THE EFFECT OF LOW LEVEL CONCENTRATIONS OF 2,4 DICHLOROPHENOXYACETIC ACID ON *CHLORELLA PYRENOIDOSA*

by

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TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

I. INTRODUCTION

Many communities obtain their water supplies from streams, lakes and reservoirs. These waters contain algae which may cause nuisance problems when growing in excessive amounts. Algal blooms may be the result of nutrient influx from runoff or wastewater discharges. The social and economic consequence of algal blooms depends largely upon the purpose for which a given body of water is used.

Algal blooms may be responsible for several water supply problems. Algae release gases and excretory products which may impart taste and odor to water. Color in water may result due to excessive algal growth. Algae attach to submerged rocks, wood and soil and on the inside of water supply lines sometimes forming continuous carpets of growth (1). Growth of algae on surfaces may become detached due to water turbulence. Detachment prior to filtration in the water treatment process may cause head loss and shorter filter runs. If detachment occurs following filtration, turbidity in water supplied to consumers may result. The pH of waters withdrawn for treatment may vary considerably due to the presence of algal populations. During the day algae use carbon dioxide from the water for photosynthesis which consequently raises the pH. Respiration at night lowers the pH due to the release of carbon dioxide back into the water. Changing pH requires changes in the dosages of chlorine,

alum and other chemicals added to the water at the treatment plant. Chlorine reacts with water to produce hypochlorous acid and hypochlorite ions, which are the strongest oxidizing and disinfecting agents of chlorination. Hypochlorous acid, which is the better disinfecting agent, is more prevalent at a pH below 7. Photosynthesis raises the pH above 7, therefore additional chlorine is required to provide equivalent disinfection power when the hypochlorite ion predominates. The hydrogen ion concentration also effects the capacity of alum to combine with negatively charged colloids in the coagulation process of water treatment. Lower pH levels tend to favor aluminum hydrolysis products of the highest positive charge which are generally the most effective in coagulating naturally occurring colloidal matter.

Blooms may be the cause for complaints by persons using the water for recreational use. The green color and odor produced by algal growth is aesthetically unpleasing and imparts an impression of a polluted body of water. The fishing industry may suffer from large algal populations because blooms may deoxygenate water and kill fish, thereby reducing the fisherman's income.

Many reports indicate that low level concentrations of auxin or auxin herbicide may stimulate the development of algae as well as higher plants. This matter is significant because herbicides may enter a body of water in several ways (2):

1. direct application for control of aquatic weeds

2. drift from aerial land applications
3. discharge from aerial land applicantions
4. percolate and runoff from agricultural land
5. discharge in wastewater from cleanup of equipment used for application.

The auxin herbicide,2,4-dichlorophenoxyacetic acid (2,4D), has been reported in lakes and reservoirs treated directly for aquatic weed control and in water from runoff of land application several days after the treatments were made. Averitt (3) applied an amine of 2,4D to water hyacinth at 4 lb 2,4D/acre. Ten parts per billion (ppb) were found after three weeks and 0.6 ppb of 2,4D were detected at twenty weeks. When 4 lb 2,4D/acre were injected into the water at two different locations, 689 and 967 ppb were detected after one day. Thirty-one days later 11 ppb was detected at the first site and 19 ppb at the other. In other tests using 4 and 5 lb 2,4D per acre, 4 and 15 ppb were detected after 29 days. Tarrant and Norris (4) sprayed a forest with low volatile 2,4D esters diluted with diesel oil at a level of 2 lb/acre. This gave residues of 0.2 to 70 ppb in adjacent waters. Maximum time for disappearance was 2 days for one station and 17 at another. Smith and Isom (5) applied 40 to 100 lbs/acre of butoxy ethanol ester of 2,4D onto a submerged aquatic plant, Myrephyllum spicatum, and found residues of 0.068 mg/l eight hours after application. Wojtalik et al. (6) treated over 18,000 surface acres of Nickajack and Guntherville reservoirs with 20 to

40 lbs/acre of 2,4D acid equivalent and found residues of 1.8 mg/1 four hours after application.

Over 400 acres of cutover land were treated with 2,4D at 2 lbs/acre to prevent broadleaf weeds (7). Several different locations were sampled three days and residues were measured in water samples and fish tissue. Concentrations ranged from 0.0022 ppm to 0.2 ppm in water samples. Sears (7) observed that spray drifted outside marked boundaries in many cases depending on wind velocity and direction and the altitude of the helicopter. Seven thousand acres along the Hillsboro perimeter canal in Loxahatchee National Wildlife Refuge, Florida, were sprayed in 1971 with 4.48 kg/ha acid equivalent of 2,4D to control water hyacinth. Stations were set up along the canal to monitor mud, fish tissue and water samples for effective levels of 2,4D. The highest 2,4D residue, 0.037 mg/1, was found one day after initial treatment. Within a few days the level decreased to less than 0.001 mg/1. The target was a thick mat of floating water hyacinths which intercepted much of the herbicide and kept it from entering the water (8).

The presence of 2,4D and other herbicides has clearly been demonstrated in water at low levels. Whether or not these concentrations are sufficient to stimulate algal growth and if these chemicals will remain in the environment long enough to cause an effect has not been demonstrated. DeOliveria and Silva (9) noted that plankton density temporarily increased following the application of a 1 percent

solution of 2,4D amine to a lake to control the aquatic weed Pontedeira. This increase in plankton was attributed to nutrients released by decaying plants killed by the herbicide. There is a possibility that the increase was not due to an increase in nutrients, but a direct effect of the 2,4D on the plankton. Recently, fish kills have been attributed to the application of an auxin herbicide, 2,4,5-T, for brush control. These "coincidental" fish kills occurred shortly after application of the herbicide. Roland (10) did not attribute the kills directly to the effect of the 2,4,5-T on the fish, but to an increase in algal density which was followed by decreasing dissolved oxygen levels (and the subsequent suffication of the fish).

It is the purpose of this paper to determine the growth response of the green alga, Chlorella pyrenoidosa, to low level concentrations of the 2,4D acid. This type of investigation is significant because algal populations dramatically affect water quality and the operation associated with the treatment of water.

II. LITERATURE REVIEW

The Test Chemical

2,4-dichlorophenoxyacetic acid (2,4D) was introduced as a selective weed killer at the end of World War II. It has high activity against many broadleaf weeds, but not against gramineous species. Thus, 2,4D is commonly used for the control of weeds in cereal crops, grass pastures and lawns. In 1969 the production of 2,4D was 47,077 thousand pounds (11) and in 1970 it was 43,576 thousand pounds (12). Production was considerably higher in the years between 1966 to 1968 because of its use as a defoliant in Vietnam. 2,4 dichlorophenoxyacetic acid is used as one of the principal herbicides because it is harmless to man and animals, reasonably inexpensive, non-corrosive to metal, inflammable, and non-explosive.

2,4D is a white crystalline substance with a molecular weight of 221.0. The melting point of this compound is 140.5°C and the heat of solution is 6.1 kcal/mole. The best analytical wavelength for spectrophotometric analysis is 283 nm and the molar extinction coefficient at this wavelength is 1900. The usual procedure for the synthesis of phenoxyacetic acid herbicides involves the reaction of an appropriate phenol with chloroacetic or 2-chloropropionic acid in alkaline, aqueous medium at a pH of 10 to 12 and at a temperature above 105°C (11).

Extensive reviews of chlorophenoxyacetic acids have been made by Audus (13), Ashton and Craft (14), Loos (11) and Audus (15). More condensed reviews on the effects of subtoxic levels of chlorophenoxy acids have been made by Wort (16), Penner and Aston (17), Moreland (18) and Hansen and Slife (19).

