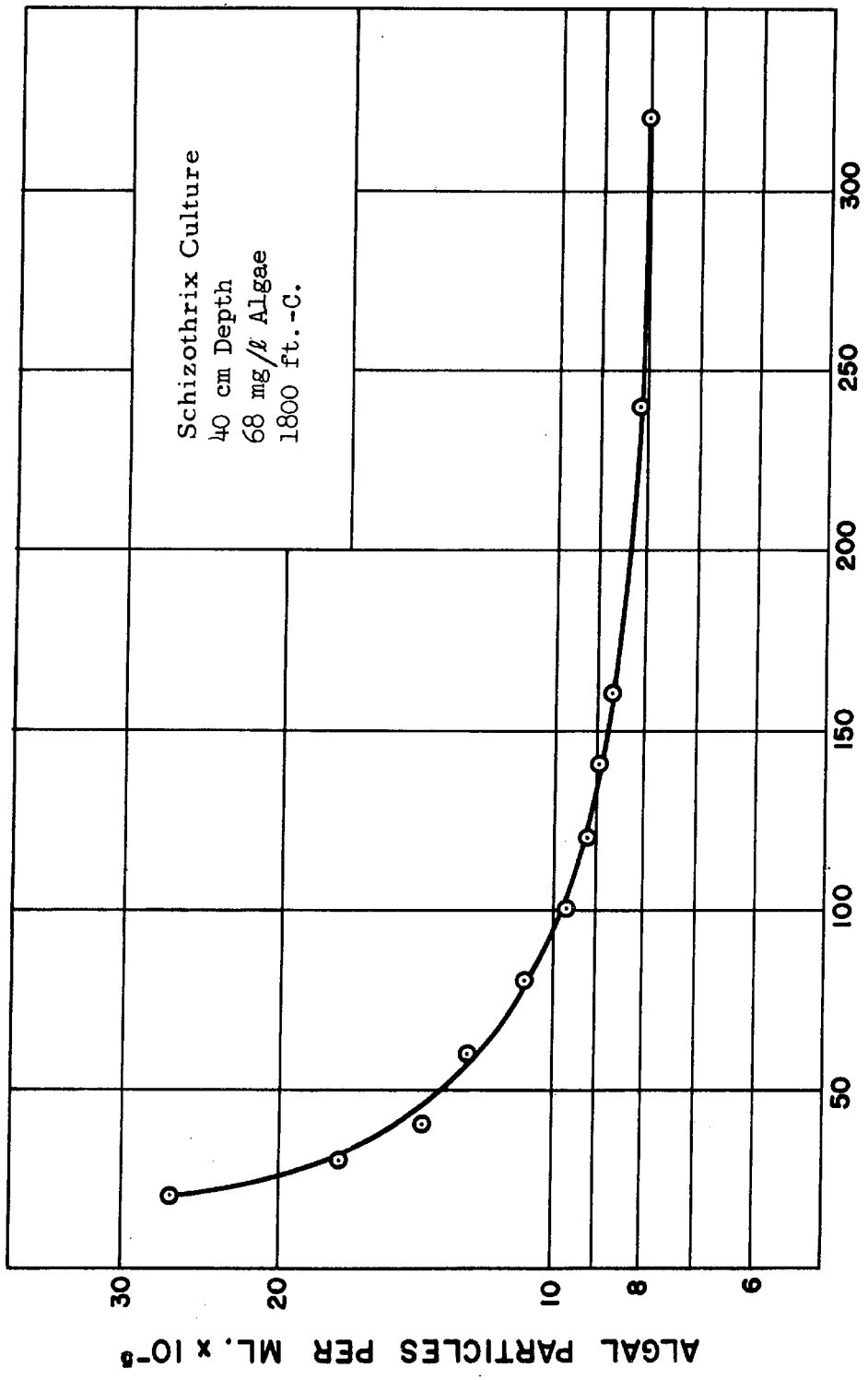


Figure 7. Algal Particle Counts as a Function of Coulter Counter Lower Threshold Settings at 1/4 Amplification and 1/8 Aperature Current

Rate of Culture Growth Activity

Evaluation of the culture growth rate or reaction rate was done by the methods of Phillips and Myers (34), Witt (2), and Gates (5). Under conditions of exponential growth, the rate, K, may be expressed as

$$K = \frac{1}{N} \frac{dN}{dt} \qquad (1)$$

where dN/dt is the increase in algal cell number per unit of time and N is the cell number. During the culture depth study, steady-state culturing conditions were maintained; so the algal density in the culture overflow was the same as that in the culturing chamber. Because of the steady-state conditions, equation (1) can be modified to read as follows (34)

$$K = \frac{1}{V} \frac{\Delta V}{\Delta t} \qquad (2)$$

where V is the constant culture volume and $\Delta V/\Delta t$ is the rate of overflow accumulation. The final operational equation that was used to evaluate the growth rate was

$$K = \frac{24}{V} \frac{\Delta V}{\Delta t} \qquad (3)$$

where 24 is the number of hours per day. The growth or reaction rate, K, was expressed as milliliter per milliliter of culture per day which is numerically equal to $\log_e$ units per day.

Overflow from the steady-state cultures was continuously collected in carboys. Periodically the overflow volume was measured

and the volume and time of measurement recorded. At the end of each phase of a specific depth study, the cumulative overflow was graphed as a function of time as shown in Figure 8. The slope of each curve is the $\Delta V/\Delta t$ used in equation (3) for the evaluation of K.

In the case of the batch culture studies the growth rate was evaluated by graphing the algal density increase on the logarithmic scale of semi-logarithmic paper as a function of time. The slopes of these curves were used for the final calculation of the growth rate in the following manner. Equation (1) can be rewritten as

$$\frac{dN}{N} = Kdt, \text{ then}$$

through integration it becomes

$$\log_e \frac{N_2}{N_1} = K(t_2 - t_1) \qquad (4)$$

or

$$\log_{10} \frac{N_2}{N_1} = K_{10}(t_2 - t_1) \qquad (5)$$

where N_2 is the algal density at the end of the time, t_2; N_1 is the algal density at the time, t_1; and $K = 2.303K_{10}$ Equation (5) rewritten as

$$K_{10} = \frac{1}{(t_2 - t_1)} \log_{10} \frac{N_2}{N_1} \quad \text{is}$$

the slope of a straight line relationship on semi-logrithmic paper. Both algal counts and dry weight concentrations were used in the evaluation of the batch culture growth rates, with the rates being finally expressed as $\log_e$ units per day.

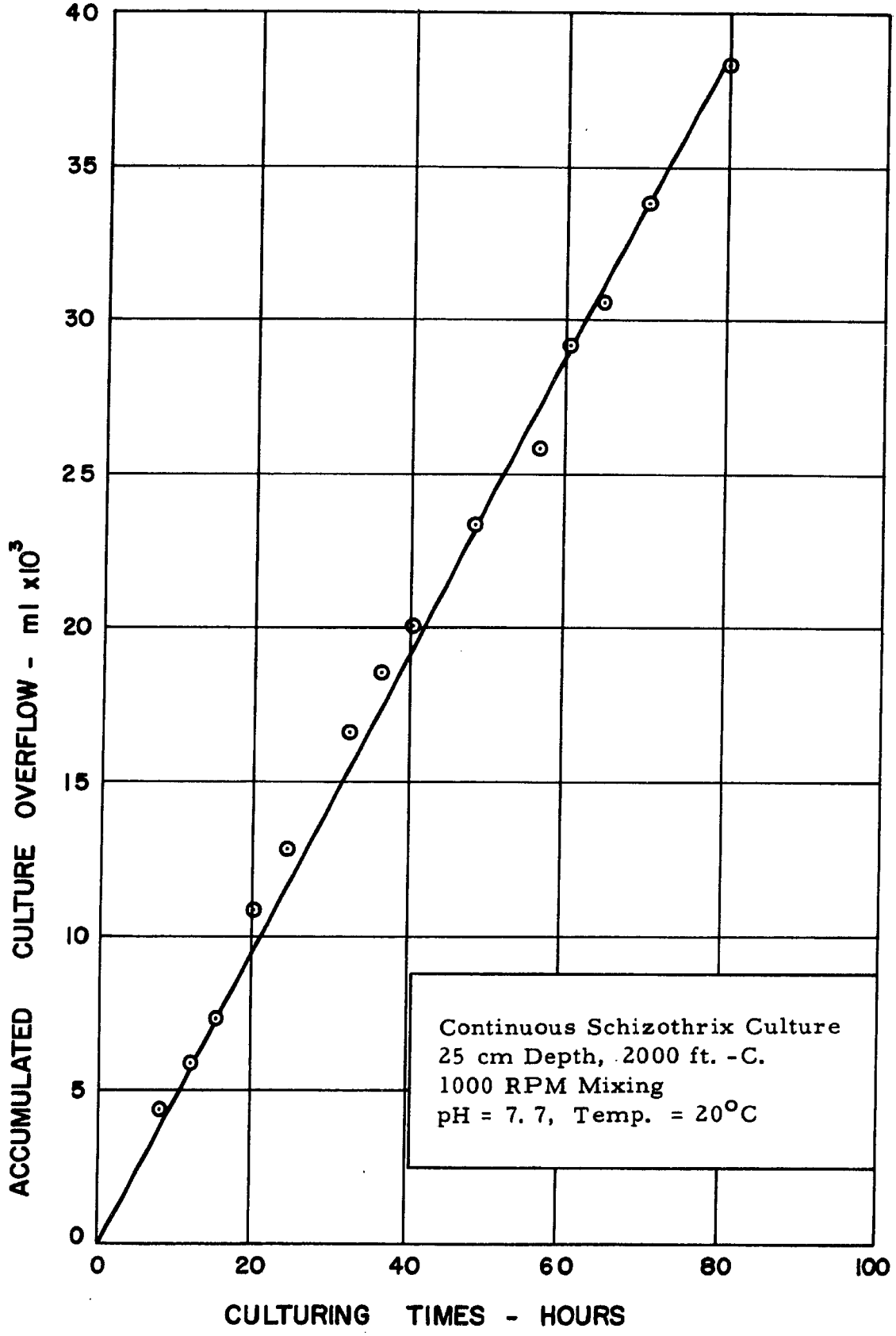


Figure 8. Accumulated Culture Overflow as a Function of Culturing Time

CHEMICAL

pH Determinations

Daily pH determinations were made on samples of the influent sewage to the constant density cultures and upon the culture filtrate obtained from the dry weight determination. Also, daily pH check readings were taken on the culture liquid for the purpose of evaluating the accuracy of the continuous pH controls. In the case of the batch cultures, pH readings were made on the culture liquid to determine whether or not the continuous carbon dioxide addition was satisfactory. The pH readings were the only chemical tests performed on the batch cultures. All of these pH readings were made with a line voltage operated pH meter that was standardized against a pH 7 buffer solution daily.

Alkalinity

Total alkalinity determinations were made daily on the influent substrate to the constant density cultures and on the culture filtrates from the dry weight determinations. The total alkalinity procedure described in Standard Methods (117) was employed along with a line voltage operated pH meter to detect the titration end-points.

Nitrogen

Ammonia-nitrogen, nitrite-nitrogen and nitrate-nitrogen determinations were performed daily on the samples described

in the alkalinity paragraph. The nitrite and nitrate determinations followed Standard Methods procedures (117), but the modified ammonia determination used in previous research (2) (5) was again used during this study. The exact nitrogen test procedures used are listed in Appendix IV. In these tests volumetric glassware and techniques were used so as to obtain accurate results.

Phosphate

Daily ortho-phosphate determinations were made on the samples mentioned in the previous two paragraphs. A slight modification of the Standard ammonium-molybdate procedure was used with good success. The exact procedure is described in Appendix IV.

The nitrogen and phosphate determinations were colorimetric. A Coleman Model 14 Spectrophotometer was used to evaluate the intensity of the developed colors, with the final concentration results being obtained from laboratory prepared standard curves. The validity of these standard curves was periodically checked by the analysts.

IV. EXPERIMENTAL OBSERVATIONS AND DISCUSSION

The experimental portion of this total algal culture investigation was divided into three major categories; batch culture studies, culture liquid depth studies, and culture pH studies. As for the reporting and discussing of the experimental observations, the same major categories are followed with the observations from the culture liquid depth studies sub-categorized into algal culture growth activity and algal culture nutrient extraction. The observations are presented through graphical illustrations, which are supplemented by narative description and discussion. In many cases the observations from the various experimental categories are inter-evaluated, so as to develop more representative conclusions.

BATCH CULTURE STUDIES

Batch algal culture studies were carried out for the purpose of evaluating the influence that an intense light source has on the growth characteristics of algae in cultures of different depths. The algal form cultured was Chlorella pyrenoidosa and the growth medium used is described in Appendix III. Culture liquid depths of 20 and 40 centimeters were used, and these provided culture volumes of 4.64 and 9.28 liters, respectively. Each culture was started with an organism concentration of around 2.5 millions per milliliter, which gave a dry weight concentration of approximately 42 milligrams per

liter. Daily Coulter Counter determinations of the organism concentration and millipore filter dry weights were made, in order to obtain sufficient data, so that the algal growth patterns under the different culturing conditions could be adequately described.

Prior to obtaining a sample from the culture chamber each day, the mixer was stopped and the liquid loss due to evaporation was made-up with distilled water. Depending upon which study was in effect, the liquid loss was accurately returned by reestablishing the manometer liquid level that corresponded with either the 20 or 40 centimeter depth. In addition to this volume adjustment, the sample remaining after the analytical determinations were completed plus the filtrate from the dry weight determination were returned to the culture chamber. All of these precautions were taken so that the data collected could be related to a constant culture volume.

Figures 9 and 10 illustrate the pattern of algal organism concentration increase as a function of time. As can be seen from these illustrations, the algal organism increase followed a typical logarithmic growth pattern, with the increase taking place at an ever decreasing rate, toward an eventual maximum organism concentration. In the case of the 20 centimeter culture, the initial lag phase was somewhat longer than that for the 40 centimeter culture. Under both culturing conditions the first phases of logarithmic growth activity were approximately the same, and in both cultures this initial log phase lasted about 1 1/2 days. In the 20 centimeter culture, a second log phase, with an activity of

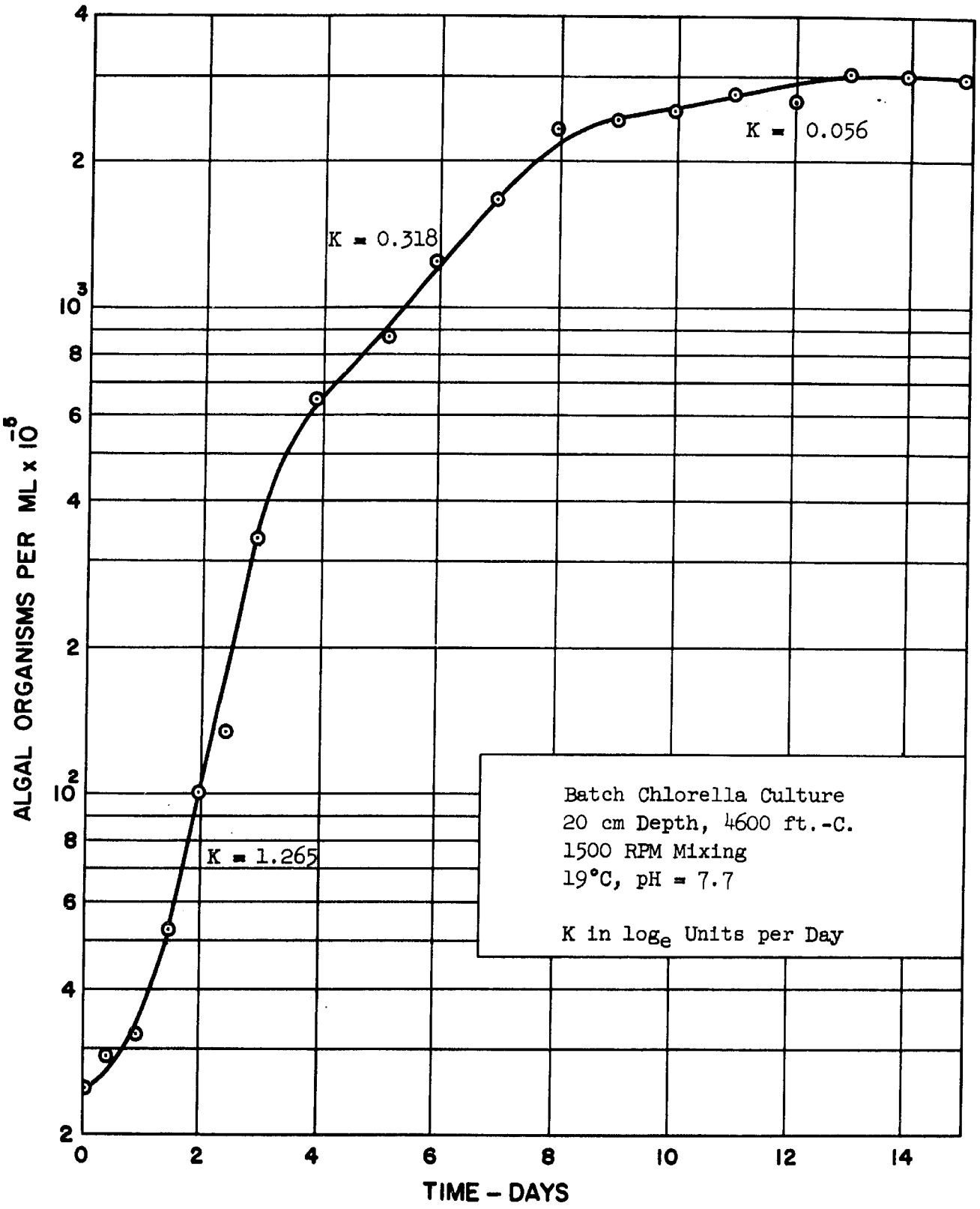


Figure 9. Pattern of Algal Organism Increase for a Batch Chlorella Culture as a Function of Time

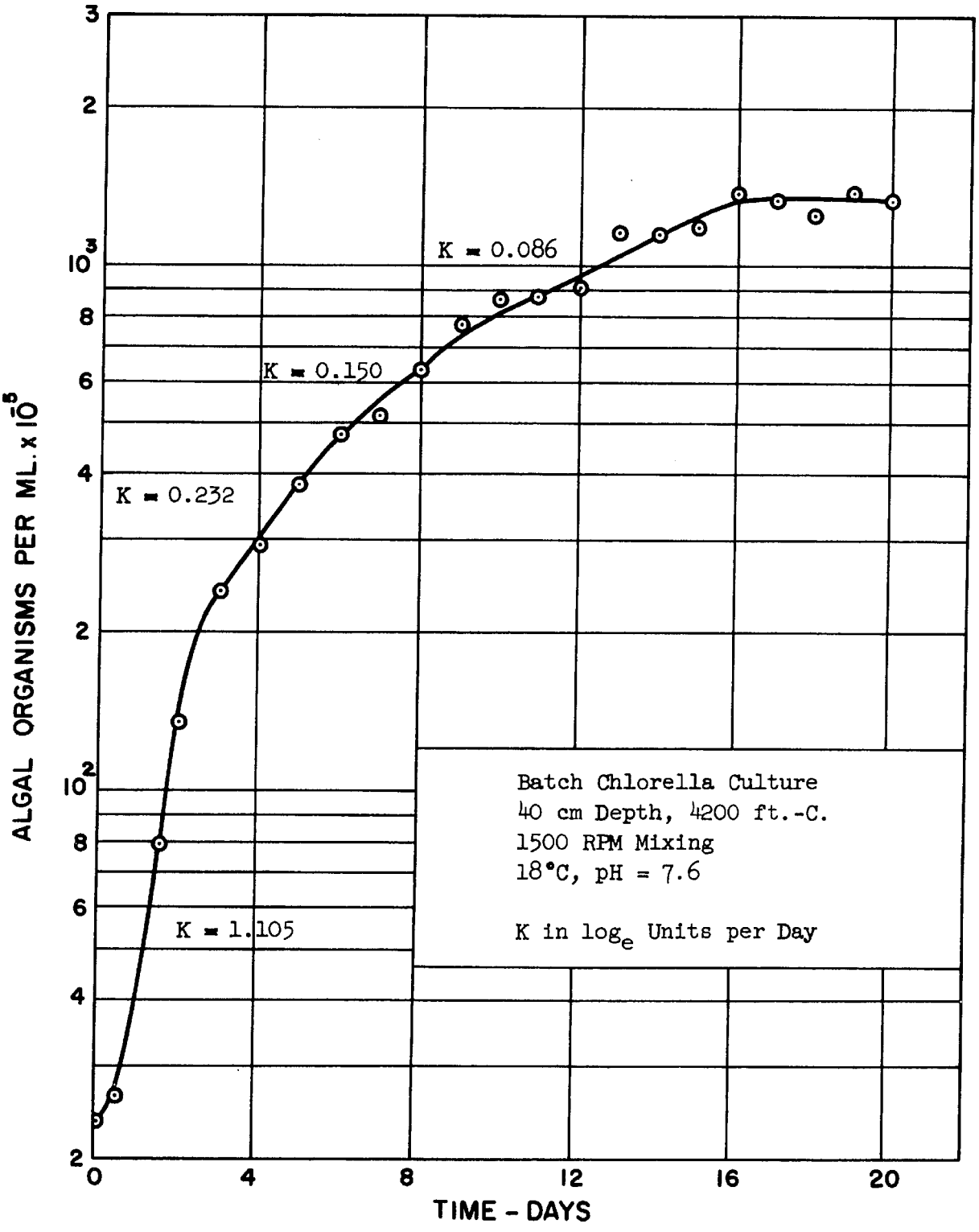


Figure 10. Pattern of Algal Organism Increase for a Batch Chlorella Culture as a Function of Time

approximately one-fourth of the initial activity, lasted for about 4 days. A third log phase followed with a much lower activity and ended at the level of maximum organism concentration. The maximum organism concentration attained in the 20 centimeter culture was 300 millions of algal organisms per milliliter of culture in a time period of 13 days. Following the initial log growth phase in the 40 centimeter culture, there were three log phases, each with a lesser rate of activity, before the maximum organism concentration was reached. The decrease in activity after the initial log phase was greater than that in the 20 centimeter culture, but the total log growth phase covered a longer time period in the 40 centimeter culture. The maximum organism concentration obtained in the 40 centimeter culture was 135 millions of algal organisms per milliliter of culture in a time period of 16 days.