2,4D is a herbicide of the auxin class. Auxins may be defined as organic substances that promote growth at concentrations less than 10^{-3} M along the longitudinal axis of shoots and inhibit elongation of roots (11). It may promote or inhibit cell expansion or cell multiplication dependent on specific properties of the cells concerned and the position of the cells within organs. The sensitivity of a tissue to the stimulus of growth substances depends greatly on the stage of its differentiation. Tissues with less differentiation seem to be more sensitive. The auxin herbicides are analogs of the natural auxin, indoleacetic acid (IAA), with the capacity to take the place of IAA in reactions controlling the growth of the plant. The herbicides do not interact with the cell mechanism controlling growth stimulated by IAA. The enzyme, IAA-oxidase, breaks down IAA rapidly in the environment. 2,4D is more active and persists for a longer time since it is not broken down by this enzyme. Auxin herbicides, when used in the same concentrations as IAA, seem to show more activity than IAA. Audus (20) found 2,4D to be 450 times more active than IAA in the inhibition of root growth. Higher concentrations of both IAA and 2,4D must be used to activate cell division than

to promote cell elongation. Linser (21) demonstrated that 0.1 percent 2,4D caused a 20 percent promotion of cell elongation, while IAA at the same concentration caused 40 percent promotion. These results appeared to contradict earlier results until Van Overbeek et al. (22) noted that the activity of IAA and 2,4D should be calculated on an undissociated molecule basis. Activity seems to be the same below the concentration of 0.0015 percent for both 2,4D and IAA as measured by an auxin curvature in the tomato petiole. However, if the concentrations are corrected for dissociated molecule levels and then compared to activity curves, the activity of 2,4D is much greater. To produce a 35 percent increase in length in the pea test, a concentration of IAA 10,000 times that of 2,4D is needed.

Effect of Exogenous Auxin on Algae

Van Overbeek (23) found auxins in many types of marine plants. Red and brown algae contained from 0.05 to 0.5 gamma equivalents IAA/kg fresh weight. Van Overbeek (24) found auxin in the brown alga, Macrocystis, in a concentration of approximately 0.5 gamma equivalents IAA/kg fresh weight. The green algae, Bryopsis, had up to 80 gammas/kg and in Elodea, 50 gammas/kg fresh weight were found. The evidence that auxin is a growth hormone in Macrocystis was also observed. Buggelin and Craigie (25) discovered IAA auxin in extracts of algae treated exogenously with IAA. The levels were below 0.1 µg IAA per gram of fresh weight. Conrad and Saltman (26) discussed literature on the early work of exogenous IAA on algae. Both stimulatory and

inhibitory reports have appeared in the literature. Yin (27) reported increases in the average cell diameter of Chlorella vulgaris using 1 to 40 ppm IAA and sugar. Evidence to show that growth of treated and control cultures differed was not conclusive. Pratt (28) obtained growth stimulation of Chlorella vulgaris at 10, 50 and 100 ppm IAA. The optimum growth was at 50 ppm (2.85×10^{-4} M). It was found that cell numbers per milliliter increased up to 32 times greater than the control. No growth occurred at 200 ppm (1.14×10^{-3} M) and there was no detectable increase in cell diameter. Brannon and Bartsch (29) using a concentration of 10 ppm IAA found a 119 percent increase in growth over the control and at 0.10 ppm found a 15 percent increase in the growth of C. vulgaris as measured by dry weight of cells. The presence of dextrose increased the yield of dry weight of cells per culture from 3 mg to 19 mg (30). Algeus (31) obtained growth promotion with added auxin in 3 out of 10 species of green algae (mostly Scenedesmus and Chlorella sp.). Davidson (32) found when using concentrations between 10^{-4} to 10^{-9} M of IAA that growth was stimulated in the vegetative thali of Fucus evanescens and Ascophyllum. Ahmad and Winter (33) obtained modest growth stimulation (50 to 70 percent) of several blue green algae with 0.17 µg/l to 1.7 µg/l of IAA, but these cultures were not axenic. A slight increase in growth (approximately 20 percent) of Nostoc was reported by Fernandez et al. (34) at 100 µg/l of IAA and Iwaski (35) found a four-fold stimulation of growth by adding 50 µg/l IAA to axenic cultures of Exuviaella, a

dinoflagellate. The green algal coenocyte Caulerpa prolifera was assayed for the presence of IAA using the Avena coleoptile test and a fluorescence assay. The results were negative. Growth studies were carried out using concentrations of exogeous IAA between 10^{-3} to 10^{-7} M. Significant increases in elongation of the rhizomes were noted at 5 x 10^{-6} M and 10^{-6} M (36).

The great importance of pH in exogenous auxin experiments was first pointed out by Algeus (31). Scenedesmus and a strain of C. vulgaris were shown to increase in cell number upon the addition of IAA only at acid pH, the effect of IAA increasing with decreasing pH. Bonner (37) indicated that the effectiveness of a growth hormone is dependent upon the hydrogen ion concentration of the medium in which the growth substance is applied. Marmer (38) obtained similar results and reported that IAA is 15,000 times more effective at pH 4.6 than at pH 7.5. Albaun et al. (39) found that the penetration of IAA into cells of Nitella takes place more rapidly at pH 3.65 than at pH 7.94. The hydrogen ion concentration affects the degree of dissociation of molecules of the growth substances. It was observed that the heteroauxin enters Nitella in a molecular form.

The Test Organism

Chlorella pyrenoidosa is a green alga of the nonmotile Chlorophyceae. The alga is referred to as a pollution type of algae because it commonly reacts to domestic sewage or effluent and it is

prevalent in highly organically enriched waters. Chlorella pyrenoidosa are spherical cells with a diameter that ranges from 3-5 microns (1). Their cell wall consists largely of celluose and hemicelluose and has no projections or markings. The cells are non-vacuolate and the protoplast contains one prominent pyrenoid inside or at the base of the plastid. Chlorella are unicelluar or in loose, irregular colonies with no outer matrix. Reproduction is asexual by autospores. Chlorella pyrenoidosa are commonly responsible for clogging of pipes, cooling towers and sand filters (1). Chlorella may color water green and cause turbidity. Taste and odor problems also have been a result of large Chlorella populations.

The use of Chlorella pyrenoidosa to test herbicides in an assay has been reviewed by Wright (40). Stimulation and inhibitory results were presented. Brannon (41) noted that concentrations between 10^{-5} to 3.3 x 10^{-6} M IAA were stimulatory to the growth of Chlorella vulgaris and Chlorella pyrenoidosa. Lethal concentrations were found to be greater than 10^{-4} M. Some evidence suggested that growth substances accelerated the rate of reproduction and increase in the size of the cells. Mowatt (42) with differential extraction and chromotographic separation detected a variety of Avena active substances of the auxin type in Chlorella pyrenoidosa. Ahmad and Winter (43) stimulated the growth (increase in dry weight) of the green alga, Chlorella pyrenoidosa, using 10^{-3} M IAA. There was an increase in dry weight of 19 mg (100 percent) over the control. Other concentrations

of IAA were also growth promoting. At 10^{-6} M concentration the dry weight increased by 31.6 percent. Wedding et al. (44) found using a concentration of 2 x 10^{-3} M 2,4D and a pH above 7.0, that photosynthesis in Chlorella was stimulated slightly and inhibited at pH levels of 3.1 and 4.1. Respiration at this concentration was inhibited at lower pH values and stimulated at pH values above 5.5. Photosynthesis and respiration both were shown to be inhibited by concentrations above 4 x 10^{-3} M at pH 4.5. Erickson et al. (45) using Chlorella pyrenoidosa found that the concentration of undissociated 2,4D to inhibit photosynthesis by 50 percent increased over the pH range of 4.0 to 5.5. Wedding et al. (46) found a progressive increase in uptake of 2,4D relative to the concentration of undissociated molecules as the external pH increased above the pK of the acid. At a constant cell pH the permeability of Chlorella cells to 2,4D molecules is 800 to 1000 times as great as the permeability to anions. An increase in permeability was noted with increasing pH. Wedding et al. (47), using Chlorella, found that 2,4D levels ranging from 10^{-7} to 10^{-2} M influenced the uptake of uncharged entities such as glucose, mannitol and undissociated phosphate. It was concluded that auxins act primarily on those properties of the membrane which permit passage of uncharged molecules. Permeability and phosphate uptake were stimulated at the lowest levels and inhibited by upper levels of 2,4D concentrations.

Effects on Plant Constituents

Carbohydrates

Many contradictory results concerning the changes in carbohydrate constituents due to 2,4D applications have been reported in the literature. These differences stem from the varied effects of different concentrations employed as well as the time elapsed between herbicide treatment and the time of plant analysis.

Payne (48) treated potato tubers with 0.5 lb/acre of sodium 2,4D at early bloom stage which resulted in increased specific gravity of the tubers. This is indicative of a higher starch content. Payne and Fults (49) later showed that early treatments to the bloom stage resulted in decreased reducing sugars and increased starch. Late treatments caused an increase in reducing sugars. Wort (50) found that the sugar content in buckwheat increased by as much as 37 percent over that of untreated plants when measured 3, 8, 12 and 24 hours after treatment. There was a steady decline in the sugar content after 24 hours. Buckwheat plants were treated at 14 inches with 50-1000 ppm of ammonium 2,4D. Sugar and carbohydrate depletion was followed by an increase in protein and amino acids. Claeys (51) studied the changes in food reserves following the treatment of peas with ammonium 2,4D. After 24 hours, treated peas showed an increase in dry weight, reducing sugars and sucrose. Later stages showed a decrease in the amount of reducing sugars. Starch-dextrin contents varied inversely with sucrose content. Using concentrations of

0.002 to 0.4 mg/l of 2,4D on leaves of cotton plants in prebloom stage, the concentrations of sucrose, hemicelluose and celluose in the leaves of the main stems increased with increasing concentrations of 2,4D. Reducing sugar levels varied inversely to the amount of 2,4D applied. The highest concentrations of 2,4D affected leaf starch reducing it by about 40 percent (52). Smith (53) reported that there was a decrease in soluble sugars four hours after treatment on the base of each primarly leaf in seedlings of red kidney bean with two drops of 0.1 percent 2,4D. By the third day starch-dextrins in the leaves decreased rapidly. Total sugar in the leaves rose slightly by the third day, then continually decreased up to seven days. Compared to the controls, the 2,4D treated plants contained only 46 percent of the soluble sugars and 7 percent of the starch-dextrins in the leaves.

The percentage of hemicelluose was higher in leaves and stems of soybean plants growing in nutrient solutions of various nitrogen levels when these solutions contained 20 ppm of 2,4D. Fourteen days after the addition of 2,4D the treated plants contained 3.36 percent while the controls contained 2.34 percent hemicellulose. Starch was shown to be lower in the 2,4D treated plants (54). Carbohydrate reserves, hydrolysable polysacharides, reducing sugars, and non-reducing sugars were decreased in the stems of red kidney bean plants following applications with 1 drop of 1000 ppm 2,4D. A decrease in sugars and carbohydrates followed by an increase in protein and amino acids suggest that carbohydrates are used for protein synthesis (55).

Nitrogen Content

The chlorophenoxy herbicides appear to affect the distribution of nitrogenous compounds through different plant parts. 2,4D prevents immature cytoplasm from changing into mature cytoplasm. The mature cytoplasm appears to revert to the immature stage (56). With the reversion of specific plant parts or organs to a meristematic state following application of 2,4D the total nitrogen levels appear to often increase at the expense of the nitrogen from other plant parts. Remobilization of phosphorous and nitrogen from leaves to stems has been noted by Penner and Aston (17).

In buckwheat treated with 1000 ppm of the sodium amine of 2,4D the total nitrogen in the stems and roots increased, while nitrogen in the leaves decreased 4 to 8 days after application (57). Similar results were obtained using 100 ppm of 2,4D (58). Smith (53) showed that the level of nitrogenous compounds in a plant vary with the period of time elapsed following treatment with 2,4D. Four hours after treatment of bean stem tissue with two drops of 0.1 percent 2,4D there was a decrease in the total nitrogen of the stem. After seven days, the total nitrogen in the stem was 4.6 times greater than in the control. A rapid mobilization of carbohydrate reserves, a diminished production of leaves and an accumulation of nitrogen was observed. Sell (55) observed an increase in total nitrogen per plant with an increase in the protein and amino acids accumulated in the stems of kidney beans treated with 2,4D. Gruzdev (59) found nitrogen

increased in wheat grains treated with 0.3 kg 2,4D/ha. There were increased percentages of glutamic acid and proline with no changes in starch and nutrient content. Lundkist (60) reported that 2,4D acid inhibited nitrogen fixation by Nostoc puntiforme, N. muscorum and Cylindrospermum sp. when applied at concentrations used in weed killing (approximately 0.01 M). Nitrogen fixation was stimulated at 1.0×10^{-4} to 10^{-5} M concentrations.

Freiberg and Clark (61) discovered that 4 ppm of Na-2,4D applied to the roots of soybeans caused a decrease in nitrates in the leaves and an increase in the roots and stems. An increase in soluble organic nitrogen forms in the plant organs was also observed. Stahler and Whitehead (62) found that nitrate levels in sugar beet leaves were increased 20-fold following the addition of 2,4D. The leaves of the control contained 0.223 percent KNO_3 but leaves of 2,4D treated sugar beets contained 4.5 percent KNO_3. At 1.5 percent, potassium nitrate becomes toxic to cattle on a dry weight basis. Berg and McElroy (63) observed that following the application of 2 to 8 ounces of 2,4D per acre, only moderate increases in nitrate content were recorded for six weed species. Sudan grass sprayed with 2,4D showed a higher level of nitrate shortly after herbicide application. But in time the nitrate levels fell below the control and finally rose to a level present in the controls (64).

Different concentrations of 2,4D have been shown to have varying effects on the reported levels of proteins and amino acids. Faludi and Faludi-Daniel (65) treated potato tubers with 2,4D concentrations

ranging from 10^{-3} to 10^{-7} M and observed a decrease in the amino acid content. Payne (66) found an increase in free glutamic acid and a decrease in 11 other amino acids in potato tuber harvested from treated plants. When 50 µg of 2,4D were applied to the primary leaf of a bean plant Akers and Fang (67) observed a decrease in aspartic and glutamic acid three days after treatment. Sell (53) found that protein in treated bean plants doubled and the amino acids, leucine, isoleucine, valine, phenyalanine, histidine, arginine and threonine also increased. The lysine and methionine were discovered to be approximately three times the control. Rasmussen and Lawrence (68) found that low levels of 2,4D caused significant increases in the free amino acids and proteins of Canadian thistle. Fults and Payne (69) found that levels of amino acids in sugar beets and potato tops significantly increased after application of 1000 ppm of sodium 2,4D. However, amino acids in pinto bean tops showed a significant decrease after application. It was noted that both the sugar beet and potato have underground storage organs whereas the pinto bean does not and that the manner in which plants use their protein fraction may be related to the selective action of 2,4D. Livingston et al. (70) observed a reduction in glutamine, alanine, lysine, and tyrosine content in the roots of treated sugar beets. However, sixty days after 2,4D treatments the content of all free amino acids, except aspartic acid, had increased.

Payne (48) observed a 27.2 percent increase in protein of potato tubers using 0.5 lb 2,4D per acre spray. Yasuda (71) noted that protein content in potato tubers was greater at harvest and after sixty days storage following the application of 0.5 lb/acre of 2,4D. Wort (50) showed that protein nitrogen increased in the roots and stems of 2,4D treated buckwheat plants. No change in protein nitrogen in the leaves was discovered. Freiburg and Clark (72) found increased protein content in soybean plant roots and stems using 5 ppm of 2,4D three days after treatment. The protein content of the leaves was found to decrease. The hydrolysis of leaf protein was followed by translocation of products to the stem and roots where additional protein was synthesized. Proteolytic enzyme activity in treated plant tissue was correlated to protein nitrogen content. A 5 ppm 2,4D treatment reduced the proteinase and peptidase activity in the leaves and a subsequent increase in protein nitrogen content and activity in the stem and roots. Coble and Slife (73) observed a 22 to 36 percent decrease in the starch content of honey-vine milkweed treated with 0.56 kg/ha of the dimethyamine salt of 2,4D and a 35 to 80 percent increase in protein. An increase in protein content and yield in wheat was reported by Khripunova (74) and Huffaker et al. (75).