As illustrated in Figures 11 and 12, the increase in algal dry weight concentration as a function of time followed the characteristic logarithmic growth pattern under both the 20 and 40 centimeter culturing conditions. In the 20 centimeter culture, the rate of dry weight increase during the initial log phase was greater than the initial rate of organism concentration increase. Following the initial log phase there were two additional logarithmic phases of dry weight increase, each with a lesser degree of activity. The maximum algal dry weight concentration produced in the 20 centimeter culture was 1550 milligrams per liter of culture in 13 days of culturing. Following the 13 day culture period, the dry weight concentration level experienced a significant decrease.

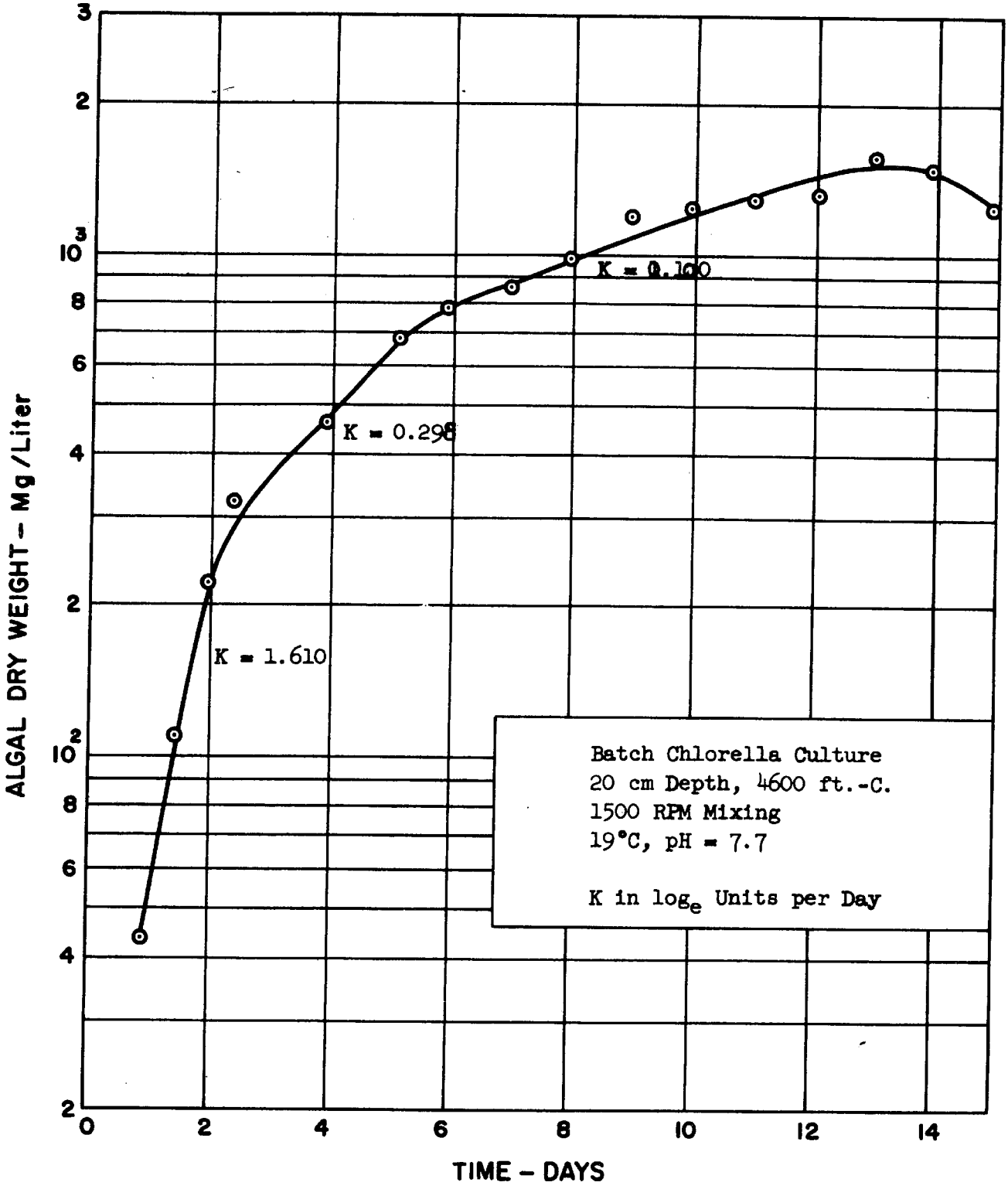


Figure 11. Pattern of Dry Weight Increase for a Batch Chlorella Culture as a Function of Time

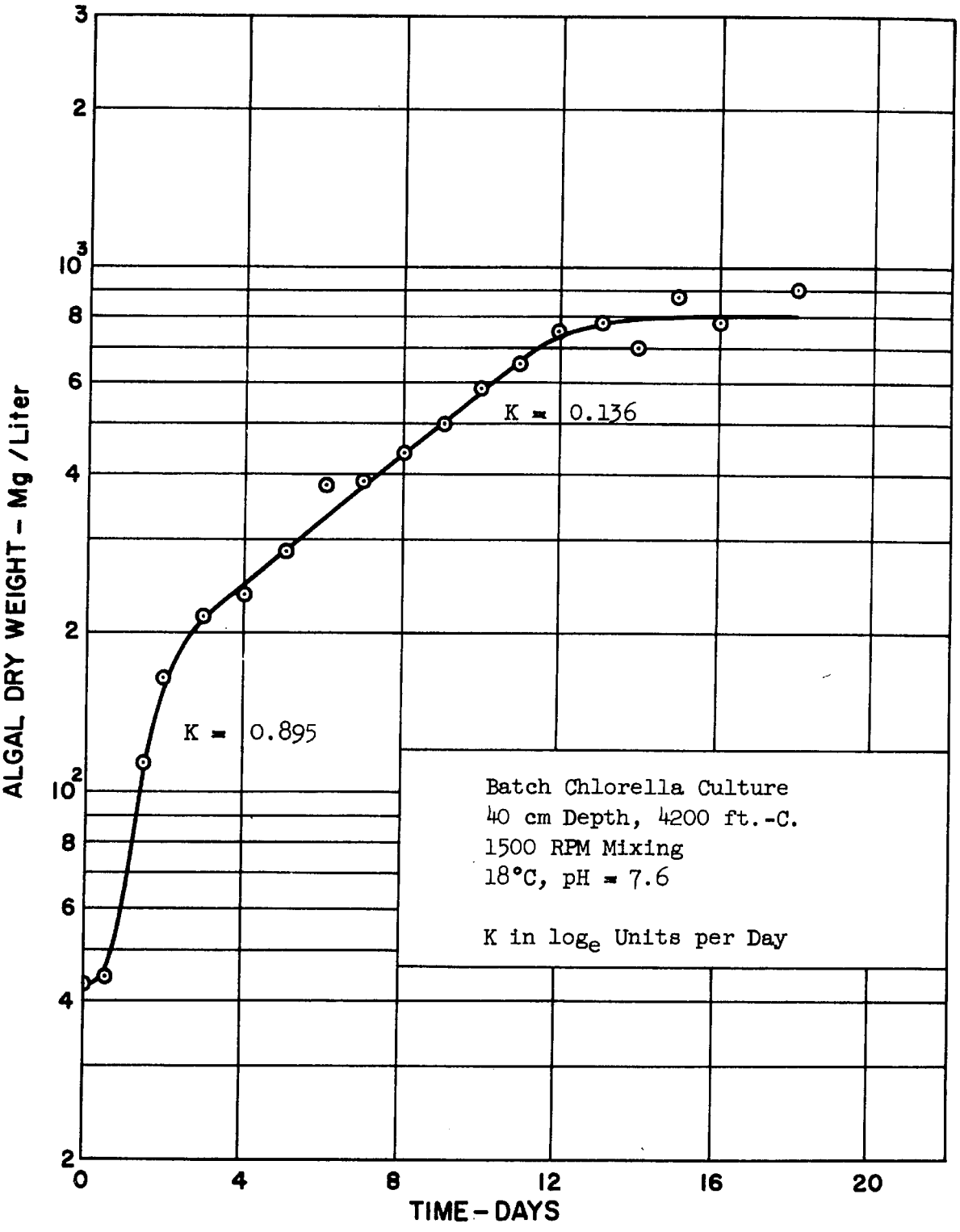


Figure 12. Pattern of Dry Weight Increase for a Batch Chlorella Culture as a Function of Time

The rate of dry weight concentration increase during the initial logarithmic phase in the 40 centimeter culture was less than the initial rate of organism concentration increase. This initial log phase was followed by a single log phase that experienced a constant rate of increase for a culturing period of about 10 days. The maximum algal dry weight concentration reached in the 40 centimeter culture, in 13 days time, was 800 milligrams per liter of culture.

As can be seen from the above discussion, the batch culturing facility and the intense light source were capable of producing extremely dense algal cultures. The maximum organism and dry weight concentrations produced by the 20 centimeter culture are greater than any other such values so far experienced in the University of Michigan Sanitary Engineering Laboratories. The ability with which each culture utilized the incident light energy can be compared by evaluating the production of algal material, which is a function of culture density and rate of culture growth. For the 20 centimeter culture the surface productions associated with the maximum dry weight concentration and the maximum rate of dry weight increase are respectively, 31 and 87 grams of algae per square meter of illuminated surface per day. Comparable values for the 40 centimeter culture are 44 and 68 grams of algae per square meter of illuminated surface per day. In evaluating these surface production values for the two cultures, it can be seen that under the culturing condition of high density and low rate of growth the 40 centimeter culture provided the

larger value, and thus, utilized the incident light energy with greater efficiency. While under the culturing conditions of low density and high rate of growth, the 20 centimeter culture made more efficient use of the incident light energy. Further observation of the curves describing the algal activity patterns in both cultures reveals that the conditions throughout the 40 centimeter culture appear to be somewhat more desirable, since the period of logarithmic growth was sustained over a longer time in the 40 centimeter culture. The fact that the rates of increase for the organism and dry weight concentrations are different is in agreement with the batch algal culturing characteristics reported by Gates (5).

Another purpose of the batch culture studies was to confirm the existence of the previously mentioned relationship between culture depth and culture density, that has been suggested to occur in algal cultures (46). An evaluation of the depth-density relationship can be accomplished by calculating a depth-density factor for each culture. As considered here, this factor has no dimensions and is obtained by multiplying a maximum numerical value associated with culture density by the numerical value of culture depth corresponding to the density value. Utilizing this technique to calculate depth-density factors associated with the maximum organism concentration values, a factor of 600 (i. e., 300 x 2) is obtained for the 20 centimeter culture and for the 40 centimeter culture the depth-density factor is 540 (i. e., 135 x 4). When considering the maximum dry weight concentration

values, the depth-density factors are 3100 (i. e., 1550 x 2) for the 20 centimeter culture and 3200 (i. e., 800 x 4) for the 40 centimeter culture. The depth-density factors associated with the organism concentration differ somewhat for the two cultures, but the factors based upon the algal dry weight concentration agree very closely with each other. As a result of these findings, it is agreed that the depth-density relationship, as suggested by Davis, et al (46), does exist in actively growing algal cultures.

CULTURE LIQUID DEPTH STUDIES

Algal culture liquid depth studies were performed for the purpose of discerning whether or not a variation in liquid depth significantly influenced the growth characteristics of the algae and their ability to extract the fertilizer nutrients from waste treatment plant effluent. In an attempt to detect a truer indication of how culture depth variation affected the biological activity of the algae, an effort was made to maintain the same lighting conditions within the various depth cultures. This effort was put into effect through stabilization of the algal cell, self-shading influence by maintaining a relatively constant depth-density factor for the different culturing depths. The results obtained from the batch algal culture studies verify that the previously mentioned depth-density relationships do exist in active algal cultures.

For purposes of convenience it was decided to adopt a

control depth-density factor of 2500 for the various culturing conditions considered in this investigation. The information obtained from the batch culture study indicated that the algal density parameter of dry weight provides very close agreement between the depth-density factors for algal cultures of different liquid depth. As a result of this finding, the algal dry weight concentration was used as the controlling culture density value incorporated in the relatively constant depth-density factor that existed throughout the liquid depth investigation. The control depth-density factor was approximated for each culturing condition by decreasing the algal dry weight concentration when the liquid depth was increased. Once the appropriate culture dry weight concentration was established for a particular liquid depth, a per cent light transmission was associated with the culture density. This per cent transmission was then used to routinely monitor the constant density control systems during the study of a specific culture depth. In addition, daily algal dry weight determinations were made, so as to be certain that the control depth-density factor was being maintained. The daily analytical results revealed an over-all average depth-density factor for the complete liquid depth study of 2635 which is in fairly close agreement with the established control factor.

The results of the lighting conditions and culture liquid depth study are presented and discussed in two sections. First the influence that the varied lighting conditions and liquid depths had on the growth activity of the algal cultures is considered; and secondly,

the influence that these varied environmental conditions had on the ability of the algal cultures to extract the fertilizer nutrients from the waste treatment plant effluent is evaluated.

ALGAL CULTURE GROWTH ACTIVITY

Throughout the liquid depth study, the growth activity of the various algal cultures was controlled by altering the incident light intensity. The influence that the increasing incident light intensity had on the growth activity of the different depth algal cultures is depicted in Figure 13. As can be seen in this illustration, the influence that the increasing incident light intensity had on the rate of growth activity of the algal cultures follows the general pattern of environmental influence that was described in a previous section. There is a range of light intensity, from 800 to 2050 foot candles, that was rate limiting to the growth activity of the various algal cultures. Within this rate limiting range, the increase in the rate of algal growth activity was directly proportional to the increasing incident light intensity. The range of light intensity beyond 2050 foot candles was non-limiting to the algal growth, since the rate of growth activity remained constant for lighting increases beyond the 2050 intensity level. As a result of these findings, the incident light intensity level at the surface of the culturing unit of 2050 foot candles can be regarded as the light saturation intensity for the algal cultures that were studied.

Figure 13 also illustrates the influence that various

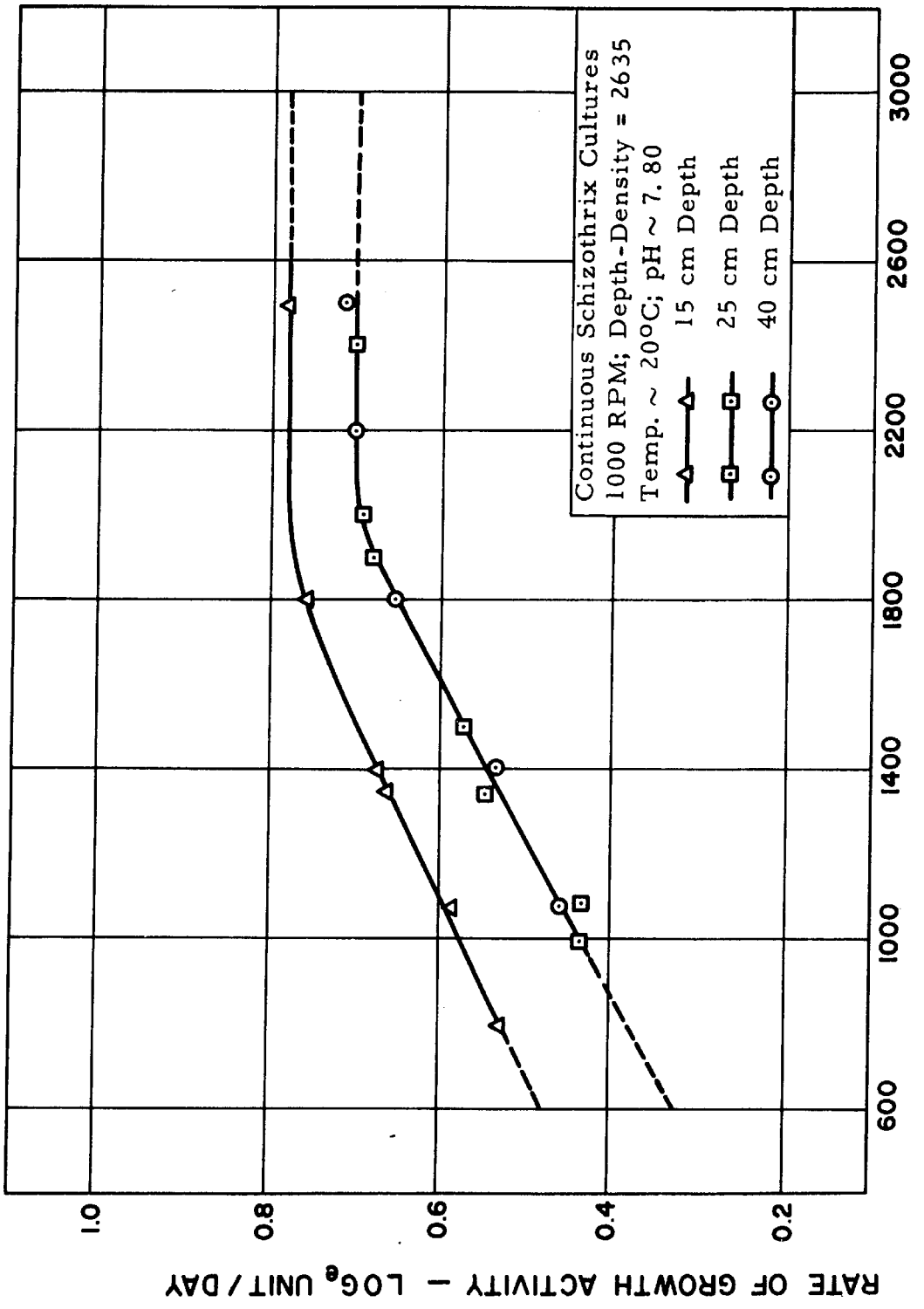


Figure 13. Rate of Algal Growth Activity as a Function of Incident Light Intensity for Cultures of Varying Depths

culture liquid depths had on the rate of algal growth activity. The 15 centimeter culture was the most active culture, with the growth activity of the 25 and 40 centimeter cultures being essentially equal. From this it can be seen that the 15 centimeter culture offered the optimum algal environment of this investigation and stimulated a maximum rate of growth activity of 0.78 $\log_e$ unit per day. The 25 centimeter culture was less than optimum and produced a maximum growth activity rate of 0.70 $\log_e$ unit per day. Surprisingly enough, an added increase of culture liquid depth to 40 centimeters did not further decrease the ability of the culture to stimulate algal growth. Throughout the range of light intensity influence, the patterns by which the rate of growth activity increased with increasing incident light intensity, for the different depth cultures, were very close to paralleling each other. This indicates that the variation in culture liquid depth had little to no influence on the rate at which the algal growth activity increased as the incident light intensity increased to the level of light saturation.

Further evaluation of Figure 13 indicates that the lighting conditions within the various depth algal cultures were closely stabilized through the maintenance of a relatively constant depth-density factor in steady state cultures, for the duration of the liquid depth study. This is confirmed by observing that the light saturation intensity level is identical for the different cultures and that the two rate of growth activity curves essentially parallel each other.

An attempt was made to discern why the algal growth

activities in the 25 and 40 centimeter cultures were identical, by evaluating the light attenuating characteristics within the two cultures. It was felt that if the rates of light attenuation within the two cultures were the same, this would possibly be the reason why the two cultures failed to reveal a difference in growth activity. The light intensity measuring equipment used was the same as that described by Beeton (68). The exposed portion of the photosensing cell was centrally located in the culture, and intensity readings were taken at 5 centimeter intervals from front to as far back in each culture as the thickness of the sensing cell would allow. As expected, the light attenuating pattern within the two cultures was semi-log linear, with the light intensity decreasing as liquid depth increased. Aside from this finding, the data were very inconclusive toward providing information to explain why the growth activity of the 25 and 40 centimeter cultures was identical.

Another indication of how increasing incident light intensity and varying culture liquid depths influenced the growth activity of the algal cultures is shown by Figure 14. This Figure illustrates how the generation time for the algal cultures was affected by altering the indicated environmental conditions. In this consideration the generation time is the time that it took the algal cultures to double themselves when growing at the various rates indicated in Figure 13. The generation time curves follow a pattern that is essentially opposite to that of the previously illustrated rate of growth activity curves. A minimum generation time of 0.89 day was computed for

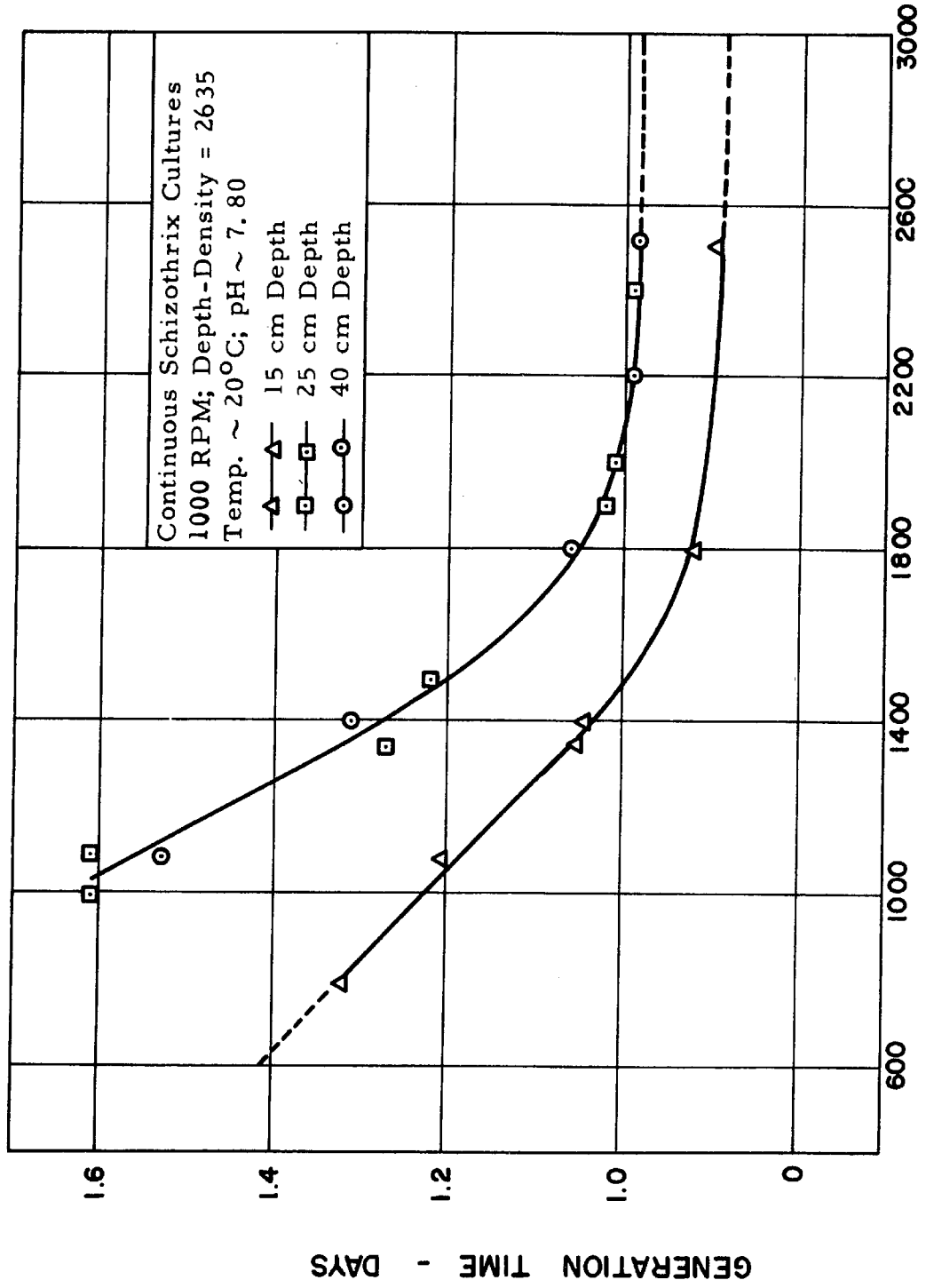


Figure 14. Algal Culture Generation Time as a Function of Incident Light Intensity for Cultures of Varying Depths

the 15 centimeter culture; and for the 25 and 40 centimeter cultures, the minimum generation times were identical at 0.98 day.

In an attempt to assess the effectiveness with which the algal cultures utilized the incident light energy, culture production values, at the various light intensities, were computed for the different depth cultures. The production value computations involved multiplying the rate of growth activity by the respective algal culture density, and then expressing the production as total grams of algae produced per unit of surface area or culture volume per day. That quantity of algae produced per unit of surface area is designated as surface production, while the total amount of algae produced per unit of culture volume is the volume production of the algal culture.

The influence of increasing incident light intensity on the surface production of the various depth cultures is illustrated in Figure 15. In general the surface production for each culture increased with increasing light intensity toward a maximum production value. For each depth culture, there was a range of light intensity through which the surface production increased at a constant rate, followed by a light intensity range in which the algal production increased at an ever decreasing rate to the maximum production level. From this illustration it appears that the 25 centimeter culture utilized the incident light energy more effectively than the other cultures. The maximum 25 centimeter culture production was 21.2 grams of algae per square meter of surface per day, with the maximum surface

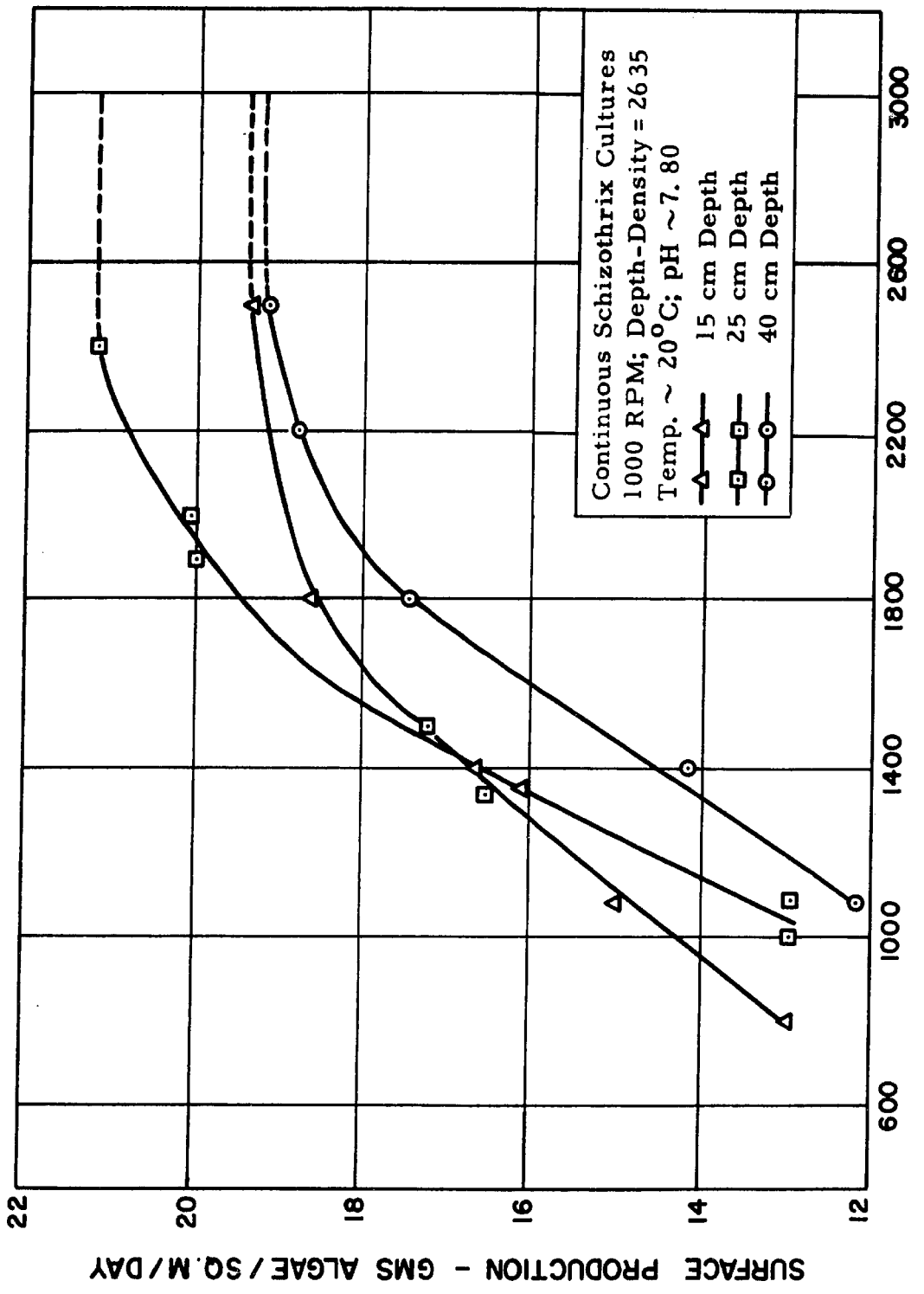


Figure 15. Algal Culture Surface Production as a Function of Incident Light Intensity for Cultures of Varying Depths

productions for the 15 and 40 centimeter cultures being almost the same at 19.4 and 19.2 grams of algae per square meter per day, respectively. Variations in culture liquid depth did not have a regular pattern of influence on the rate of surface production increase with increasing light intensity. The rate of surface production increase was greatest for the 25 centimeter culture, while the rates of production increase for the 40 and 15 centimeter cultures were of successively lesser magnitudes.

The effectiveness with which the various depth algal cultures utilized the growth substrate, through inducement from the variation of incident light intensity, is indicated in Figure 16. As illustrated in this Figure, the volume production for the different cultures generally increased to a maximum level with increasing light intensity. The 15 centimeter culture utilized the growth substrate most effectively. A maximum volume production of 0.125 grams of algae per liter of culture per day was attained with the 15 centimeter culture; while the same values for the 25 and 40 centimeter cultures were, respectively, 0.079 and 0.048 grams of algae per liter of culture per day. Increasing the culture liquid depth beyond 15 centimeters reduced the rate of volume production increase throughout the growth limiting range of light intensity. This happening reveals that the influence of incident light intensity on the volume production of the algal cultures decreased as the culture liquid depth was increased. Also from Figure 16, it can be observed that for any incident light intensity level within the scope of this study, the culture volume production decreases with an

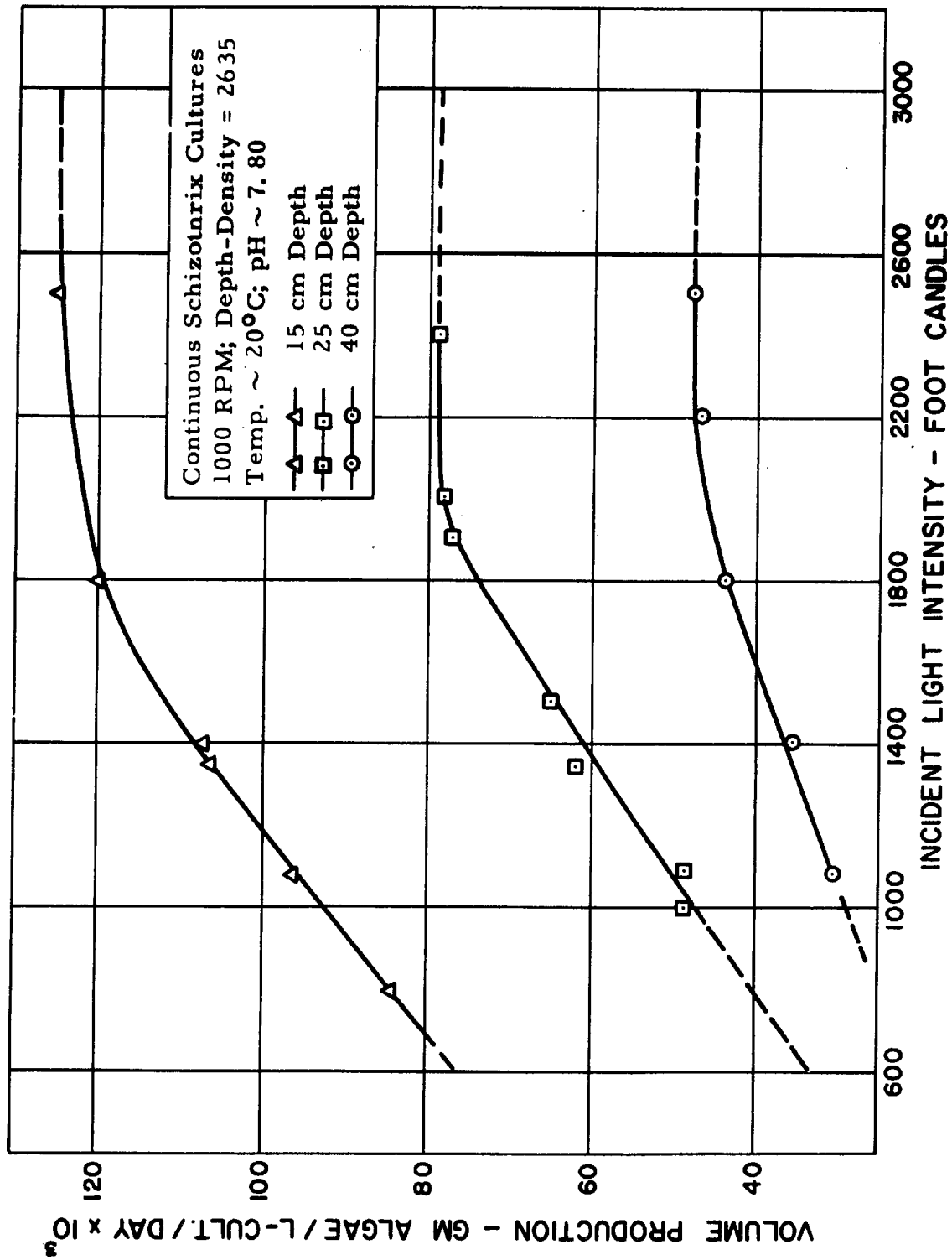


Figure 16. Algal Culture Volume Production as a Function of Incident Light Intensity for Cultures of Varying Depths

increase in culture depth, when a relatively constant depth-density relationship was maintained for the varying culture conditions. A comparison of Figures 15 and 16 indicates that if an algal environment is developed to effect maximum utilization of the growth substrate, maximum utilization of the incident light energy is sacrificed. Since the ultimate purpose of an environmentally controlled algal waste treatment process is the effective bio-extration of fertilizer nutrients from waste treatment plant effluents, it is strongly felt that information such as present in Figure 16 should govern the design and development of this treatment process.

In an attempt to establish trends of how various other environmental parameters influenced the maximum culture volume productions within the scope of this study, Figures 17, 18 and 19 were prepared. In Figure 17 it is shown by Curve 1 that the maximum volume production decreases with increasing culture liquid depth along a concavely downward path. These data were then linearized by plotting the reciprocal of the maximum volume production against increasing culture depth as shown by Curve 2. From curves of the nature presented in Figure 17, definite trends to the influence that increasing culture depth had on maximum volume production can be established. Also, it is evident that a constant rate of maximum volume production variation was involved within the limits of this culture liquid depth study.

Figure 18 illustrates the influential trend that the increasing ratio of surface area to liquid depth exerted on the maximum

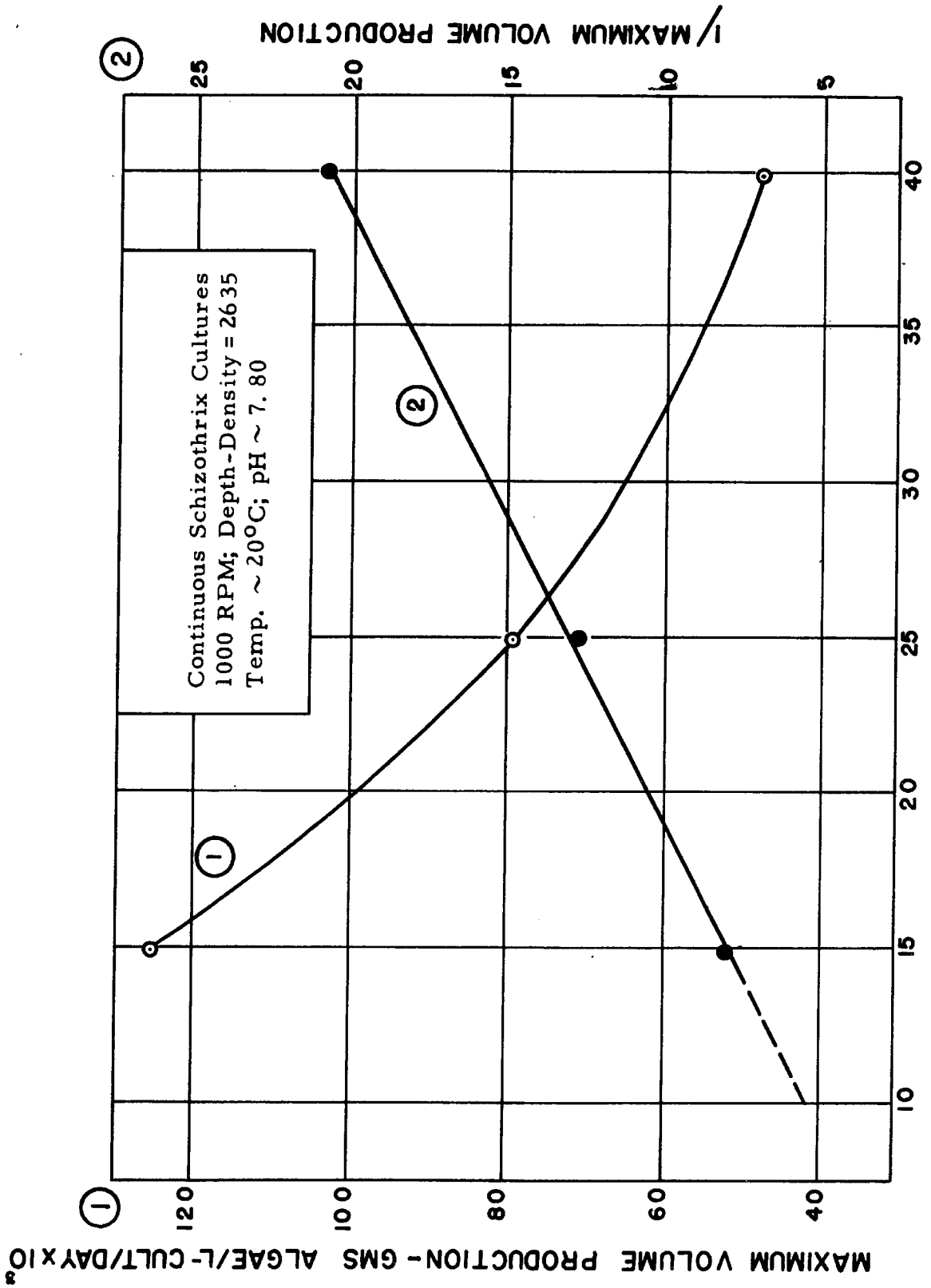


Figure 17. Maximum Culture Volume Production as a Function of Algal Culture Liquid Growth

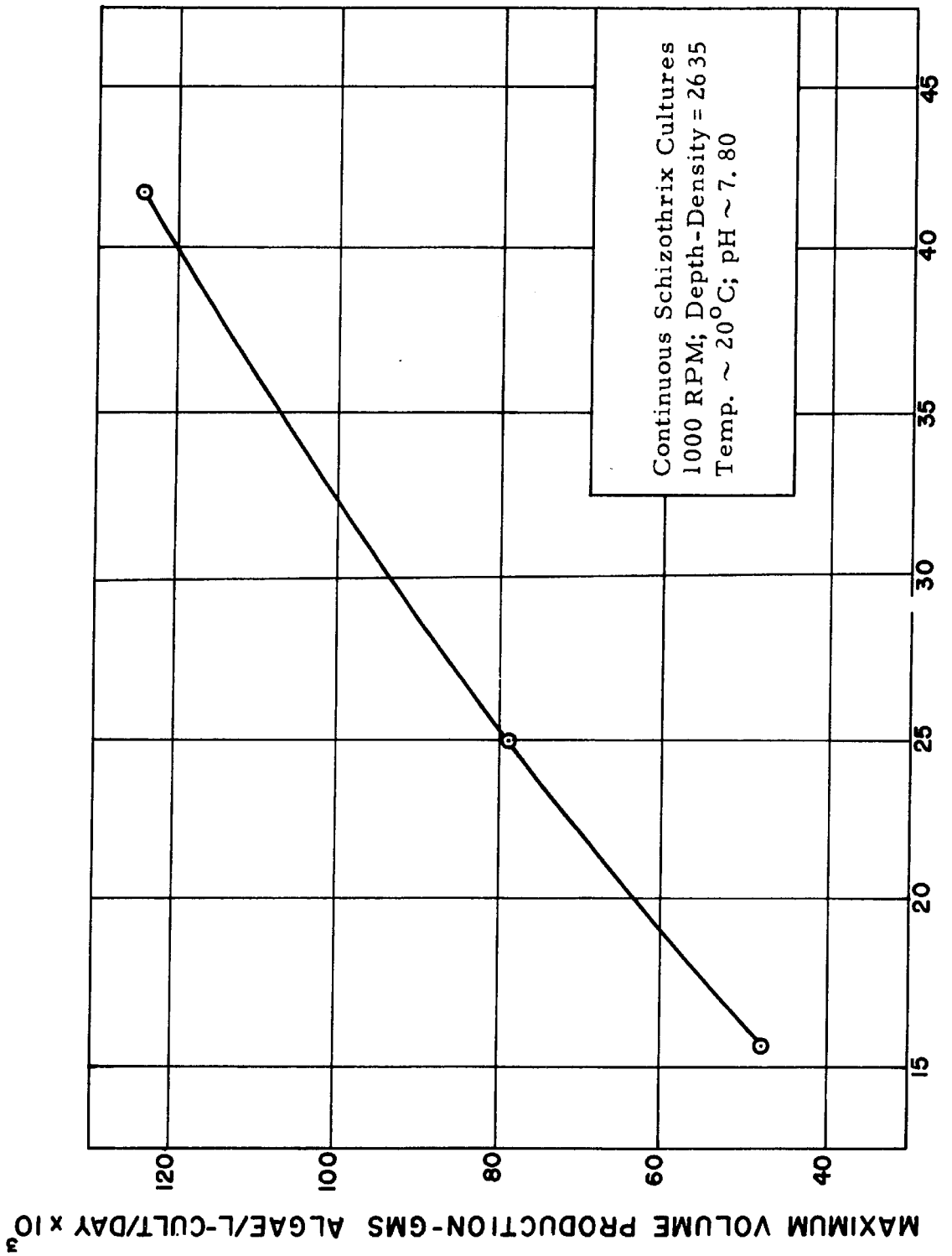


Figure 18. Maximum Culture Volume Production as a Function of Surface Area to Liquid Depth Ratio

volume production of the three algal cultures that were investigated. As depicted in this Figure, the increasing ratio of surface area to liquid depth positively affected the maximum volume production in a slightly curvilinear fashion. From this Figure it appears that it might be possible to suggest a so-called "rule-of-thumb" criterion for the design of environmentally controlled algal processes for the bio-extracting of fertilizer nutrients from waste treatment plant effluents. A statement of this suggested criterion would be that a trend towards a larger surface area to liquid depth ratio would provide an algal environment more conducive to the effective utilization of the growth substrate. In order for this criterion to remain valid, it must be remembered that the influence of algal cell, self-shading must be stabilized for varying culture conditions through the maintenance of a relatively constant depth-density relationship. This suggested criterion would also possibly be worthy of consideration in the design of the lighted volume associated with the development of light-time, dark-time algal culturing techniques.