Untreated samples of cotton cotyledon tissue showed a loss of RNA, of protein and of phosphatase activity in the soluble proteins after 48 hours. Basler and Nakazawa (76) found that concentrations of

10^{-4} and 10^{-3} M 2,4D prevented these changes in RNA and protein content. Higher RNA and protein contents were observed in 2,4D treated plant than in controls. The respiration rate for the untreated tissue was discovered to be lower when measured 24 hours after 2,4D application. Osborne and Hallaway (77) showed that local applications of 2,4D to leaves of Euonymus japonica caused an increase in the nitrogenous compounds in the 2,4D treated areas and a decrease in the non-treated areas. The 2,4D treated area maintained a high rate of respiration and carbon compounds moved into the treated areas of the leaf. No net protein breakdown occurred in the 2,4D treated area. The 2,4D apparently caused an increased amino acid pool and an increase in protein synthesis thereby delaying leaf aging or acting in a stimulatory manner on the basic metabolic rate of the cells. West et al. (78) found that 5 ppm of 2,4D applied to the cucumber mesocotyl caused a decrease in the level of RNA. At concentrations of 400 ppm the RNA level and protein content were increased over that of the control. They concluded that the action of 2,4D may be more directly related to RNA synthesis than protein synthesis. Key and Hanson (79) using 5×10^{-4} M 2,4D on soybean seedlings found that the protein to RNA ratio decreased up to 48 hours and then increased. Schröder (80) found that 10^{-3} M 2,4D increased the DNA concentration of Neurospora crassa by 40 percent greater than control. Protein content was 17 percent greater. Key (81) found that 10 ppm 2,4D increased the amount of leucine C^{14} incorporated into

soybean hypocotyl sections. Using a variety of auxin inhibitors he found that the enhancement of cell elongation by 2,4D requires active RNA synthesis and protein synthesis. The rate of formation of RNA is enhanced by an auxin which increases the supply of a limiting enzyme or enzyme system. Actinomycin D inhibited enhanced amino acid incorporation.

Vitamins

Treatment of plants with 2,4D appears to alter the vitamin content of plants. Yakushkina and Kravtsova (82) found that the application of 10 ppm of 2,4D to pepper, tomato, and egg plant flowers increased dry weight, fruit sugar content and vitamin C content. Luecke (83) applying 0.05 ml of 0.1 percent 2,4D to the primary leaves of the red kidney bean observed that levels of thiamine, riboflavin and nicotinic acid decreased in the leaves and increased in the stems. The amount of pantothenic acid increased in both stem and leaves.

Minerals

The distribution and mobiility of minerals follows a similar pattern to that observed for amino acids and sugars. The mineral content decreases in leaves but increases in the stem. The uptake of several ions may be effected, but Wort (16) pointed out that generally the absorption and content of many ions are depressed or unaltered by the application of 2,4D. Concentrations of 2,4D greater than 3 ppm have been found by Blackman (84) to depress the absorption of the total amount of potassium, phosphorous and nitrogen by

Lemna minor. The effect was a function of both time and concentration. However, on either a dry or fresh weight basis the contents of nitrogen and phosphorous were increased from 3 to 48 ppm. Wolf et al. (54) discovered a decrease in the percentage of potassium and calcium in soybean plants treated with 20 ppm of 2,4D. Potassium levels were considerably lower in the leaves, while calcium levels were considerably higher in the leaves. Cooke (85) found an initial increase in the mineral uptake using 2,4D concentrations of 100 mg/l. An increase was noticable in potassium, chloride and calcium ions. Hanson and Bonner (86) observed that the rate of $Rb^{86}Cl$ uptake was considerably lower in artichoke tubers that had been treated with 2,4D than the untreated plants. Sodium and potassium in the leaves of cranberry bean plants were shown to increase following 2,4D treatment. Calcium in the leaves decreased and varying results on the change in mineral content of the stem were observed (87).

Loustalot (88) treated white bean plants with an aqueous spray of 0.1 percent Na-2,4D when they were 15 inches high. The inorganic phosphorous in the leaves and stems of treated plants determined 4, 10, 24, 48 hours and seven days after treatment fluctuated in most instances like the controls, but was significantly higher in roots, stems, and leaves of the treated plants. The same author found that treatment of Commelina sp. and Xanthosoma sp. with 2,4D resulted in consistently higher percentages of water soluble phosphorous, measured 24 hours and 7 days after treatment. These situations seem to be exceptions rather than the rule for mineral uptake.

Fang and Butts (89, 90) found that upward movement of P^{32} to the leaves is reduced proportionally to the amount of 2,4D applied. Rebstock (91) observed that 1000 ppm of 2,4D caused a large increase in the phosphorous content of the stem of the cranberry bean plant with a concurrent decline of phosphorous in the leaves. Fang and Butts (89, 90) obtained results similar to Rebstock (91) and concluded that the 2,4D treated area acted as a metabolic sink. Penner and Aston (17) stated that 2,4D causes a reversion in plant tissue which acts as a metabolic sink, thus accounting for increased protein, amino acids, phosphorus and vitamin content.

Water Content

The effect of 2,4D on plant water relations depends upon the concentrations applied, the plant organ it is applied to and the species of plant that is observed. Brown (92) found that 1000 ppm of 2,4D applied to bean seedlings increased the moisture content of the stems, but decreased the content in the leaves. Freiburg and Clark (61) reported that the water content of nasturtiums and soybeans was significantly increased by 4 ppm of Na-2,4D. However, dry matter content decreased initially, but again increased after the plant had wilted. Plants treated with high concentrations of 2,4D did not have an initial increase in water content and wilted much earlier. Hanson and Bonner (86) showed that the uptake of water by slices of Jerusalem artichoke tubers was markedly increased by bathing the slices in a solution of 0.5 mg 2,4D per liter. Wort (50) observed

an overall increase in the moisture content of 12 to 14 inches high buckwheat when using sublethal doses of 2,4D. Eight days after spraying plants treated with 50 to 1000 ppm 2,4D contained between 103 and 138 percent of the moisture content of the controls. Commoner and Mazia (93) and Higinbotham et al. (94) have observed that auxin induced water uptake is paralleled by increased ion absorption. However, the converse has been observed by Steward (95) and Hanson and Bonner (86). Higinbotham et al. (94) relate increased water and cation uptake of auxin treated tissue to the increased respiration caused by auxin application. The results of Steward (95) and Hanson and Bonner (86) indicate that the two processes involve different mechanisms but are related in their dependence upon the cellular energy pool.

Nucleic Acids

Silberger and Skoog (96) were the first to notice nucleic acid increases in the tabacco pith callus following auxin treatment. This gave rise to the hypothesis that the mechanism of herbicide action might be linked with nucleic acid metabolism. Key (97) applied 5×10^{-4} M 2,4D to 2.5 day old soybeans and found that 24 to 48 hours after the application there were increases in proteins, nucleic acids and acid soluble nucleotides in the hypocotyl. ATP increased in the acid soluble nucleotide fraction. Key and Hanson (79) found increases in the soluble nucleotides: cystidine mono- and triphosphates; adenosine mono-, di- and triphosphates; guanosine mono- and

triphosphates; and uridine mono-, di- and triphosphates. It was reported that at certain concentrations, 2,4D would stimulate the incorporation of ATP into the RNA of excised soybean hypocotyls.