An indication of how the varying algal dry weight concentration influenced the maximum volume production for the different depth cultures is revealed in Figure 19. As the dry weight concentration increased with each culture depth decrease, the maximum culture volume production assumed an increasing trend. From this illustration, it can be observed that a constant rate of maximum volume production increase occurred as the algal culture density increased, since this relationship assumed a semi-log linear pattern. Again, the validity

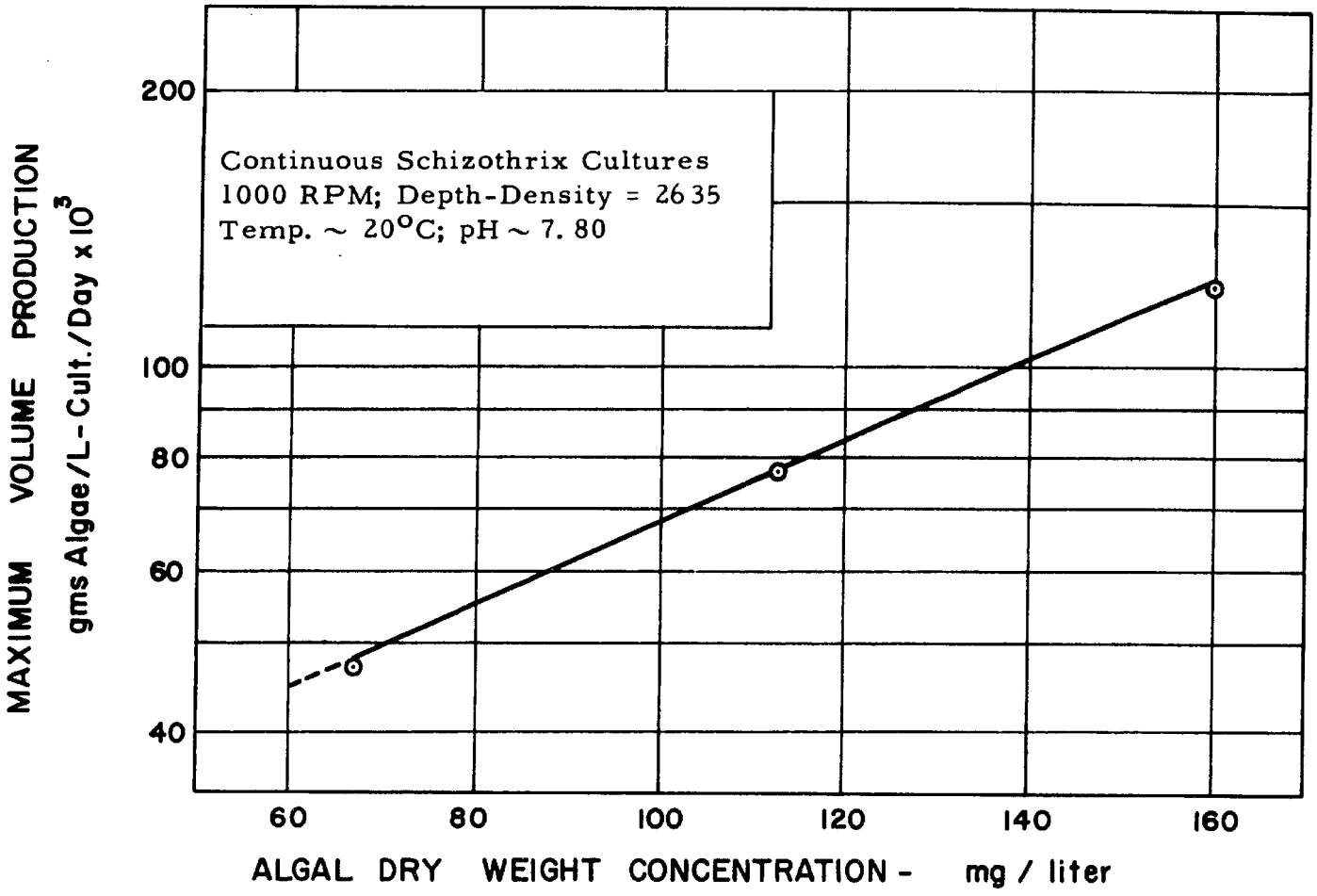


Figure 19. Maximum Culture Volume Production as a Function of Algal Dry Weight Concentration

of the trend established in Figure 19 is restricted to the limitations of this culture liquid depth study, and projections beyond these limitations would be subject to question, unless verified through experimental observations.

As a result of this liquid depth study, it was observed that equal lighting conditions can be maintained within different depth cultures through the maintenance of a relatively stable depth-density relationship for the varying environmental conditions. The shallowest culture depth established an environment that stimulated the highest rate of algal growth activity. This investigation also revealed that the environment which stimulated the most active algal cultures was also very conducive to effective bio-utilization of the growth substrate.

ALGAL CULTURE NUTRIENT EXTRACTION

As part of the total culture liquid depth study, an evaluation was made of the effectiveness with which each algal culture, under the varying environmental conditions, bio-extracted fertilizer nutrients from the waste treatment plant effluent. The inorganic nutrient extracting characteristics of each environmentally controlled algal culture were determined by routine chemical analyses of the total inorganic nitrogen (TIN) and orthophosphate concentrations in the culture influents and culture effluents following removal of the algae by membrane filtering. From this analytical information, percentage extractions of the inorganic nutrients were computed and used to indicate

the nutrient extracting abilities of the various algal cultures. In this discussion section, the nutrient extracting percentages are evaluated as functions of those parameters influencing the algal growth and the growth activity results that were reported in the preceding discussion section.

The influence that the increasing incident light intensity had on the precentage of TIN extracted from the waste effluent by the various depth cultures is illustrated in Figure 20. As can be seen, the TIN extraction increased with increasing light intensity, along a curvilinear pattern that appears to tend toward an eventual maximum level. The 15 centimeter culture was the most effective TIN extractor with a maximum extraction percentage of 97.6 being developed. For the 25 and 40 centimeter cultures, the maximum developed TIN extraction percentages were successively less at 65.2 and 55.7, respectively. The first and last percentage values for the 25 centimeter culture were somewhat erratic; but it is felt that the indicated curve extrapolations are valid, since the established trend of the 25 centimeter curve agrees with the 15 and 40 centimeter curves. Figure 20 also indicates that for a specific light intensity value, within the range of this study, the TIN extraction percentage increased as an inverse function of the varying culture liquid depth. The variation in culture liquid depth did not appear to significantly affect the manner in which the changing light intensity influenced the TIN extraction percentage, since the three curves are essentially parallel. The TIN extraction data, as reported in Figure

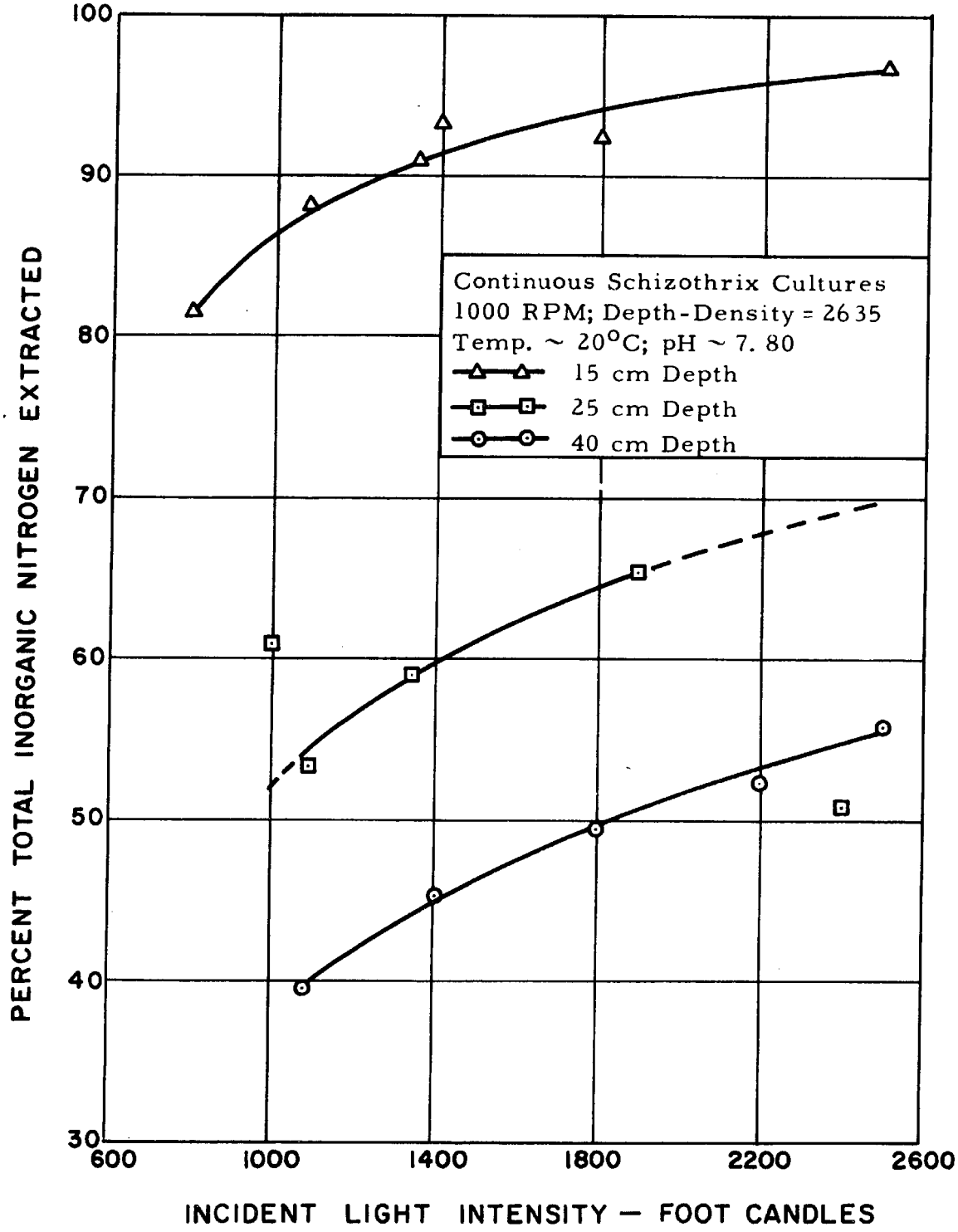


Figure 20. Algal Extraction of Total Inorganic Nitrogen as a Function of Incident Light Intensity for Various Depth Cultures

20, are in consistent agreement with the algal culture volume production information, illustrated in Figure 16.

In an attempt to determine how the algal culture growth activity information influenced the TIN extraction characteristics of the algal cultures, Figures 21, 22 and 23 were developed. Figure 21 indicates the pattern of influence that the increasing rate of algal growth activity exerted on the TIN extraction percentages for the different depth cultures. In all three algal cultures the TIN extraction percentage was positively influenced as the rate of culture growth activity increased. The increasing extraction percentages for the 15 and 40 centimeter cultures followed a curvilinear path, while those for the 25 centimeter culture assumed a linear trend. Further evaluation of Figure 21 reveals that as the rate of culture growth activity increased and the culture liquid depth decreased, the effectiveness of the algal cultures as TIN extractors improved.

As would be expected, Figure 22 illustrates that the variation in algal culture generation time influenced the TIN extraction abilities of the algal cultures in a manner almost exactly opposite to that shown in Figure 21. Figure 22 reveals that, under the conditions of this investigation, the effectiveness of an environmentally controlled algal culture as a TIN extractor increases as the culture generation time and liquid depth decrease.

Figure 23 depicts the relationship between algal culture volume production and TIN extraction. For the 15 and 40 centimeter

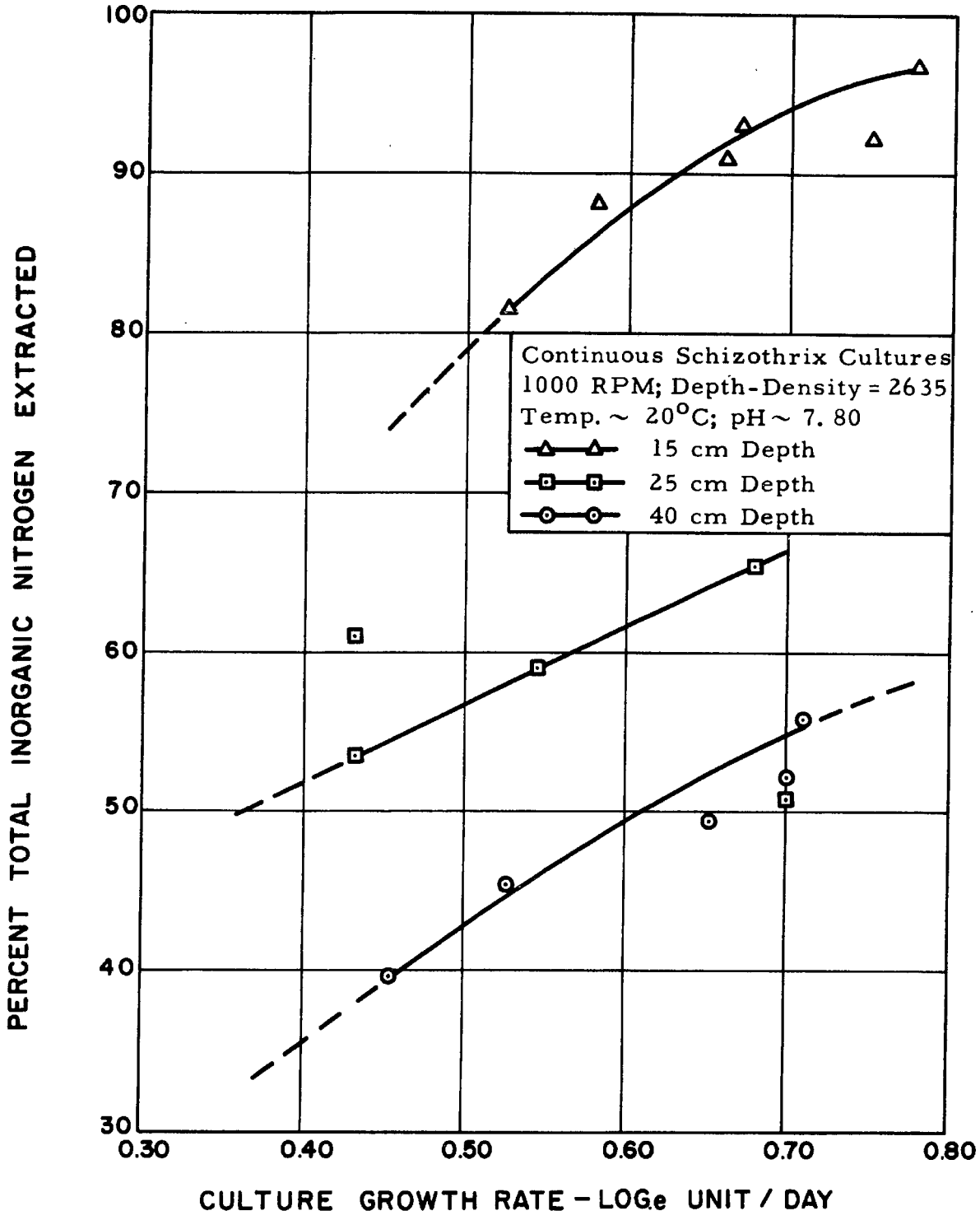


Figure 21. Algal Extraction of Total Inorganic Nitrogen as a Function of Algal Growth Activity for Various Depth Cultures

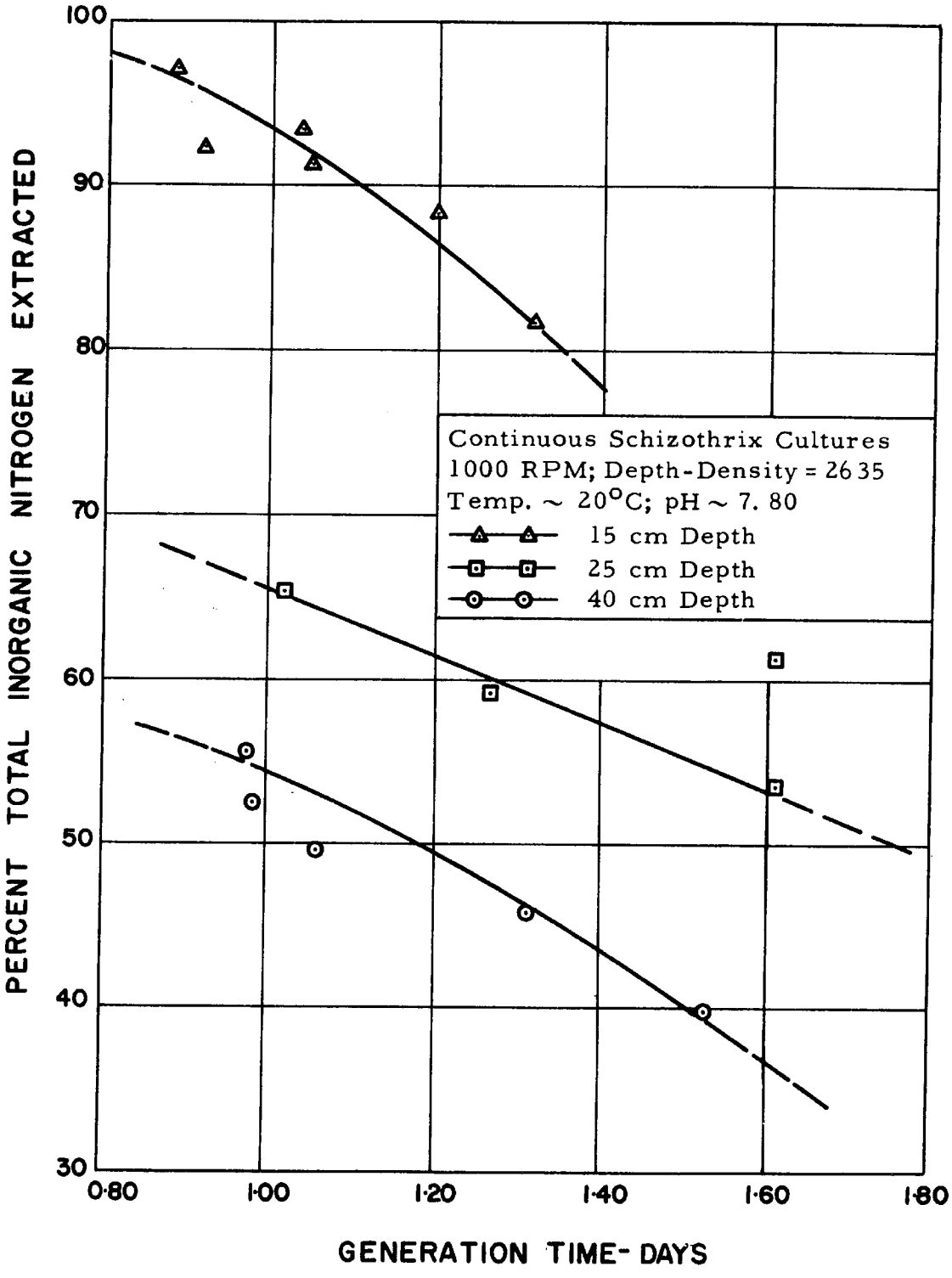


Figure 22. Algal Extraction of Total Inorganic Nitrogen as a Function of Culture Generation Time for Various Depth Cultures

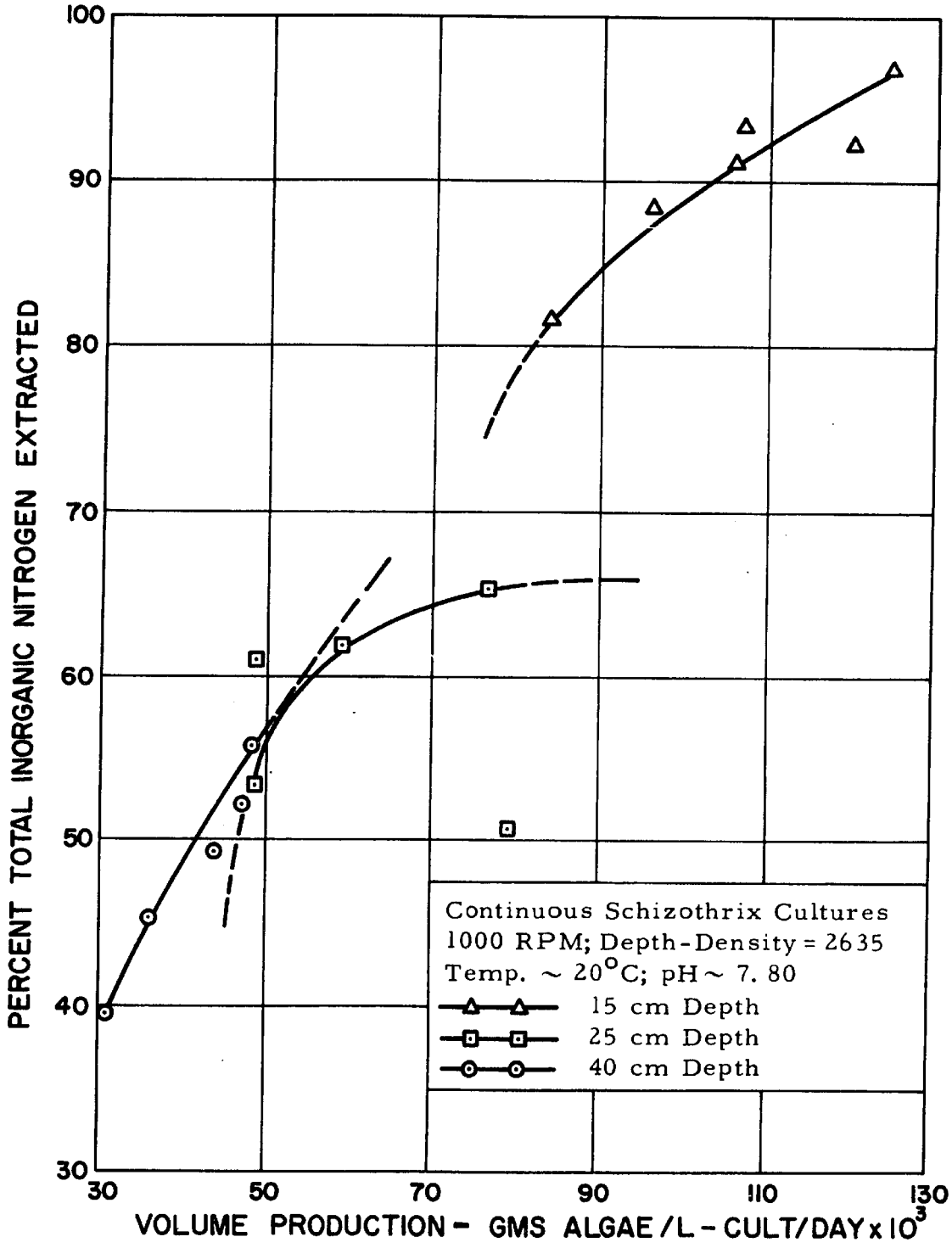


Figure 23. Algal Extraction of Total Inorganic Nitrogen as a Function of Culture Volume Production for Various Depth Cultures

cultures, the percentage of TIN extracted increases with increasing volume production along a similar curvilinear trend, with the 15 centimeter curve appearing to almost be an extension of the 40 centimeter curve. The information for the 25 centimeter culture follows the same general trend, but is somewhat erratic and assumes a curved pattern different from the other two cultures. A general statement concerning the algal cultures of this investigation, based upon an evaluation of Figure 23, is that as the culture liquid depth decreased, an increase in culture volume production and bio-extraction of TIN were stimulated. An illustration of this nature further substantiates the concept that the volume production of an algal culture indicates how effectively the algal culture is utilizing the growth substrate.

The occurrence of nitrogen conversion problems in the 25 and 40 centimeter cultures tended to retard the ability of these two cultures to effectively extract TIN from the culturing substrate. These two algal environments also appeared to be satisfactory for the active growth of nitrogen converting bacteria. Under the low algal growth activity and long residence time conditions in the 25 and 40 centimeter cultures, the bacteria had adequate opportunity to develop significant amounts of nitrate-nitrogen in the culture liquid. Since algae appear to practice a nitrate-nitrogen sparing reaction in the presence of ammonia-nitrogen, the high nitrate-nitrogen concentrations remained in the culture effluents. A suggested and investigated process control technique to eliminate and control this nitrogen conversion problem

is presented and discussed in the next experimental section of this chapter.

As in the case of the algal extraction of TIN, the environmental changes, throughout this investigation, also significantly affected the effectiveness of orthophosphate extraction from the waste treatment plant effluent. Figure 24 indicates the way in which the increasing incident light intensity altered the percentage extraction of orthophosphate by the various depth algal cultures. In the case of all three cultures, the percentage extraction of orthophosphate increased with increasing light intensity along a concavely positive pattern, with the 15 centimeter culture again being the most effective nutrient extractor. Maximum percentage extractions of orthophosphate indicated in Figure 24 are 66.9 for the 15 centimeter culture, 39.5 for the 25 centimeter culture and 43.1 for the 40 centimeter culture. The manner in which the increasing light intensity altered the orthophosphate extraction in the 15 and 40 centimeter cultures appears to be essentially the same, since both curves closely parallel each other. As for the 25 centimeter culture, this extraction variation did not follow the same pattern. A possible reason for this discrepancy in the 25 centimeter culture is presented in a later paragraph.

Since the bio-extraction of TIN was a function of the rate of culture growth activity, it was expected that the extraction of orthophosphate would also be influenced by this algal growth parameter. The extent to which the algal-extraction of orthophosphate from the

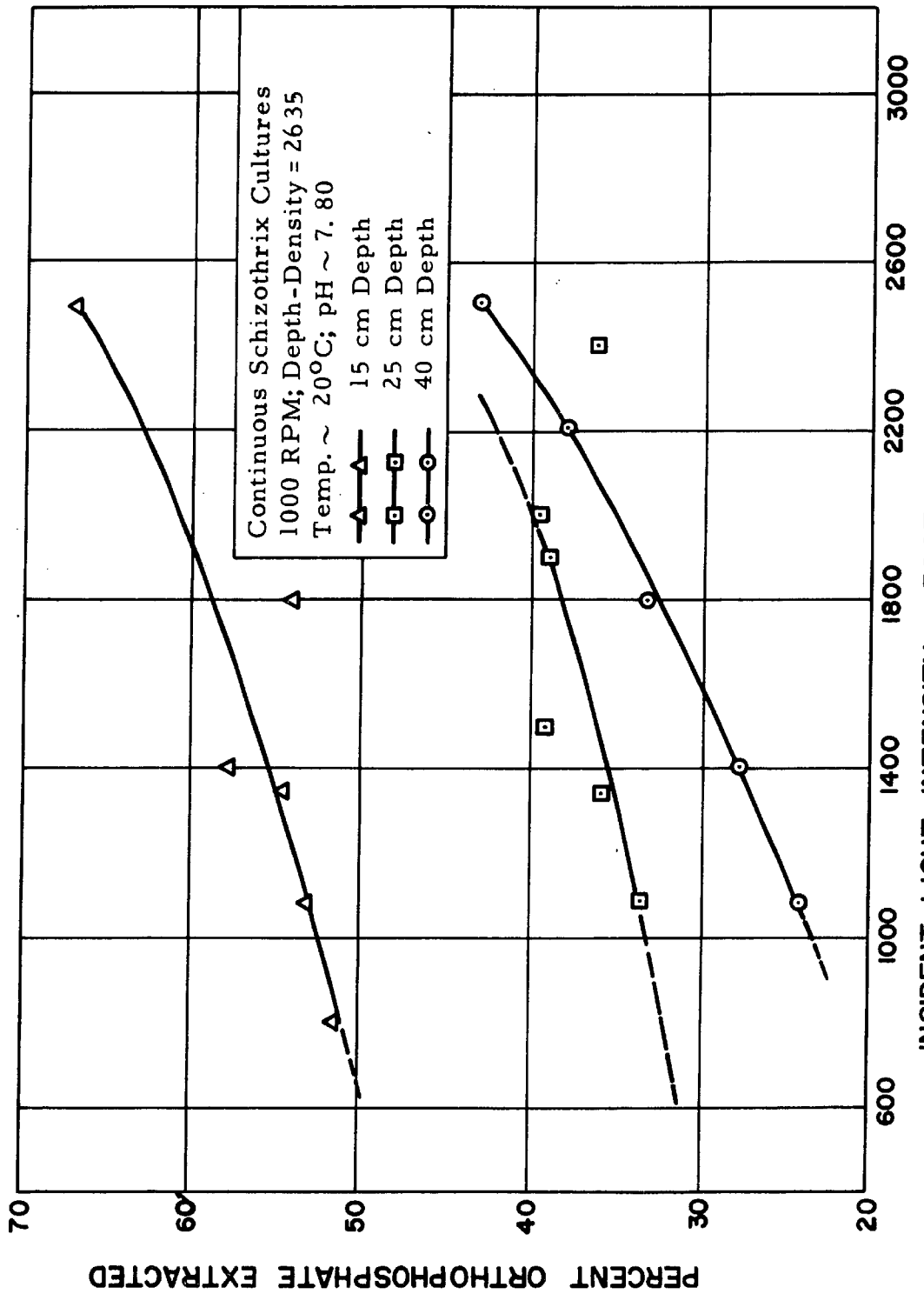


Figure 24. Algal Extraction of Orthophosphate as a Function of Incident Light Intensity for Various Depth Cultures

growth substrate was a function of the rate of culture growth activity is shown in Figure 25. In the case of the 15 and 40 centimeter cultures, the percentage of orthophosphate extracted increased with increasing culture growth rate in a concavely upward fashion, which tends toward a region where orthophosphate extraction is no longer a function of increasing culture growth rate. Again, the information presented for the 25 centimeter culture is erratic and does not conform with the growth rate influenced extraction trend of the other two cultures.

Figure 26 indicates the manner in which the algal extraction of orthophosphate from the growth substrate was influenced by the culture generation time. As was expected, the curves in this illustration are very close to being exact mirror images of the curves in Figure 25. An illustration of this nature is useful, since it allows the observation of how effectively an algal culturing process operates as a nutrient extractor per culture generation, under specified culturing conditions. Information of this form would be useful when considering the physical design of process facilities.

An illustration of the extent to which the bio-extraction of orthophosphate was a function of the culture volume production is revealed in Figure 27. The pattern of volume production influence is generally the same as that of the rate of culture growth influence which was illustrated in Figure 25. The volume production influence on the precentage of orthophosphate extracted was more dramatic in the 40 centimeter culture than in the 15 centimeter culture. A general

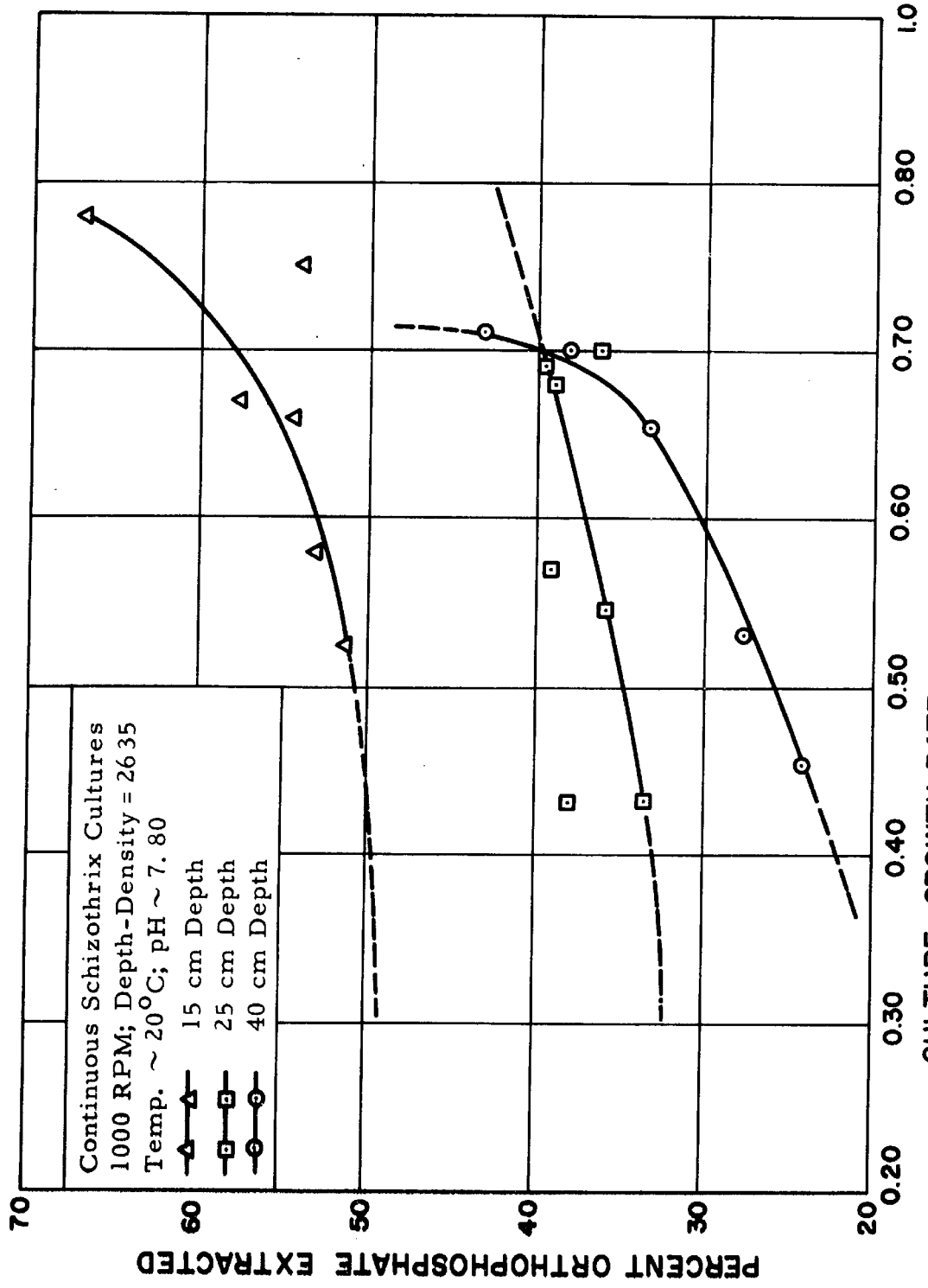


Figure 25. Algal Extraction of Orthophosphate as a Function of Algal Growth Activity for Various Depth Cultures

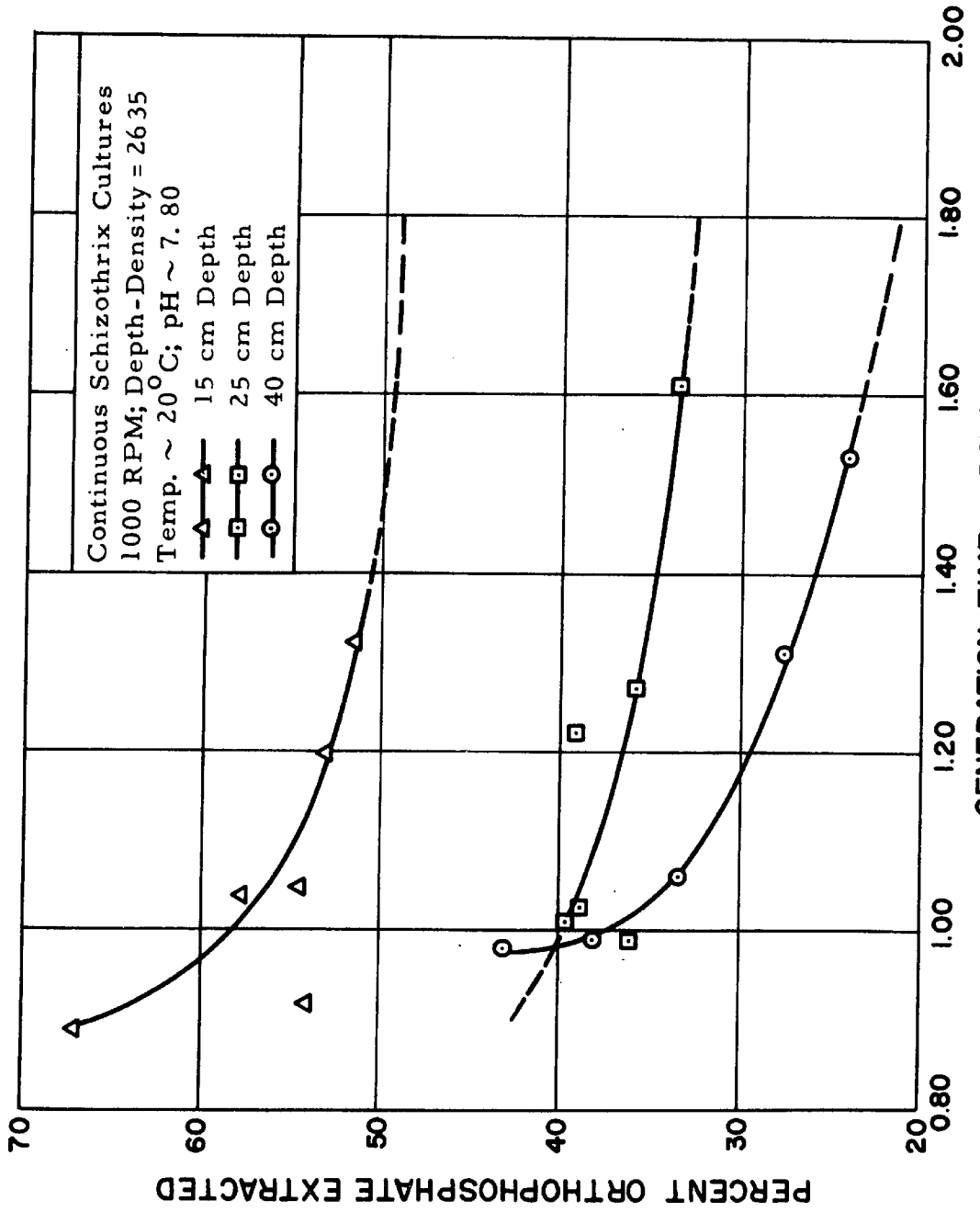


Figure 26. Algal Extraction of Orthophosphate as a Function of Culture Generation Time for Various Depth Cultures

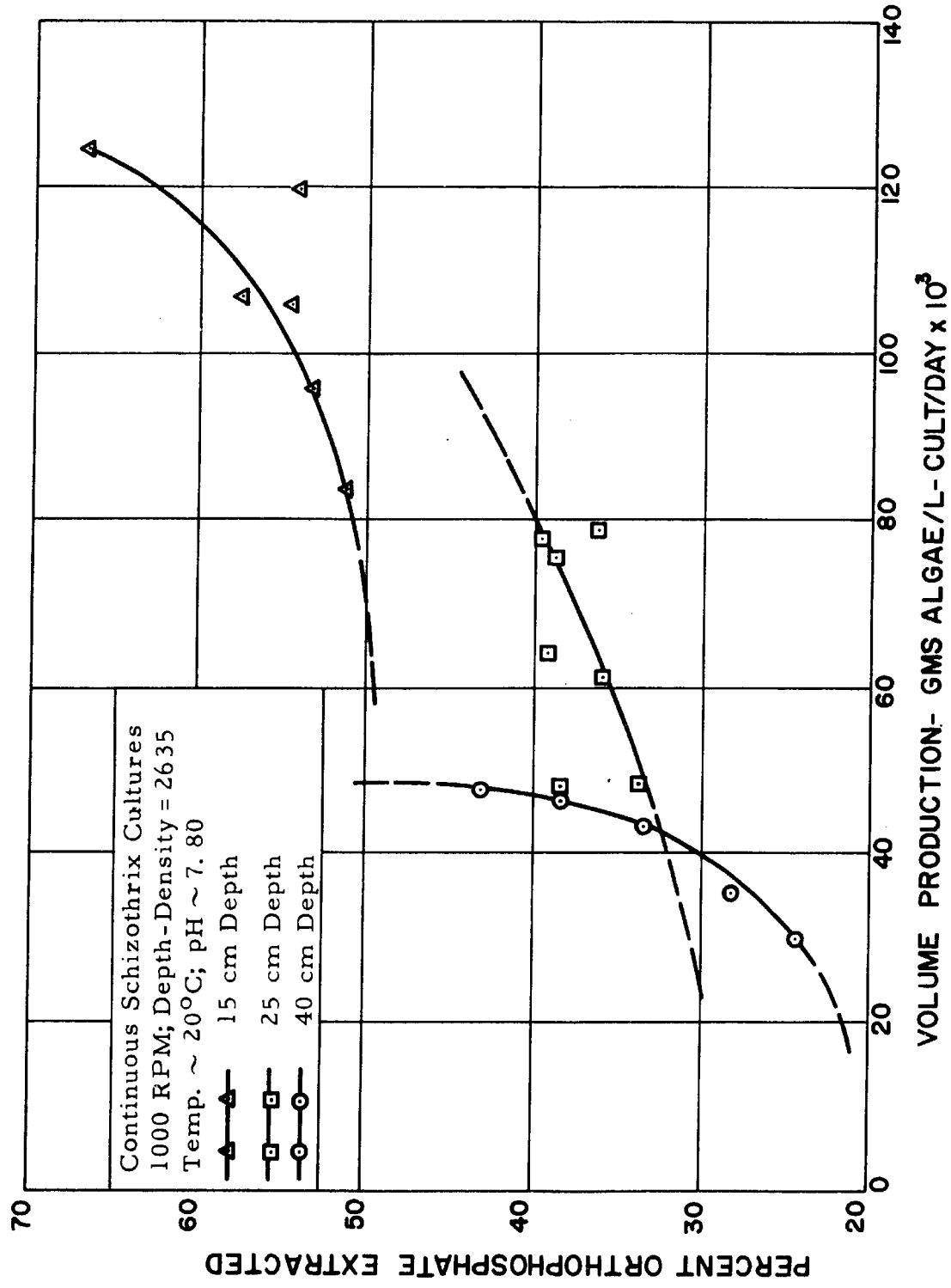


Figure 27. Algal Extraction of Orthophosphate as a Function of Culture Volume Production for Various Depth Cultures

evaluation of Figure 27 is that the algal culturing environment which stimulates the highest culture volume production, also stimulates the most effective algal-extraction of orthophosphate from the culturing substrate. Once again, the information for the 25 centimeter culture is somewhat erratic and does not agree with the other two cultures.