During a 48-hour period after spraying with 5×10^{-4} M 2,4D, the RNA in subcelluar particles of soybean hypocotyl tissue doubled. Over half of the increase (175 percent of the control) appeared in the microsomal fraction and one quarter in the soluble fraction. Crispeels and Hanson (98) noted that an increase in synthesis of RNA and protein in the region of rapid cell proliferation is preceeded by increased nuclear activity. Since RNA and protein synthesis are controlled by DNA, the primary site of action was apparently in the nucleus. Key and Shannon (99) enhanced C^{14} nucleotide incorporation into RNA of excised soybean hypocotyl tissues with concentrations of IAA and 2,4D which promote cell elongation (5 to 25 ppm 2,4D). Inhibitory levels (100 to 500 ppm) decreased C^{14} nucleotide incorporation. Actinomycin D inhibited RNA synthesis and prevented increases in ribosomal RNA. A net increase in RNA of 25 to 30 percent occurred primarily in the ribosomal fraction following auxin application in fully elongated cells. Bendana et al. (100) isolated labeled messenger RNA from green pea stem sections following short term incubations with 10^{-5} M C^{14} carboxyl labeled IAA. Labeling was inhibited by 10 ppm of actinomycin D. The incorporation of the labeled auxin and CO_2 into RNA was stimulated by light, indicating that some of the effect results from decarboxylation and the recycle of carbon dioxide. Key (101) showed that low concentration of 2,4D (10 ppm)

enhanced RNA loss in excised corn mesocotyl whereas high concentrations (800 ppm) inhibited the apparent degradation of RNA. Decreases in RNA content occurred entirely at the expense of microsomal and soluble RNA with no measurable change in nuclear and mitrochondrial RNA. Low concentrations promoted RNA loss by enhancing the metabolic breakdown of preexisting and newly synthesized RNA. Ribonuclease activity was shown to increase at low concentrations and decrease at high concentrations. Sorokin et al. (102) found that applying 2,4D (7×10^{-7} M) and IAA (1.7×10^{-5} M) to the isolated segment from the second internode of etiolated pea epicotyls resulted in cambium tissue activation and abnormal growth. When kinetin was added, the growth became more normal, callus did not develop and xylem elements became regular and apparently functional. The effect of kinetin therefore gave a more orderly arrangement to cell divisions and it appeared that an auxin-kinetin imbalance could induce growth of a 2,4D treated plant.

Ribonuclease activity increases as corn mesocotyl cells grow. At low concentrations (up to 50 ppm) accelerated growth is paralleled by stimulated ribonuclease activity. At higher 2,4D concentrations both were inhibitory. High levels of 2,4D were shown to induce synthesis of nucleic acids and proteins (103). Arens and Stout (104) observed a linear enhancement of RNA polymerase activity in maize treated with concentrations of 10^{-7} to 10^{-3} M 2,4D 48 hours prior to harvest. Basler and Hanson (105) showed that 10^{-3} M 2,4D inhibited both RNA synthesis and degradation. Sucrose appeared to be necessary

for increased RNA synthesis to occur in the particulate nucleic acid fraction. Sucrose also stimulated loss of nucleic acid in the RNA containing supernatant. The investigators concluded that 2,4D reduced movement of RNA from cell particulates to the cytoplasmic fraction or that 2,4D caused preferential incorporation of ^{14}C orotic acid into DNA. The latter possibility might be due to the renewed nuclear activity following 2,4D treatment (98). Key (106) sprayed 2,4D on soybean seedlings and found an initial suppression of synthesis of DNA, RNA, protein, growth and cell divisions up to 48 hours in the apical region of the hypocotyl. The basal tissue showed swelling by 12 hours due to cell divisions and began synthesizing nucleic acids and proteins. Synthesis of RNA slightly preceeded protein synthesis, DNA synthesis and cell division. This work is valuable because it shows nucleic acid metabolism to be suppressed where growth is suppressed and accelerated where growth is accelerated. Both Moreland (18) and Hanson and Slife (19) have observed that enhancement of growth by 2,4D was accompanied by RNA synthesis with subsequent increase in protein synthesis.

The Effect on Plant Metabolism

Increased Yields

The phenoxyalkanoic herbicides have been experimented with to produce varying effects on crop yields. Rojas-Garcidueñas *et al.* (107) using concentrations of 0.01 to 10 ppm 2,4D stimulated the

germination of pigweed seeds. A concentration of 0.1 ppm 2,4D was optimal for the induction of germination and higher concentrations showed a herbicidal effect. Growth of plumule and radicle in wheat was stimulated by lower 2,4D concentrations (0.001 to 1 ppm). Wedding et al. (108) treated bean seedlings with 10 to 20 ppm of 2,4D acid equivalent and discovered an increase of approximately 35 percent in the yield of shelled green beans. More pods per plant were found and the size of the individual beans increased. This phenomenon was time dependent. Applications of 5 to 20 ppm applied in different years at different locations resulted in lima and snap bean yields which increased by 70 percent or decreased as much as 75 percent. Wort (58) obtained increased yields of green beans as well as beets, maize and potatoes with foliar applications of sub-toxic levels of 2,4D alone and in combination with iron. Payne (109) and Wort (110) reported increased yields and increased quality in potatoes. Miller (111) obtained increased growth of field beans with 0.5 to 1.0 ppm of 2,4D with and without the presence of $FeSO_4$. 2,4D increased both the size of the beans and number of beans per pod. Low concentrations of 2,4D also increased seed yield whereas high concentrations reduced seed yield. Beste and Schreiber (112) demonstrated that 4.5×10^{-5} M 2,4D increased the fresh weight and RNA per gram of fresh weight of soybean hypocotyls within 20 hours.

Respiration

Both stimulation and inhibition seem to be caused by the application of 2,4D. The effect is mainly due to the concentration of 2,4D applied. Smith (53) treated bean stem tissue with two drops of 0.1 percent 2,4D on the base of each primary leaf in seedlings. A marked increase in respiration was observed using 1 to 10 ppm 2,4D at 48 hours. Avery and Kelly (113) reported an increase of 20 percent in O_2 uptake by coleoptile segments using concentrations ranging from less than 1 to 100 mg/l of 2,4D. In further investigations it was found that a concentration of 10^{-3} M was needed to give 20 percent stimulation of Avena. Avery (114) has reviewed several papers which report increases of up to 80 percent in O_2 consumption or CO_2 evolution as the result of 2,4D application to a variety of plants. The respiration of excised corn roots growing in sterile nutrient was increased sharply when 2,4D was added to form a 10^{-7} M solution. French and Beevers (115) found stimulation in both growth and respiration using 2,4D in concentrations ranging from 10^{-7} M to 10^{-3} M with a maximum at 3×10^{-5} M. Growth and respiration were both stimulated greater than 200 percent of the control. Phosphate uptake and oxygen consumption measured 18 and 24 hours after application were greater than the control. Inhibitory concentrations caused a lowered respiration rate. Wedding et al. (44) showed that respiration appeared to be stimulated following application of low to intermediate concentrations of 2,4D, whereas high concentrations inhibited respiration.

Photosynthesis

It appears that a reduction in photosynthesis follows the application of 2,4D. Freeland (116) showed that 100 ppm of 2,4D reduced the apparent rate of photosynthesis in bean leaves by 40 percent over a four day period. Freeland (117) measured photosynthesis and respiration for Anacharis canadensis before and after treatment at concentrations of 2,4D at 30 and 100 ppm. The higher concentration was more effective. At either 30 or 100 ppm there was first a decrease in the rate of respiration followed by a partial or complete recovery at the end of 48 hours. Wedding et al. (44) showed stimulation of photosynthesis with a 2,4D concentration of 2×10^{-3} M at a pH above 7.0. At this concentration photosynthesis was inhibited at pH 3.1 and 4.1. Photosynthesis and respiration were inhibited at higher concentration above 4×10^{-3} M at pH 4.5. Erickson et al. (45) noted that 2,4D was 200 times more effective than acetic acid in inhibiting photosynthesis in Chlorella, based on the concentration of undissociated molecules at pH 4.0.

Cell Permeability and
Protoplasmic Streaming

Veldstra and Booij (118) reported increases in cell permeability under the influence of the auxin herbicides 2,4D and MCPA. Von Guttenberg and Beythien (119) showed that growth promoting concentrations of IAA increased the permeability of epidermal cells of Rhoea discolor to water. High concentrations inhibited growth and reduced

the permeability of the cells. Wedding et al. (46, 47) showed that the permeability is directly affected by the pH and later showed that 2,4D affected the rate of soluble uptake. Low concentrations stimulated uptake and high concentrations decreased uptake.

Thimann and Sweeney (120) stimulated protoplasmic streaming in epidermal cells of Avena coleoptile at concentrations of IAA, ranging from 0.5 to 0.002 mg/1. The maximum effect was found at 0.01 mg/1 with concentrations greater than 0.5 mg/1 being inhibitory. It was concluded that action of auxin is directed toward the protoplasm and that streaming takes place at the same concentrations that produce growth. Currier (121) treated several plant cells with Na-2,4D at concentrations of 10^{-2} to 10^{-8} M. Stimulation of protoplasmic streaming could be obtained with several representative cells and was greatest at 10^{-4} to 10^{-8} M. An increase in cell survival time was also noted over the controls. Carrol (122) reported that low concentrations of 2,4D immediately reduced protoplasm viscosity in Spirogyra and Elodea. Increased duration of immersion at these concentrations resulted in increased viscosity. Kelso and Turner (123) noted that low concentrations of auxin stimulated protoplasmic streaming in Tradescantia, whereas high concentrations depressed the rate. Concentrations of 0.5 to 50 mg/1 2,4D brought about a slight and apparently permanent stimulation of streaming. A 10 percent maximum stimulation was recorded at 10 mg/1. An increase in pH reduces the effect over a wide range of concentrations.

Ethylene Production

Hansen (124) has found that 2,4D treatments of premature Bartlett pears resulted in increased rates of ripening, respiration and ethylene production. Carbon dioxide evolution was enhanced 1.3 times and ethylene production was 3.6 times greater than the controls. Hall and Morgan (125) showed that ethylene production was stimulated by 2,4D in cotton plants sensitive to 2,4D injury, but not in sorghum plants which were insensitive to 2,4D. Holm and Abeles (126) linked 2,4D activity to ethylene production. Ethylene and 2,4D inhibited the growth of etiolated soybean seedlings, causing tissue swelling and increases in RNA, DNA, and protein in the subapical hypocotyl tissue. Ethylene production was increased by 2,4D and some of the response of soybeans to 2,4D results from ethylene production.

Degradation in the Environment

Selectivity

Chlorophenoxy herbicides such as 2,4D may persist in the treated plants for varying periods of time depending upon the environment, the species examined and the physical state of the plant. Interpretation of different physiological responses by 2,4D resistant plants and susceptible varieties must take into account internal concentrations of the herbicide. The concentration used in the treatment process is again very important. At low concentrations it may stimulate or effect the response, whereas in high concentrations the response may be completely different. Consequently, an effect

observed between a susceptible and a resistant species may only be a reflection of the different internal concentrations of the herbicide. Williams (127) found that algae containing high concentrations of endogenous auxin are injured by amounts of exogenous auxin that have a stimulatory effect on low auxin producing plants. Fang and Butts (89, 90) demonstrated that growth regulators were absorbed and translocated much slower by monocots than dicots following uniform treatments with 2,4D. Part of the 2,4D is incorporated in other complexes. Differences in the internal 2,4D concentrations of susceptible and resistant species could also result from a more rapid detoxification in resistant species. Slife et al. (128) found that 2,4,5T is mobile in plants for a much longer time than 2,4D. The amount of $^{14}CO_2$ evolved from biological decarboxylation was 10 times greater for 2,4D than 2,4,5T. Craft and Yamaguchi (129) found that 2,4D is not freely mobile in plants and moves faster in some parts of the plant than others. Within the same plant, differnt organs, different tissue in the same organ and different cells in the same tissue may not respond to herbicide treatments in the same way. In addition different parts of plants generally react independently. Leonard and Crafts (130) found that 2,4D was absorbed at different times of the year by various plants by a variety of mechanisms.

The hypothesis that 2,4D and a protein or amino acid interact to aid in detoxification has been proposed by Andreae and Good (131), Fang and Butts (89, 90), Fang (132) and Freed et al. (133). Fang and Butts (89, 90) observed that part of the 2,4D treatment was

incorporated into unknown complexes which were recovered in alcohol extracts from monocots. It was concluded that these complexes (conjugations) may play a role in the speed of detoxification. Andreae and Good (131) discovered the formation of conjugates using IAA and 2,4D. After 24 hours, several different products were reported with only 20 percent recognizable as indole compounds. From 2,4D breakdown three products were found. The most abundant was 2,4-dichlorophenoxyacetyl aspartic acid. Toxicity was explained on the basis that susceptible plants are not able to form these complexes. Fang (132) described the formation of conjugates called Unknown I and Unknown III which were found in 80% ethanol extracts from both peas and tomatoes. The difference in inhibition between susceptible and non-susceptible species was not due to the manner in which 2,4D reacts with plant constituents. Freed et al. (133) also felt that breakdown of 2,4D by plant metabolism may involve the formation of conjugates with proteins, such as amino acids.

Plants apparently are also capable of hydrolysing the long chain esters of 2,4D. Weintraub et al. (134) showed that $^{14}CO_2$ was evolved by bean plants treated with 2,4D labeled in the carboxyl or methylene position. The rate of decarboxylation was greater from carboxyl labeled 2,4D. Basler (135) found only one percent of the carboxyl labeled 2,4D was decarboxylated. Bach and Fellig (136) considered the decarboxylation of 2,4D and the release of $^{14}CO_2$ from carboxyl labeled 2,4D only a minor pathway of 2,4D breakdown.

Luckwill and Lloyd-Jones (137) attributed the toxic action of 2,4D to the presence of free 2,4D in the tissues or the ability of the tissues to oxidize the carboxyl and methylene carbons from the side chain. Red currants, a nonsusceptible species, oxidized up to 50 percent of the carboxyl carbon and 20 percent of the methylene carbon from the side chain over a one week period. Black currants, a susceptible species, oxidized only 2 percent. Later (138) it was shown that Cox applies a resistant form, decarboxylated 57 percent of the 2,4D in 92 hours in excised leaf experiments whereas the susceptible variety Bramley seedlings, only decarboxylated 2 percent of the 2,4D in this same time. Oxidative destruction of 2,4D in certain tolerant species may partially explain selectivity, but other mechanisms are also involved.

Soils and Microorganisms

Herbicides may enter the soil either by using excess amounts of 2,4D, by direct application to the soil or from decaying or dead plants treated with the herbicide. Herbicides are leached into drainage water at a rate which is influenced by the degree of adsorption between herbicide and soil colloids and by their chemical destruction in the soil. Barnett et al. (139) measured, 2,4D run-off and residues from soil using 2.2 or 4.4 lb 2,4D per acre with simulated rainfall intensities of 1, 10, 80 and 100 year storms. Formulations of 2,4D used included isooctyl and propylene glycol butyl ether ester and an alkanolamine salt of the ethanol and

isopropanol series. Concentrations of runoff were greatest at the start of each storm and decreased with storm duration. The isooctyl ester was as high as 4.2 ppm. When a direct comparison was made between the butyl ether ester and the amine salt form, it was found that 13 and 4 percent of the 2,4D formulations were lost in a one year storm and 26 and 5 percent of the respective ester and salt form were lost in a one hundred year storm. Most of the 2,4D remained in the top 3 inches of soil. Akamine (140) found that soil types had an effect on the rate of 2,4D dissipation, which varied from 2 to 14 weeks. 2,4D toxicity disappeared most rapidly in soil with high pH values. Kries (141) tested 6 soil types and foun no toxicity after 4 weeks. Hank (142) observed that inhibitory effects of 20 ppm 2,4D persisted longer in naturally alkaline, sandy clay soil at pH 8.4 than any other of six soils tested.

Some physical, chemical, and biological factors that might influence the persistence of 2,4D compounds in natural waters were investigated. The amounts of 2,4D (sodium salt) isopropyl butyl and isooctyl esters sorbed on bentonite, illite and kaolinite ranged from 0.02 to 0.14 mg/gram (2). Kries (141) found that lime seemed to make 2,4D toxicity persist for longer periods of time. Hernadez and Warren (143) discovered that soils with low organic content are more likely to leach 2,4D. It was also found that 2,4D persisted for longer periods of time at lower temperature. Akamine (140) observed that the persistence of 2,4D toxicity was related to the mean

daily soil temperature. The higher the temperature the more rapid the rate of 2,4D dissipation. The fact that warmer temperatures enhance 2,4D breakdown was discovered to be due to the greater proliferation of soil bacteria which degrade 2,4D. Kries (141) discovered that after 18.5 weeks in air dried soil, 2,4D was present when applied at concentrations of 2 and 20 ppm. Hernandez and Warren (143) showed that in air dried peat and in water saturated peat that 2,4D was degraded in 16 and 4 weeks respectively. 2,4D disappeared after 12 weeks in sterilized soil and after 4 weeks in unsterilized soil. Mitchell and Marth (144) also concluded that organic matter, moisture content and temperature effect 2,4D breakdown. Audus (145) perfused concentrations of 10 to 1000 ppm 2,4D through garden loam. It was discovered that detoxification of 2,4D is due almost entirely to microorganisms. Detoxification of 10 ppm 2,4D required 10 days in previously untreated garden loam. A second trial with 10 ppm required only 5 days. Audus (146) later found that bacteria play a role in 2,4D detoxification. Very low concentrations of the bacterial poison, sodium azide, were shown to lower the rate of 2,4D detoxification in soils through kinetic experiments. Audus successfully isolated a 2,4D decomposing bacterium known as Bacterium globiforme. Subsequently, other investigators discovered several organism can use 2,4D as a carbon source. A suggested mechanism for degradation of 2,4D by Arthobacter and Pseudomonas was proposed by Loos (11).