Since, on an individual basis the percentage TIN extracted and the percentage orthophosphate extracted from the algal growth substrate were dependent upon the same growth stimulating and growth characteristic parameters, it was felt that these two extraction properties should be dependent upon each other. Therefore, Figure 28 was developed to illustrate the extent to which the percentage extractions of the two algal nutrients were dependent on one another. For the three different cultures, the percentage of TIN extracted increased with increasing percentage of orthophosphate extracted toward a maximum level where further increases in orthophosphate extraction no longer stimulated additional increases in TIN extraction. In the case of the 15 and 25 centimeter cultures, decreases in percentage TIN extracted were accompanied by decreases in orthophosphate extraction that tended toward minimum levels where further decreases in TIN extraction had no additional affects on the orthophosphate extractions. Whereas, for the 40 centimeter culture, the decreasing trend in extraction of nutrients tends toward zero nutrient extraction. The information presented in Figure 28 definitely confirms the observation that throughout this liquid depth study the shallowest algal culturing

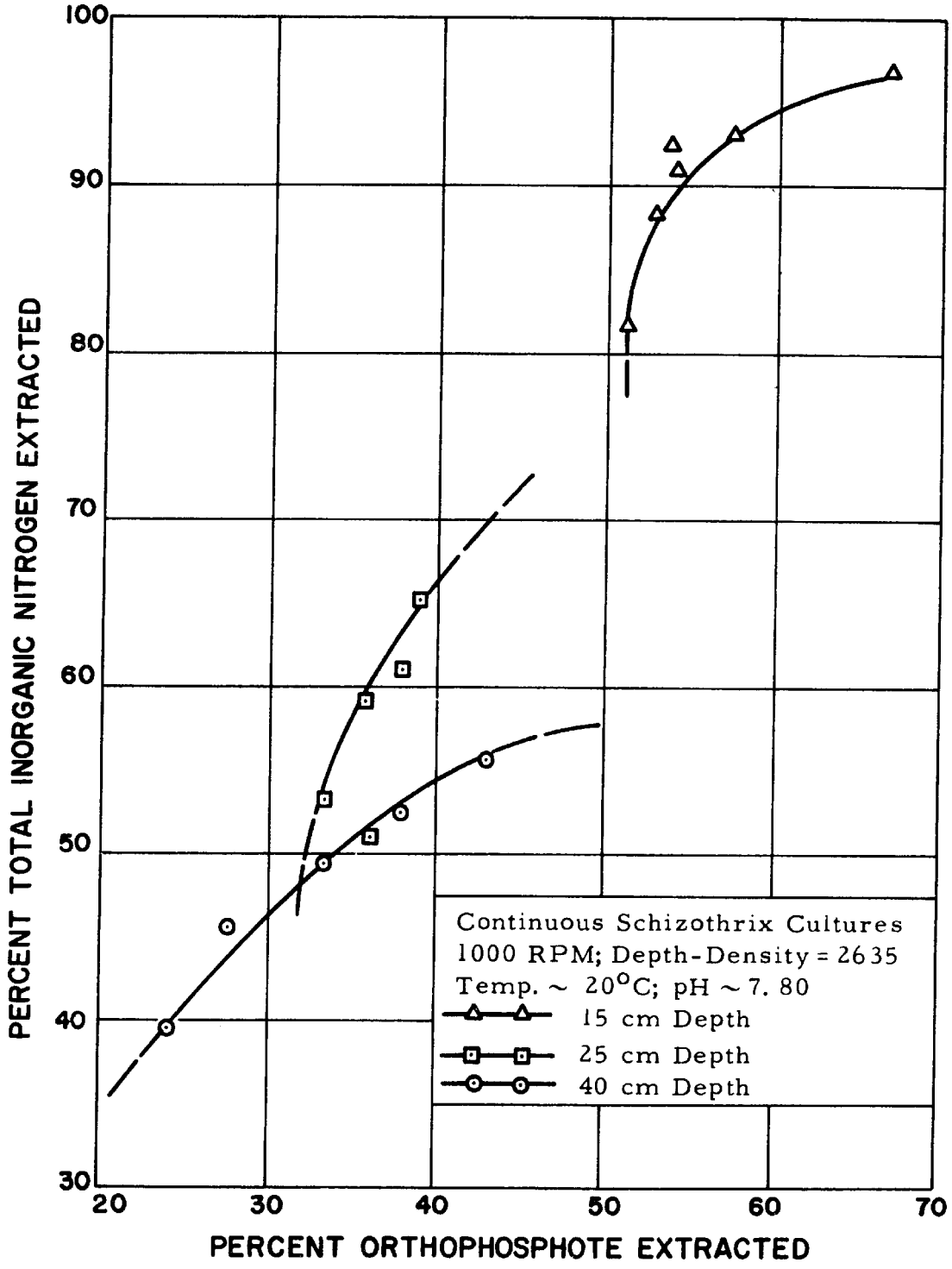


Figure 28. Algal Extraction of Total Inorganic Nitrogen as a Function of Orthophosphate Extraction for Various Depth Cultures

environment stimulated the most effective extraction of algal nutrients from the culturing substrate.

Along with the description and discussion of most of the illustrations in this section, it was indicated that the information concerning the 25 centimeter culture was somewhat erratic and did not conform with the general trends associated with the other two cultures. The exact reason for this unusual behavior of the 25 centimeter culture is not positively evident from the research data that were collected. The only immediately obvious physical difference between the three cultures was liquid depth, since lighting conditions within the cultures were stabilized by the relatively constant depth-density relationship and turbulent mixing was maintained in all cultures. Strangely enough, the 25 centimeter culture must have possessed some differences, since it was stimulatory to the development of a significant population of diatoms. An investigation to determine the quality of light at depth in the different cultures was not made, but it is highly possible that the light quality within the 25 centimeter culture was conducive to diatom growth. Therefore, it is possible that this is the reason why the 25 centimeter culture was a less effective extractor of orthophosphate than the 40 centimeter culture, and why its pattern of nutrient extraction, as related to different algal growth characteristics, differed from the other two cultures. From this it appears that the diatoms might be less effective utilizers of culturing substrate than the blue-green and green algal forms that were dominantly and

subdominantly present throughout this investigation. Specific research to confirm these suspected characteristics of the diatoms would be necessary, since the literature appears to be quite lacking on this subject.

This phase of the total culture liquid depth study has conclusively revealed that the culturing environment which is the most desirable for algal growth is also highly conducive to effective bio-extraction of algal nutrients from waste treatment plant effluent. It is further evident from this study that any information pertaining to the volume production characteristics of an environmentally controlled algal culture is indicative of the culture's potential abilities as an algal nutrient extractor.

ALGAL CULTURE pH STUDIES

The occurrence of a high nitrate-nitrogen concentration in the effluents from the 25 and 40 centimeter cultures was discussed in the previous section. If this nitrate-nitrogen concentration increase is allowed, then the purpose of an algal treatment process for the bio-extraction of fertilizer nutrients from waste treatment plant effluents is obviously defeated. Assuming that this nitrogen conversion happening was instigated through bacterial activity, it was decided that attempts should be made to alter the algal culturing environment in such a way to discourage the activity of the nitrogen converting bacteria. An analysis of the nitrogen pathways in nature revealed that the nitrosification

bacteria (Nitrosomonas), which oxidize ammonia-nitrogen to nitrite-nitrogen, have an optimum growth pH of 8. 6 (120); while the nitrifying bacteria (Nitrobacter), which oxidize the nitrite-nitrogen to nitrate-nitrogen, have an optimum growth pH just below 7. 7 (121). Salle (121) further indicates that the nitrifying bacteria are so sensitive to pH that at a level just above 7. 7, little to no nitrite-nitrogen is oxidized to nitrate-nitrogen; while at a pH level just below 7. 7, this nitrogen conversion is so rapid that the biological validity of the transformation is questionable. Based upon these pH characteristics of the nitrogen converting bacteria, it was decided to poise the culture pH at a level, high enough to effectively discourage the bacterial activity, in hopes that this environmental change would reduce the nitrate-nitrogen concentration in the algal culture effluent.

The previously described culturing conditions, which are conducive to the production of a high nitrate-nitrogen effluent, were established and the culture was operated as a steady-state system until a relatively high nitrate-nitrogen concentration developed in the culture effluent. When the high nitrate-nitrogen level in the culture effluent was attained, the culture pH was allowed to increase to 9. 5 and then the pH control system was set so as to maintain the culture pH at approximately 9. 5. Daily chemical determinations were performed on the culture influent and effluent in order to evaluate the algal nutrient removal characteristics of the high pH algal culture. Also, the routine physical determinations of algal dry weight, microscopic count, Coulter

Counter count and per cent light transmission were made for the purpose of monitoring the steady-state culturing system controls, and to discern whether or not the high pH environment adversely affected the physiological characteristics of the algae. The microscopic counts were also used to determine if this environmental change had any influence on the organism concentration distribution for the various algal species within the culture.

It was found that the nitrate-nitrogen concentration in the effluent from the algal culture decreased quite dramatically during a steady-state culturing period at the high pH level of 9.5. Figure 29 illustrates the pattern of nitrate-nitrogen concentration reduction in the culture effluent throughout the period of study. It is shown that the nitrate-nitrogen concentration decreased from a high value of 8.00 milligrams per liter to a low value of 1.90 milligrams per liter over a culturing period of 3 days; and that this concentration decrease was a logarithmic function of the culturing time. The line of best fit through the reported data provides a negative slope of 0.205 $\log_{10}$ unit per day, which also can be used to express the rate of nitrate-nitrogen concentration change in the culture effluent during this particular culturing period.

Following the culture study at the pH level of 9.5, the influence of various other pH levels on the algal culture growth activity was investigated. The affect of the different culture pH levels on the rate of algal growth activity in the culture is pictured in Figure 30.

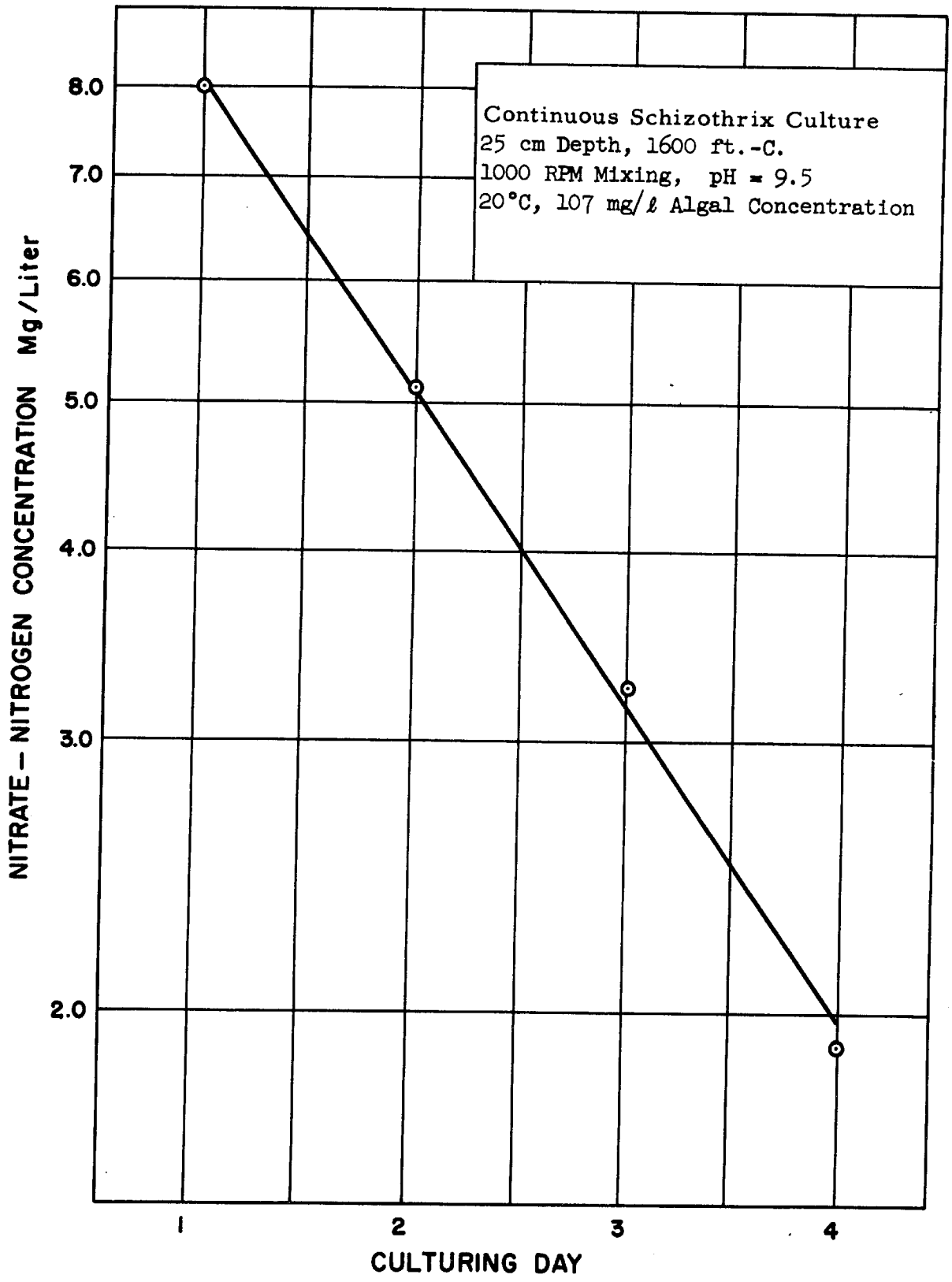


Figure 29. Culture Effluent Nitrate-Nitrogen Concentration as a Function of Culturing Day at a Constant pH of 9.5

As can be seen, the culture pH range from 7. 0 to 8. 5 had a positive influence on the growth activity, while the range from 8. 5 to 10. 0 negatively influenced the algal growth to a greater extent than the positive influence. The maximum rate of growth activity occurred with the culture pH being maintained at the 8. 5 level. This pattern of growth activity variation, due to an environmental change, does not conform with the generalized environmentally stimulated growth activity trend for algae, which was previously described. In this situation, which is depicted in Figure 30, there are two areas of growth limitation on either side of a maximum rate of growth value. This indicates that during the culture pH study there was an optimum culture pH value that stimulated maximum algal activity instead of a range of pH values that were non-limiting to the growth characteristics of the algal culture.

Under the varying culture pH conditions, the nitrate-nitrogen concentration in the culture effluent experienced further changes. This concentration decreased from the low value of 1. 90 milligrams per liter at pH 9. 5 to 1. 15 milligrams per liter during the culturing period at the pH level of 9. 0. Further decrease in culture pH level resulted in an increase in effluent nitrate-nitrogen concentration to a value of 2. 48 milligrams per liter at the culture pH of 7. 0. The logarithmic trend of this nitrate-nitrogen concentration increase as a function of decreasing culture pH is illustrated with Figure 31. The concentration values reported are the average

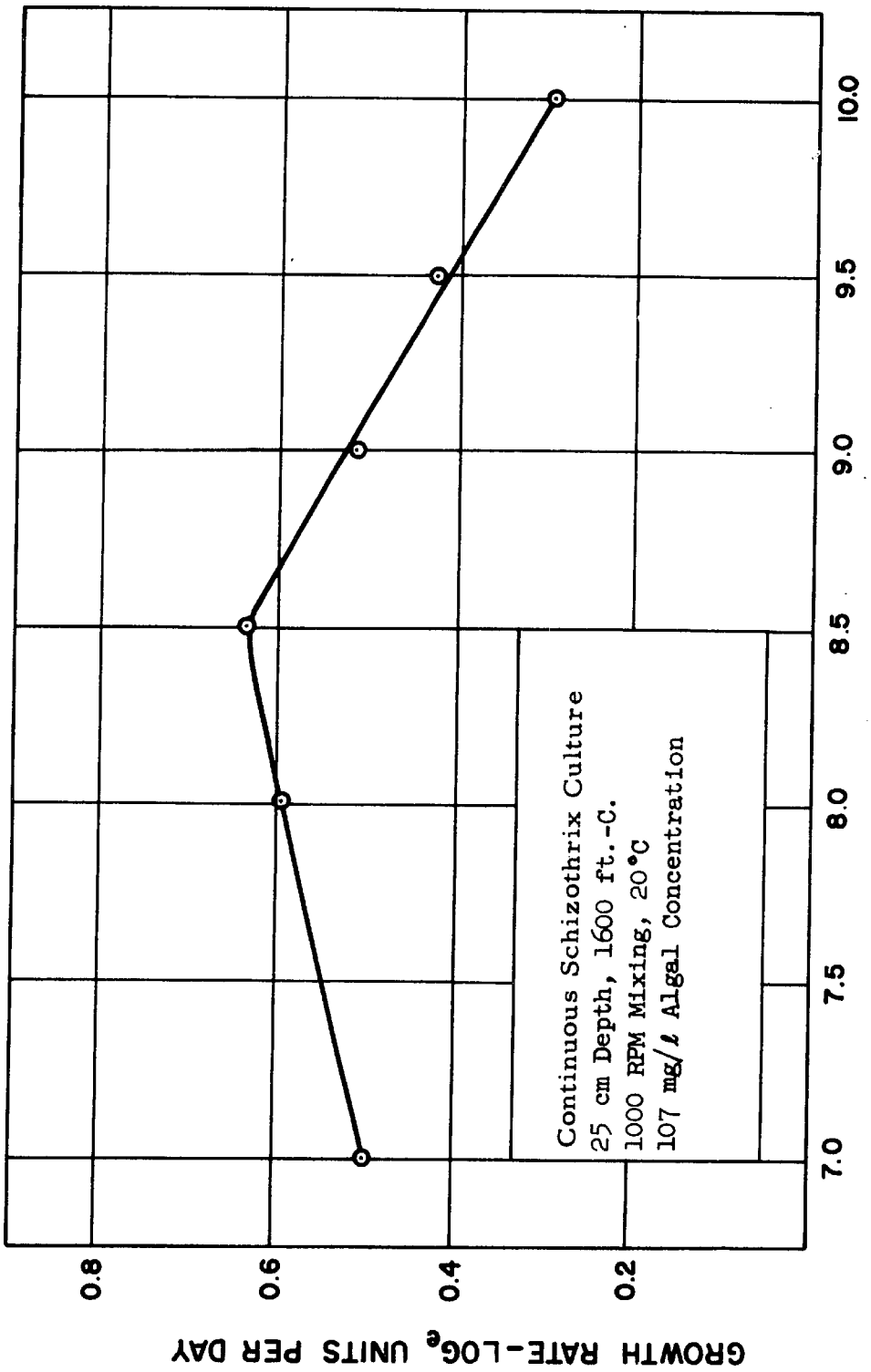


Figure 30. Rate of Algal Growth Activity as a Function of Culture pH

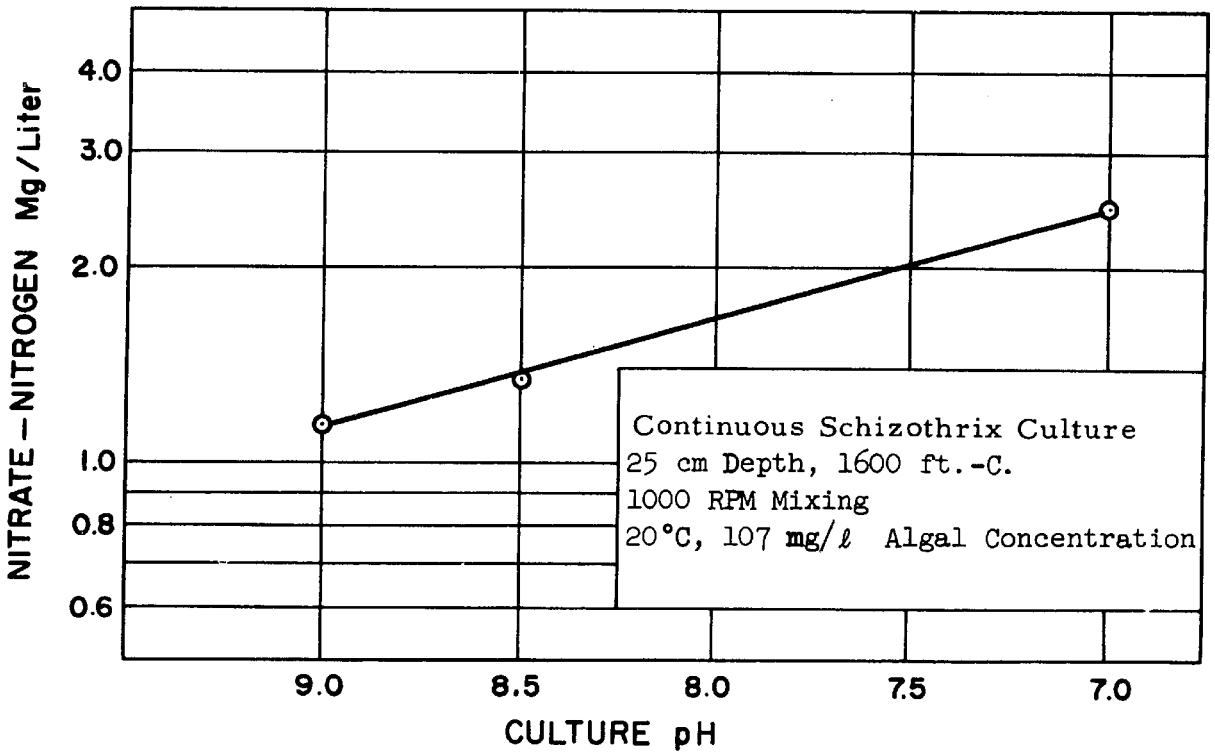


Figure 31. Culture Effluent Nitrate-Nitrogen Concentration as a Function of Decreasing Culture pH

effluent nitrate-nitrogen concentrations during the culturing periods at the pH levels of 9. 0 and 8. 5, and the maximum effluent concentration attained during the culturing period at pH 7. 0. Further evaluation of this increasing trend indicates that the rate of effluent nitrate-nitrogen increase, during the course of this study, was 0. 159 $\log_{10}$ unit per decreasing unit of pH.