Trichell et al. (147) treated sod and fallow plots of varying slopes with 2,4D. After 24 hours, the plots were given the equivalent of 0.5 inches of rainfall. The maximum amount of herbicide lost was 5.5 percent and averaged about 3 percent. Four months after application the losses of herbicides averaged less than 1 percent of that lost 24 hours after application. Faust and Aly (2) sampled lake bottom sediments from two lakes. One lake had been treated with 2,4D the summer before and one was previously untreated. Through laboratory systems to study the degradation of 2,4D it was found that mud of the previously treated and untreated lakes degraded 2,4D after 35 days and 65 days, respectively. In subsequent treatments it was discovered that 2,4D decreased 81 to 85 percent in lake muds within 24 hours. From these studies it can be seen that decomposition rates of 2,4D depend upon the degree to which bacteria are acclimiated to the herbicide. Akamine (140) observed more rapid 2,4D breakdown in soils with greater numbers of aerobic bacteria.

III. MATERIALS AND METHODS

The algal assay is used here to evaluate the potential effects of 2,4D on algal growth in receiving waters. In general, the methods described were developed by the Environmental Protection Agency (148).

Medium Preparation and Inoculation

The culture vessels used were Pyrex 250 milliliter flasks. Each culture flask was filled with 60 ml of medium. This surface to volume ratio insures the availability of carbon dioxide and maintains the pH below 8.5 (148). A pH below 8.5 will favor the formation of inorganic carbon species that algae readily utilize. Culture flasks were stoppered with cotton covered by cheese cloth, thereby permitting proper gas exchange. Experiments were conducted in a constant temperature room capable of providing 20 $\pm$ 1.0°C. The culture box was illuminated by a "cool-white" fluorescent lamp which provided 140, 200 and 220 foot candles measured adjacent to the flasks at liquid level. The light intensities reported are average values for adjacent flasks grown under continuous lighting. Standard deviations were 140 $\pm$ 15 ft-candles, 200 $\pm$ 8 ft-candles and 220 $\pm$ 2 ft-candles. Light intensities were measured with a Weston illumination meter.

The Woods Hole MBL (Guillard Type) culture media given by Stein (149) was utilized with the addition of tris buffer at the recommended concentration. In preliminary experiments Guillard's medium was

discovered to be superior to the EPA media for growing Chlorella pyrenoidosa over a short time span. Glass distilled water was used for medium preparation. The final pH of the medium was 7.4. Stock solutions of the individual macro- and micronutrients were made 1000 times more concentrated than working nutrient concentrations. To prepare medium for a trial one milliliter of each was added to a flask and diluted to one liter. Stock solutions were stored at 4°C in a refrigerator, except for vitamins which were frozen. Medium was filter sterilized with a 0.45 μ porosity membrane filter (148). Medium sterilization by autoclaving was not possible because of the rapid breakdown of 2,4D at high temperature and pressure.

The test alga, Chlorella pyrenoidosa, obtained from Starr (149) stock cultures was maintained in Guillard's medium at 220 foot candles. Stock transfers were performed weekly to keep cells in the log phase of growth. Aseptic techniques were employed throughout the experiment in all procedures dealing directly with the growth of the test organisms. In order to prepare uniformly dense inocula, cells were mixed on a vortex mixer. Cell counts were then determined with a hemacytometer and dilutions made to provide a standard inoculum of twenty thousand cells per milliliter. The fluorometric value at this cell population was 15 fluorometric units.

Glassware was washed in Alconox and sodium carbonate, followed by soaking in a 30 percent solution of reagent grade hydrochloric acid. After the acid wash, glassware was rinsed five times in tap water followed by five rinses with distilled water. Disposable pipettes were

used throughout the experiment. Glassware was inverted on paper towels to dry, fitted with a cotton plug and autoclaved.

The 2,4D acid used in this experiment was obtained from the Dow Chemical Company. The concentrations used are those which have shown stimulatory responses in plants (less than 10^{-3} M) and which have been found to be present in the environment. 2,4D acid weighing 0.442 grams was dissolved in one liter of medium to give a concentration of 2×10^{-3} M 2,4D. The pH of this mixture was adjusted to 7.4 by the addition of 1 N sodium hydroxide. Medium supplemented with 2×10^{-3} M 2,4D was diluted with stock medium to obtain the concentrations of 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8} M 2,4D used in this experiment. Three replicates of each herbicide treatment and a control were used. Growth measurements were made at days 2, 4, 6, 8, 10 and 12.

Sampling and Analytical Technique

Growth was measured by two methods: direct cell counts by means of miscroscopic enumeration and direct fluorometric determinations. Cultures were uniformly mixed with a vortex mixer and enumerated by microscopic examination with the aid of a hemacytometer. Cell counts were made at 430 power. Fluorometric analysis of chlorophyll was made by direct determination using a modified Turner Model 111 Fluorometer. The modification included a high sensitivity door, a T-5 lamp capable of providing light in the 270 to 335 nm wavelength range and a #2-64 filter. Good correlation between *in vivo* units and cells per milliliter has been

demonstrated during logarithmic phase of growth by the EPA (148).

When possible, gravimetric analysis of dry weight is the preferred method of the EPA for determining algal growth. The interest in this experiment was in the logarithmic phase of growth usually occurring between days one and six. The relatively sparse density of algae during log growth would require large volumes of filtered water to provide any degree of accuracy using gravimetric analysis.

Data Analysis and Interpretation

Data from direct microscopic counts and from fluorometric readings were analyzed by two methods. These methods included a least squares linear regression analysis followed by comparison of the slopes and an analysis of variance for subsequent use in the Dunnett's test (151).

Linear regression lines were constructed for direct cell counts and fluorometric data obtained for seven treatments and a control. Increase in slope would indicate an increased rate of growth. Because the growth of Chlorella is characterized by an exponential function it was necessary to take the logarithm of cell counts and fluorometric measurements for linear regression analysis.

An analysis of variance was performed on direct cell counts and fluorometric measurements for each of the seven herbicide levels and a control at days 2, 4, 6, 8, 10 and 12. The effectiveness of the herbicide as a growth hormone was evaluated using Dunnett's test for the comparison of several treatments with a control. A 95 percent level was chosen to be statistically significant.

IV. RESULTS AND DISCUSSION

The data obtained in this study indicate that 2,4 dichlorophenoxyacetic acid was effective in stimulating the rate of growth of Chlorella pyrenoidosa.