During the culturing period at the pH level of 7. 0, the nitrate-nitrogen concentration in the culture effluent experienced a significant daily increase. The characteristic pattern of this effluent concentration increase is indicated by Figure 32. Again the trend is logarithmic, in agreement with the other patterns of effluent nitrate-nitrogen concentration change. The rate of effluent nitrate-nitrogen increase under the pH 7. 0 culturing conditions is less dramatic than the rate of nitrate-nitrogen decrease during the pH 9. 5 culturing period.

Variation of the culture pH conditions also significantly influenced the algal culture's effectiveness as an extraction process for the removal of inorganic nitrogen and phosphorus from the culturing substrate. During the culturing period at the pH level of 9. 5, the percentage extraction of total inorganic nitrogen from the culture influent increased from 51 to 74 per cent, due to the abrupt decrease in effluent nitrate-nitrogen concentration. Following the 9. 5 culturing period there was further increase in the culture's effectiveness as a TIN extractor. The TIN extraction percentage

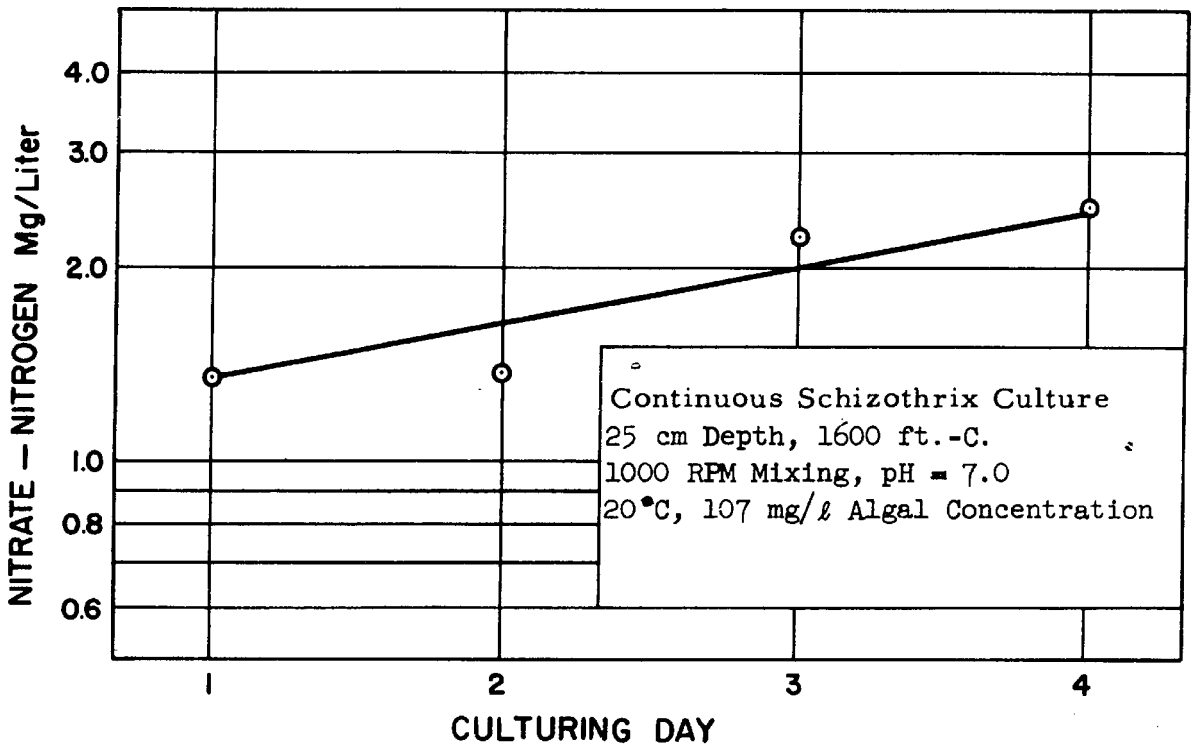


Figure 32. Culture Effluent Nitrate-Nitrogen Concentration as a Function of Culturing Day at a Constant pH of 7.0

finally attained an equilibrium of about 84 per cent, which was maintained throughout the culturing periods at the pH levels of 9.0, 8.5 and 7.0.

The influence that the varying culture pH levels had on the algal culture's ability to extract orthophosphate from the culture influent is shown in Figure 33. As can be seen, the change in the per cent of orthophosphate extracted from the culture influent is an arithmetic function which is directly proportional to the increasing culture pH levels. The percentage values reported are the average extraction percentages during the culturing periods at the pH levels of 9.5, 9.0 and 8.5, and the lowest extraction percentage during the culturing period at pH 7.0. The increase in orthophosphate extraction up to the pH level of 8.5 was no doubt due to the increased algal growth activity throughout the pH range from 7.0 to 8.5. While the increased orthophosphate removal above the 8.5 pH level was probably due to phosphate precipitation at the high pH levels, since the rate of algal growth activity significantly decreased through the pH range of 8.5 to 10.0. During the culturing period at the pH level of 7.0, the percentage of orthophosphate extracted noticably decreased with each culturing day. As shown in Figure 34, this decreasing percentage was a curvilinear function of the culturing day. When the reciprocal of the orthophosphate extraction percentage is evaluated as a function of the culturing day, a linear relationship, with a positive slope, is the result. From this linear relationship, one can observe a constant rate of

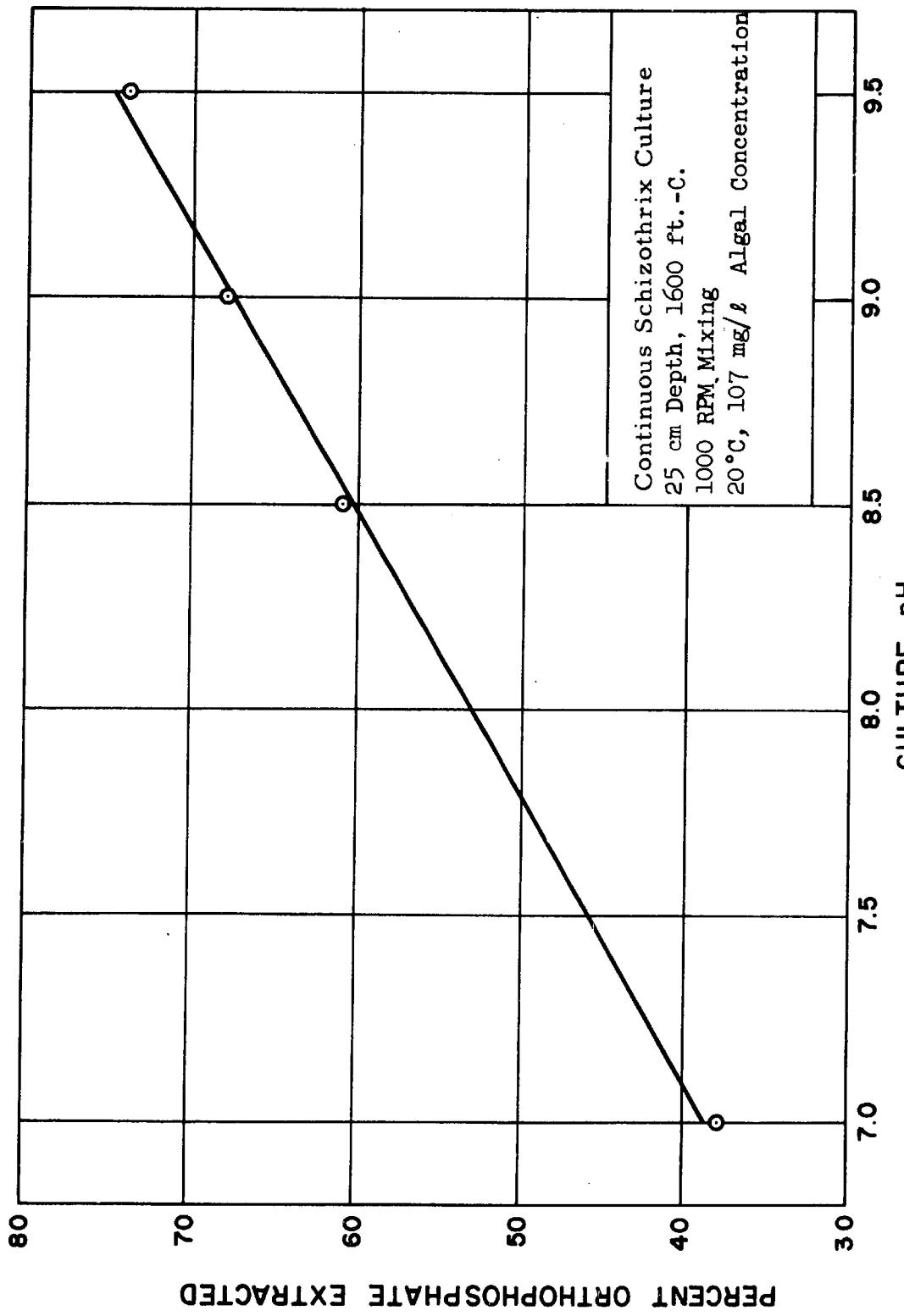


Figure 33. Orthophosphate Extraction as a Function of Culture pH

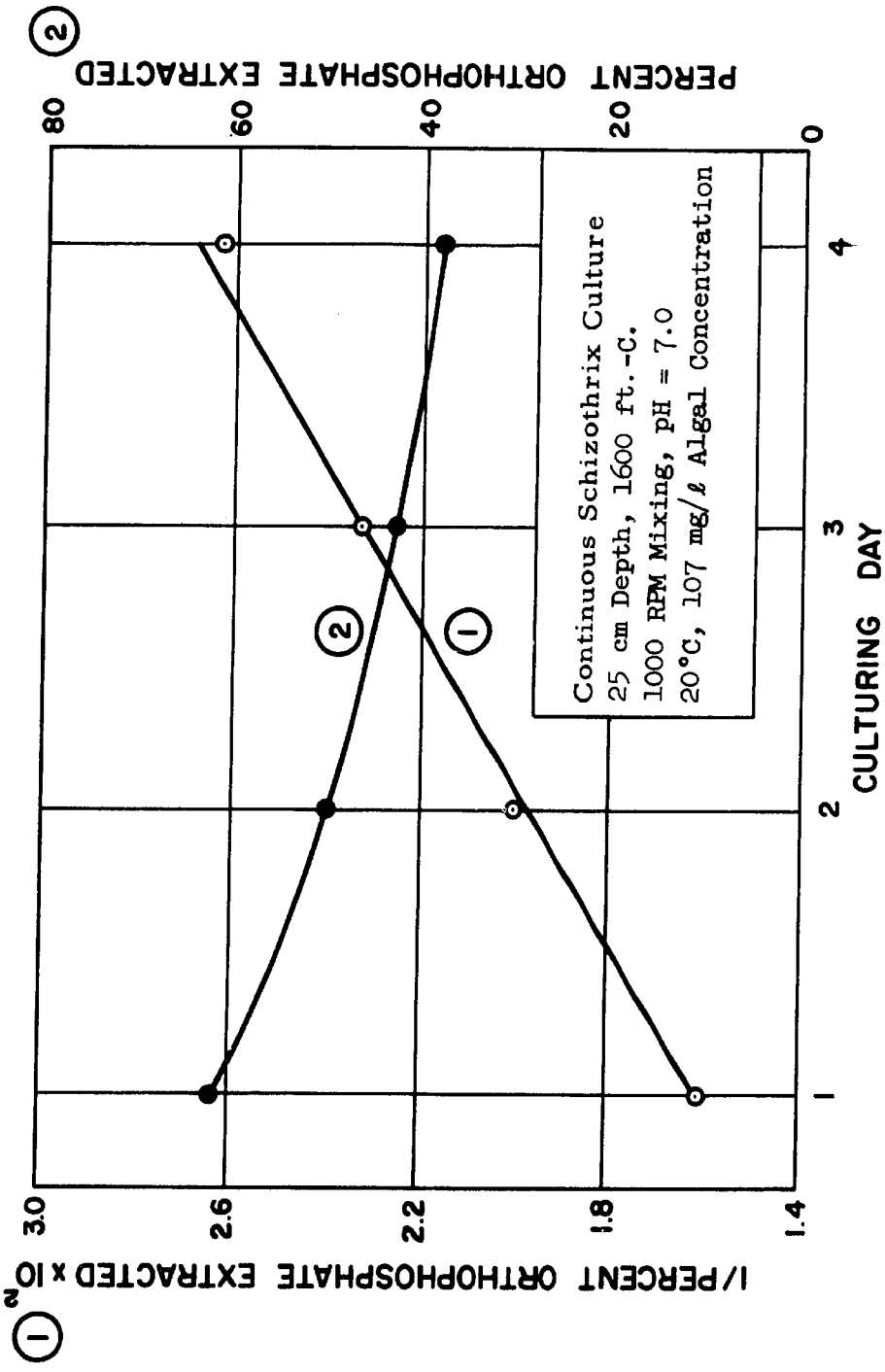


Figure 34. Orthophosphate Extracted as a Function of Culturing Day at a Constant pH of 7.0

reduction in orthophosphate extraction percentage throughout the culturing period, at the culture pH level of 7.0.

From this culture pH study it was determined that a high nitrate-nitrogen concentration in the effluent from a controlled algal culture can be effectively reduced and maintained at a desirably low level by poising the culture pH at a level within the range of 9.0 to 9.5. This pH range appears to significantly retard the growth activity of nitrogen converting bacteria, so as to eliminate the development of high nitrate-nitrogen levels in the culture effluent. It was also found that when the culture pH was returned to a level that is indicated to be desirable for the nitrifying bacteria, the effluent nitrate-nitrogen concentration significantly increased during a culturing period at this pH level. The varying culture pH conditions influenced the effectiveness with which the algal culture extracted the inorganic nitrogen and orthophosphate from the culture influent. The nitrogen and orthophosphate extraction percentages were greatly improved under the high culture pH conditions. The routine physical determinations, that were performed on a daily basis, revealed that the changing pH conditions did not significantly alter the physiological characteristics of the algae or organism concentration distribution of the various types of algae within the culture. Additional culture pH studies are planned for the purpose of developing systematic models to be used in an attempt to further define the nutrient extraction process within the environmentally controlled algal culture.

V. SUMMARY

An investigation was performed for the purpose of discerning the influencing patterns of various lighting conditions and liquid depths on the growth activity of environmentally controlled algal cultures. This study is part of an over-all effort to develop engineering design criteria for the development of optimum algal environments, to be applied as tertiary waste treatment processes. This investigation was divided into the three major categories of batch culture studies, culture liquid depth studies and culture pH studies. The first two categories were concerned with the influence that the lighting conditions and the varying liquid depth had on the algal growth activity; while the third category was involved with the use of culture pH to retard or eliminate an undesirable process problem that developed in the deeper cultures of the liquid depth study.

BATCH CULTURE STUDIES

Two batch culture studies were carried out at different culture liquid depths to evaluate the affect of a high intensity light source on the algal growth characteristics and to confirm that depth-density relationships exist in actively growing algal cultures. An available batch culturing unit was used, with the light source being a 1000-watt incandescent lamp which developed an intensity at the face of the culturing chamber of approximately 5000 foot candles. Turbulent

mixing was maintained within the culture chamber throughout the studies.

The culturing organism was Chlorella pyrenoidosa, originally obtained from the University of Indiana and maintained in the University of Michigan Sanitary Engineering Laboratories, in stock culture form, on proteose agar slants. Synthetic media was used as the culturing substrate, with nutrient concentrations adequate enough to minimize the consideration of algal growth limitation due to nutrient depletion.

Culture growth characteristics were determined by routine analysis of the per cent light transmission at a wavelength of 425 millimicrons, algal organism concentration and algal dry weight concentration. The increases in algal organism concentration and dry weight concentration were graphed on semi-logarithmic paper as a function of culturing time, in order to observe the algal growth trends in the two cultures. Culture pH was controlled by continuously feeding a small amount of 100 per cent carbon dioxide into the culture chamber.

The high intensity light source had a very dramatic affect on the algal growth activity within the two batch cultures. In general, the culture growth, as plotted on semi-logarithmic paper, followed typical biological growth patterns, with the cultures experiencing various decreasing rates of growth in their travel toward eventual maximum organism and maximum dry weight concentrations. It is felt that these maximum culture density levels were the result

of light limiting conditions that developed within the cultures. The culture density values of 135 million organisms per milliliter and 800 milligrams per liter for the 40 centimeter culture, and 300 million organisms per milliliter and 1550 milligrams per liter for the 20 centimeter culture were the highest algal culture densities so far attained in the University of Michigan Sanitary Engineering Laboratories, under culturing conditions of this nature. Therefore, it was decided that the high intensity light source had a very desirable influence on the algal cultures and was able to maintain extremely dense algal cultures in a state of growth.

Depth-density values for the two cultures were obtained by multiplying the numerical value of the liquid depth by the numerical values of the maximum culture densities that were attained. The computed depth-density values were used as dimensionless, numerical factors. It was found that when the density value of dry weight concentration was used to compute the depth-density factor, the two culture factors very closely equaled each other; whereas, when the density value of organism concentration was used, there was some disagreement between the depth-density factors. The results of these batch culture studies agreed with the suggested depth-density relationships between algal cultures, and revealed that a truer indication of this relationship between algal cultures can be obtained by incorporating the dry weight density parameter into the computing of the culture depth-density factor.

CULTURE LIQUID DEPTH STUDIES

Continuous, constant volume algal culturing studies were performed for the purpose of evaluating the influences of varying incident light intensity and culture liquid depth on the growth activity of the algae and on the ability of the algae to extract fertilizer nutrients from waste treatment plant effluents. Photo-electrically controlled, constant density algal culturing techniques were employed, with the varying incident light intensity being used to regulate the growth activity of the algal cultures. The light source was 12, 110-watt Gro-Lux fluorescent lamps, developed by Sylvania Corporation for the specific purpose of stimulating photosynthesis. The intensity output from the light source was regulated by the number of lamps in operation. An incident light intensity range from 800 to 2600 foot candles was used in this investigation. In order to overcome the problem of algal cell self-shading in the culture, a relatively constant depth-density factor was maintained throughout the liquid depth study. A controlling depth-density factor of 2500 was arbitrarily selected and was used as the guide for proper culture density adjustment whenever the liquid depth was changed. The routine dry weight concentration analyses revealed that an over-all average depth-density factor of 2635 was maintained, during the total liquid depth study.

The algal culturing substrate was sterilized effluent from the Ann Arbor Waste Water Treatment Plant. Following

sterilization the effluent was fortified with reagent grade chemicals, in order to reestablish its original chemical quality. Dominant algal cultures were maintained, with the dominant organism being the filamentous blue-green alga, Schizothrix calcicola (Ag.) Gom., which was originally isolated from the treatment plant effluent. Major algal contaminants, in the three different depth cultures, were forms of Chlorella and Scenedesmus. In addition, a significant population of diatoms developed in the 25 centimeter culture.

After determining the appropriate dry weight concentration to give the control depth-density factor at the particular depth being studied, the culture was allowed to adjust to this density level. A per cent light transmission at a wavelength of 425 millimicrons was then associated with the adjusted culture density. This per cent transmission value was then used as the basis for monitoring and adjusting the constant density control systems. The algal culture density was routinely evaluated by microscopic and Coulter Counter counts to determine the organism concentration and type distribution, by dry weight determinations, and by per cent transmission readings. In addition, periodic light intensity and mixer speed measurements were made, along with daily chemical analysis of the culture influents and effluents, in order to determine the effectiveness with which the cultures were extracting the fertilizer nutrients, under the established culturing conditions. The algal growth activity for the various culturing conditions was indicated by the rate of culture overflow.

Results of the liquid depth study indicated that the varied incident light intensity influenced the algal culture growth activity in a manner that is generally typical of how environmental changes affect algal growth. Throughout the light intensity range of 800 to 2050 foot candles, the increasing algal growth was directly proportional to the increasing light intensity for the three different depth cultures. Light intensity increases beyond 2050 foot candles, to the limits of this study, had no additional influence on the growth activity of the algal cultures. Therefore, the incident light intensity level of 2050 foot candles was selected as the light saturation intensity for the algal cultures studied. The most active algal culture was the 15 centimeter culture. A significant decrease in growth activity was experienced when the culture depth was increased to 25 centimeters. No additional decrease in algal activity occurred, when the culture depth was increased to 40 centimeters. Another observation was that the changing culture depth did not affect the trend of light intensity influence on the growth pattern for the different cultures. From this, it was decided that equal lighting conditions can be maintained within various depth algal cultures by establishing a relatively constant depth-density relationship between the different cultures.

The culture surface productions and volume productions also were influenced by the varying incident light intensity. Highest surface production of algal material was developed in the 25 centimeter culture, indicating that this culture made somewhat more effective use

of the incident light energy than the other two cultures. The 15 centimeter culture developed the highest volume production of algal material, indicating that this culture was the most efficient user of the culturing substrate. As the culture liquid depth increased, the degree of influence that the increasing light intensity exerted on the culture volume production lessened. These data observations reveal that in order to develop an algal environment which will stimulate the highest utilization of the culturing substrate, the most effective use of the incident light energy must be sacrificed. It is felt that this sacrifice is justified, since in the application of algal culturing processes to tertiary waste treatment, the effective utilization of the culturing substrate is of the highest concern.

The bio-extraction of algal nutrients from the culturing substrate by the different cultures was also influenced by the varying incident light intensity. Evaluation of the data indicated that the nutrient extracting effectiveness of the different cultures was a function of the variously determined culture growth characteristics. In the case of all data considerations, the 15 centimeter culture was the most effective nutrient extractor. For this culture, the maximum percentage extraction of TIN and orthophosphate was 96.7 and 66.9, respectively.

In all of the nutrient extraction considerations, the information presented for the 25 centimeter culture was somewhat erratic and did not conform with the established trends for the other

two cultures. As indicated previously, the 25 centimeter environment stimulated the development of a significant population of diatoms, which remained for the duration of the 25 centimeter study. It is possible that this occurrence of significant organism contamination might be the reason for the non-conformity of the 25 centimeter culture data. Nutrient extraction by the diatoms might follow a different pattern and be less effective than that by blue-green and green algae. Further research in an attempt to confirm these speculations might be a worthy endeavor, since the literature appears to be quite lacking on this subject.

During the 25 and 40 centimeter culture studies, significant nitrogen conversion from the ammonia species to the nitrate species occurred in the culture liquids. It appears that these algal environments must have induced the growth of nitrogen converting bacteria; and that under the low growth rate and long residence time conditions of the 25 and 40 centimeter cultures, the bacteria had adequate opportunity to effect significant nitrogen conversion. Along with this, the algae appeared to exhibit a nitrate-nitrogen sparing reaction in the presence of the ammonia-nitrogen species, so the high nitrate concentrations remained in the culture effluents. Some nitrogen conversion also occurred in the 15 centimeter culture, but it was of little significance. The total nutrient extraction data evaluation indicated that the algal environment which stimulated the most active algal culture was also the most effective algal nutrient extractor, when

waste treatment plant effluent was used as the growth substrate.

ALGAL CULTURE pH STUDIES

Culture pH studies were performed for the purpose of investigating the use of culture pH as a process control technique to eliminate and control the nitrogen converting problem in algal cultures. It was decided that since the nitrogen converting bacteria have an optimum pH range (7. 7 to 8. 6) for effective growth, culture operation outside of this pH range might retard the activity of these bacteria. After the culturing conditions for nitrogen convertion were established and the nitrate concentration in the culture liquid reached a significantly high level, the culture pH was allowed to increase to 9. 5 for the beginning of this study. The previously mentioned routine culture analyses were again performed in conjunction with this study.

The high culture pH level had a very dramatic affect on the nitrate-nitrogen concentration in the culture effluent. During a three day culturing period at the pH level of 9. 5, the nitrate-nitrogen concentration in the culture effluent decreased from 8. 00 to 1. 90 milligrams per liter. The effluent nitrate-nitrogen concentration experienced further decrease to a minimum of 1. 15 milligrams per liter during the culturing period at a pH of 9. 0. Upon further decrease in pH level, the effluent nitrate-nitrogen remained at a low value, until the desirable pH range for nitrogen converting bacteria was again reached. During a culturing period at the pH level of 7. 0, the effluent

nitrate-nitrogen concentration gradually began to increase, indicating that the nitrogen converting process was slowly being reactivated.

Varying the culture pH level also influenced the algal growth activity. During the course of this study, the algal culture experienced an optimum pH level which stimulated the highest rate of culture growth activity. On either side of this optimum pH level of 8.5, the varying pH levels adversely affected the rate of culture growth.

In addition to influencing the algal culture growth activity, the altered pH levels had an affect on the bio-extraction of algal nutrients from the growth substrate. An increase in the percentage extraction of TIN was experienced during the high pH culturing, due to the decreased quantity of nitrate-nitrogen in the culture effluent. The percentage of orthophosphate extracted was also favorably affected, being increased with increasing culture pH level. During the pH range up to the optimum pH level, the increased orthophosphate extraction was no doubt due to increased algal activity; while during the pH range following the optimum level, the increased orthophosphate extraction was probably the result of increased precipitation of the orthophosphate, since the algal growth activity decreased through the pH range above the optimum level. The results of this study indicate that the nitrogen converting process within algal cultures can be effectively retarded and controlled by judicious pH adjustment and control, and that this process control technique significantly improves the nutrient extracting characteristics of an algal culture.

VI. CONCLUSIONS

Based upon the results of the previously described investigation, the following conclusions are presented. These conclusions apply only to the scope of this investigation, and projections beyond the limitations of this study should not be considered valid without confirmation through experimental observation.

BATCH CULTURE STUDIES

1. The high intensity light source had a very desirable affect on batch Chlorella cultures and was able to maintain extremely dense algal cultures in a state of growth.
2. The suggested depth-density relationship between different depth algal cultures appears to exist among actively growing cultures.
3. A close agreement between the depth-density factors of different depth algal cultures can be obtained if the dry weight concentration, culture density value is incorporated into the computation of the depth-density factor.

CULTURE LIQUID DEPTH STUDIES

1. The rate of algal growth activity for the three different depth cultures was a function of the incident light intensity.
2. The 15 centimeter culture exhibited the highest degree of photosynthetic activity.

3. Algal culture growth activity was significantly decreased when the culture depth was increased to 25 centimeters, but no further decrease in growth activity was experienced when the liquid depth was increased to 40 centimeters.
4. It was possible to maintain equal lighting conditions within the different depth algal cultures by establishing a relatively constant depth-density relationship between the different cultures.
5. The culture volume production of algal material was a function of the incident light intensity and culture liquid depth.
6. For a given incident light intensity value, the culture volume production of algal material increased with decreasing culture liquid depth.
7. The percentage extraction of TIN from the culturing substrate tended toward a maximum level along a curved path as the light intensity increased; while the percentage extraction of ortho-phosphate appeared to be an exponential function of the increasing light intensity in the three different cultures.
8. The 15 centimeter culture was the most effective extractor of algal nutrients from the culturing substrate.
9. Low growth rate and long residence time culturing conditions induced significant nitrogen conversion in the culture liquid; and due to apparent nitrate sparing reactions by the algae, high nitrate-nitrogen concentrations remained in the culture effluents.

10. Under optimum culturing conditions at least 97 per cent of the TIN and 67 per cent of the orthophosphate can be bio-extracted from waste treatment plant effluent.
11. The algal environment that was most conducive to photosynthetic activity was also the most effective extractor of algal nutrients from the culturing substrate.
12. Information concerning the volume production of algal material by environmentally controlled algal cultures is of highly significant importance, when considering the application of this process to tertiary waste treatment.

ALGAL CULTURE pH STUDIES

1. The high pH culturing technique effectively retarded the undesirable nitrogen conversion process that existed in the low growth rate and long residence time algal cultures.
2. Upon return of the culture pH to the optimum range for nitrogen converting bacteria, it was evident that the nitrogen conversion process was slowly being reactivated.
3. During the culture pH studies the highest degree of algal growth activity was experienced at the pH level of 8. 5.
4. The high pH culturing conditions also desirably influenced the nutrient extracting abilities of the algal culture.
5. It is predicted that with continuous algal culture operation at a pH level of about 9.0, nitrogen conversion will not become a culturing problem and effective algal utilization of the growth substrate will prevail.

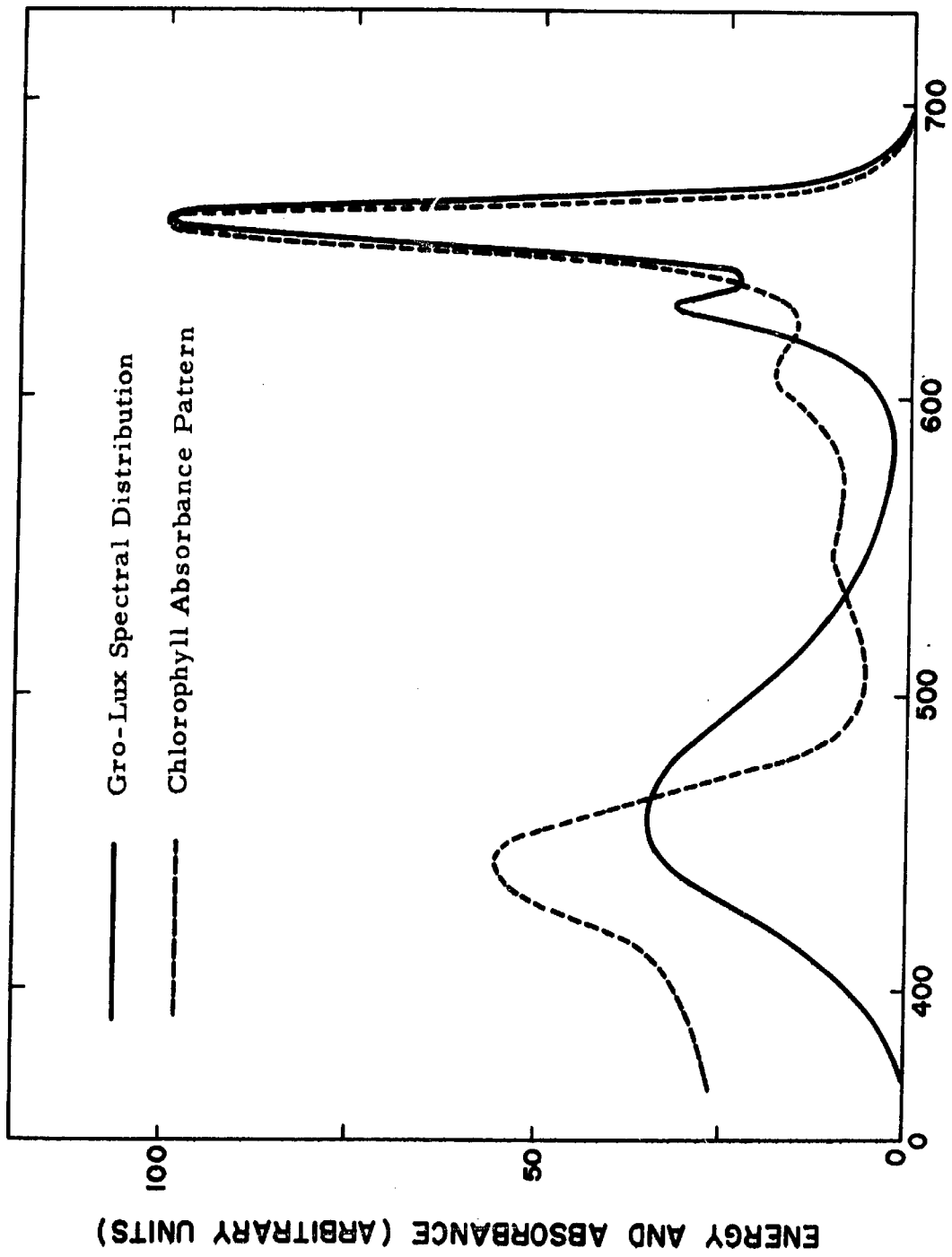


Figure 35. Spectral Energy Distribution for Gro-Lux Fluorescent Lamps and Energy Absorbance Pattern for Chlorophyll (122)

Relative Amounts of Photosynthetically Effective Light Energy From Various Fluorescent Lamps, Expressed in Arbitrary Units (123)

Lamp	Effective Energy		Total
	400 - 500 mμ	600 - 700 mμ	
Warm White	35	65	100
White	50	58	108
Cool White	64	50	114
Daylight	78	56	134
"Gro-Lux"	80	150	230

In this table the effective energy output from the warm white lamp is taken as the base value.

APPENDIX II

Chemical Characteristics of Unsterilized and Sterilized Ann Arbor Waste Water Treatment Plant Effluent

	Unsterilized mg/liter	Sterilized mg/liter
NH_3 - N	28.76	17.10
NO_2 - N	0.48	0.53
NO_3 - N	1.79	1.55
Ortho - PO_4	13.74	11.59
pH	7.54	9.12
Total Alkalinity (as $CaCO_3$)	210	176

The above chemical results are the averages of the chemical analyses performed on 23 collections of effluent, throughout the culture depth study. Appropriate quantities of the reagent grade chemicals of ammonium chloride, potassium phosphate, sulfuric acid and sodium bicarbonate were added to the effluent after each sterilization, so as to reestablish the chemical quality that the effluent had before sterilization.

APPENDIX III

GROWTH MEDIUM USED FOR BATCH CULTURE STUDY (124)

Growth Medium B:

Nutrient Chemical	grams/liter of culture
$Ca(NO_3)_2 \cdot 4H_2O$	0.025
KNO_3	1.000
$Mg\ SO_4 \cdot 7H_2O$	0.250
K_2HPO_4	1.000
$FeCl_3 \cdot 6H_2O$	0.035
Na - Citrate $\cdot$ $2H_2O$	0.222
Micronutrients	1 ml/liter

A_5 Micronutrient Stock Solution:

Nutrient Chemical	grams/liter
H_3BO_3	2.860
$MnCl_2 \cdot 4H_2O$	1.810
$ZnSO_4 \cdot 7H_2O$	0.222
MoO_3 (85%)	0.0177
$CuSO_4 \cdot 5H_2O$	0.079

Prepare stock solutions of the first four nutrients in the growth medium of such a concentration that 10 milliliters of the stock solutions per liter of medium will provide the desired nutrient concentrations. Add the nutrient solutions to distilled water in the order listed above with mixing after each addition, in order to prevent

precipitate formation. Mix the appropriate quantities of ferric chloride and sodium citrate in a separate volume of distilled water, and then add the iron-citrate solution to the medium solution and thoroughly mix. After sterilization the medium solution should be rapidly cooled, so as to redissolve precipitate formed during the sterilization. Store the micronutrient stock solution in a dark bottle.

The following iron-EDTA solution (125) is offered as an alternate to the above iron-citrate combination, as an attempt to discourage bacterial contamination of batch algal cultures--

> Dissolve 26.1 grams of EDTA (ethylenediamine tetraacetic acid) in 268 milliliters of 1.0 N KOH. Add 24.9 grams of $FeSO_4 \cdot 7H_2O$ and dilute solution to 1 liter with distilled water. Aerate solution overnight in order to form a stable ferric-EDTA complex. Store solution in a dark bottle. One milliliter of this stock solution per liter of culture medium will provide an iron concentration of 5 milligrams per liter.

This iron-EDTA solution was not used in the batch culture studies reported in this dissertation, but recent use of this solution in the Sanitary Engineering Laboratories at The University of Michigan has proven it to be quite satisfactory.

APPENDIX IV

ANALYTICAL PROCEDURES FOR CHEMICAL DETERMINATIONS

Determination: Ammonia-Nitrogen (117)

Procedure:

1. Dilute a 5 or 10 milliliter sample to 100 milliliters with ammonia-free distilled water in a volumetric flask.
2. To 10 milliliters of the above dilution add 3 drops of Rochelle salt solution and thoroughly mix.
3. Add 1 milliliter of sodium hydroxide solution followed by 1 milliliter of modified Nessler reagent. Mix thoroughly after each addition.
4. Allow 10 minutes for color development. Measure color density in a spectrophotometer at a wave length of 410 millimicrons and with a 13 millimeter light path.
5. Zero the spectrophotometer with a blank solution of ammonia-free distilled water plus reagents.

Reagents:

1. Rochelle Salt Solution - Dilute 50 grams of potassium sodium tartrate, $KNaC_4H_4O_6 \cdot 4H_2O$, to 100 milliliters.
2. Sodium Hydroxide Solution - Dilute 160 grams of NaOH to 1 liter.
3. Modified Nessler Reagent - Dilute 100 grams of HgI_2 and 70 grams of KI to 1 liter. Store in a dark bottle.

Note--Use ammonia-free distilled water for all reagent preparations.

Determination: Nitrite-Nitrogen (117)

Procedure:

1. Use a 10 milliliter sample from the dilution preparation described in item 1 of the ammonia-nitrogen procedure.
2. Add 1 milliliter of the sulfanilic acid reagent, mix thoroughly and allow to stand for approximately 3 minutes.
3. Add 1 milliliter of 1-naphthylamine hydrochloride reagent, followed by 1 milliliter of sodium acetate buffer solution. Mix thoroughly after each addition.
4. Allow 20 minutes for color development. Measure color density in a spectrophotometer at a wave length of 510 millimicrons and with a 13 millimeter light path.
5. Adjust the spectrophotometer to 100% transmission with a blank solution containing ammonia-free distilled water plus the reagents.

Reagents:

1. Sulfanilic Acid Reagent - Dissolve 0.60 gram of sulfanilic acid in 70 milliliters of hot distilled water, cool, add 20 milliliters of concentrated HCl and dilute to 100 milliliters. Mix thoroughly.

2. 1-Naphthylamine Hydrochloride Reagent - Dissolve 0.60 gram of 1-naphthylamine hydrochloride in distilled water containing 1.0 milliliter of concentrated HCl. Dilute to 100 milliliters and mix thoroughly. Store in refrigerator.
3. Sodium Acetate Buffer Solution - Dissolve 16.4 grams of $Na\,C_2\,H_3\,O_2$ or 27.2 grams of $Na\,C_2\,H_3\,O_2 \cdot 3\,H_2O$ in distilled water and dilute to 100 milliliters. Filter if necessary. Store in a dark bottle.

Note--Use distilled water for all reagent preparations.

Determination: Nitrate-Nitrogen (117)

Procedure:

1. Place a 10 milliliter sample or a 5 milliliter sample diluted to 10 milliliters in an evaporating dish and evaporate to dryness.
2. Dissolve the remaining residue in the dish in 2 milliliters of phenoldisulfonic acid.
3. Transfer acid mixture to a 100 milliliter Nessler tube. Wash the dish 3 times with distilled water, adding each wash to the Nessler tube. Adjust volume in Nessler tube to about 30 milliliters with distilled water.
4. Add approximately 6.5 milliliters of potassium hydroxide solution to the Nessler tube. Dilute mixture to 50 milliliters with distilled water and thoroughly mix.

5. Measure color density in a spectrophotometer at a wave length of 410 millimicrons with a 13 millimeter light path.
6. Adjust the spectrophotometer to 100% transmission with a blank solution containing distilled water plus the reagents.

Reagents:

1. Phenolfisulfonic Acid - Dissolve 25 grams of pure white phenol in 150 milliliters of concentrated H_2SO_4. Add 75 milliliters of fuming H_2SO_4 (15% free SO_3) and mix thoroughly. Heat for 2 hours over a hot water bath. Prepare this solution with care under a hood.
2. Potassium Hydroxide Solution - Dissolve 673 grams of KOH in distilled water, allow to cool and then dilute to 1 liter with distilled water.

Determination: Ortho-Phosphate (117)

Procedure:

1. Dilute a 5 or 10 milliliter sample to 50 milliliters with distilled water in a Nessler tube.
2. Add 2.5 milliliters of ammonium molybdate solution, followed by 5 scoops (0.5 gram) of Stanna Ver Powder. Mix thoroughly.
3. Allow 10 minutes for color development. Measure color density in a spectrophotometer at a wave length of 700

millimicrons with a 13 millimeters light path. Place red filter in front of the spectrophotometer's light sensing cell before taking readings.

4. Adjust the spectrophotometer to 100% transmission with a blank solution of distilled water plus reagents.

Reagents:

1. Ammonium Molybdate Solution - Dissolve 25 grams of $(NH_4)_6\ Mo_7\ O_{24}\ 4H_2O$ in 175 milliliters of distilled water. Carefully add 310 milliliters of concentrated H_2SO_4 tc 400 milliliters of distilled water. After acid solution has cooled, add molybdate solution to it and dilute to 1 liter with distilled water.
2. Stanna Ver Powder - Hach Chemical Company, Ames, Iowa, Catalog No. 293.

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