Cell counts were plotted on semi-log paper and are presented in Figures 1, 2, and 3 representing growth at 140, 200, and 220 foot candles, respectively. From the inspection of Figures 2 and 3 it can be seen that cell growth rates decline following day 8 at the highest 2,4D concentrations. For this reason, linear regression analysis was performed only from days 0 through 8. Increasing 2,4D concentrations from 10^{-8} to 2×10^{-3} M show higher rates of growth than the control at all three light intensities. Mean values for cell counts depicted in Figures 1, 2, and 3 can be found in Tables 1, 2, and 3, respectively. Stimulation in plants is caused by low levels of 2,4D and inhibited at higher levels. Loos (11) defined stimulatory auxin concentrations as those less than 10^{-3} M for the elongation of root shoots. Higher concentrations have been shown to be inhibitory. Figure 3 indicates that 10^{-3} M 2,4D had a greater effect on growth than 2×10^{-3} M 2,4D. This effect was also noticeable at day 8 for 140 ft-c and at day 10 for 200 ft-c. It appeared that 2×10^{-3} M 2,4D is on the border between stimulation and inhibition in Chlorella. The growth rate at 2×10^{-3} M was greater than the rates determined for all other 2,4D treatments except 10^{-3} M. Linear regression analyses for cell counts are presented in Table 4 for 140, 200,

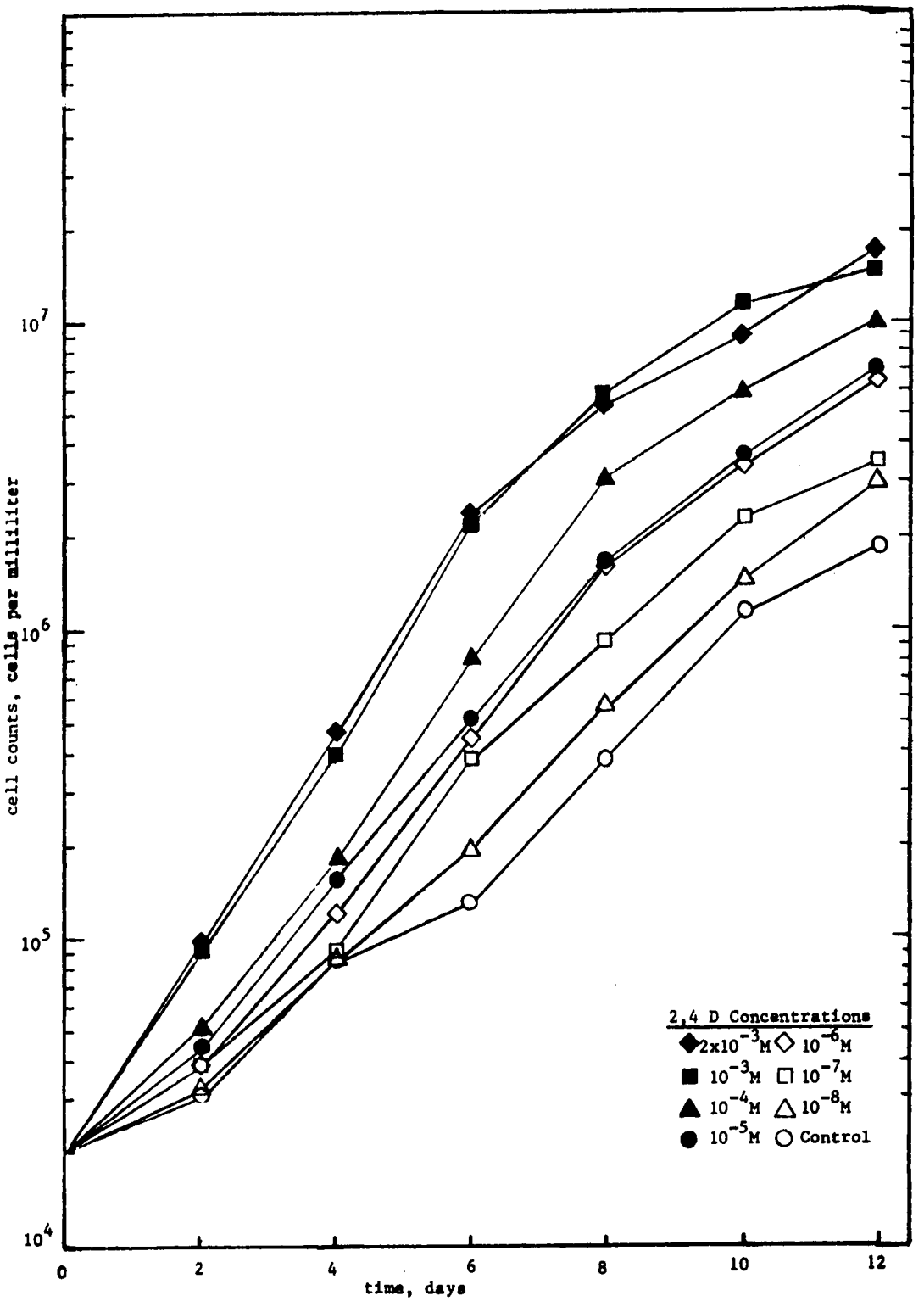

Figure 1. Effect of 2,4D on cell density at 140 ft-c and 20°C.

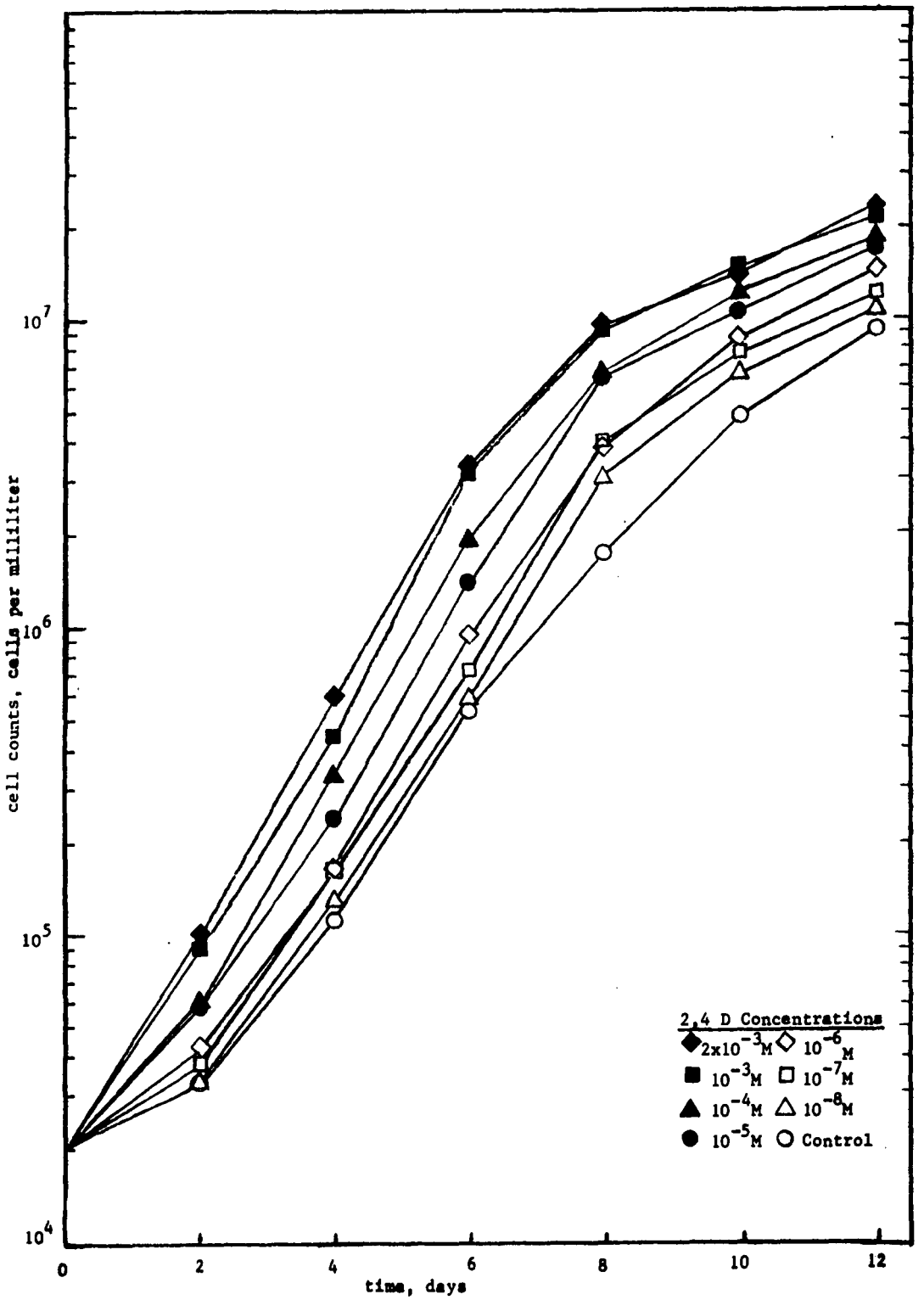

Figure 2. Effect of 2,4D on cell density at 200 ft-c and 20°C.

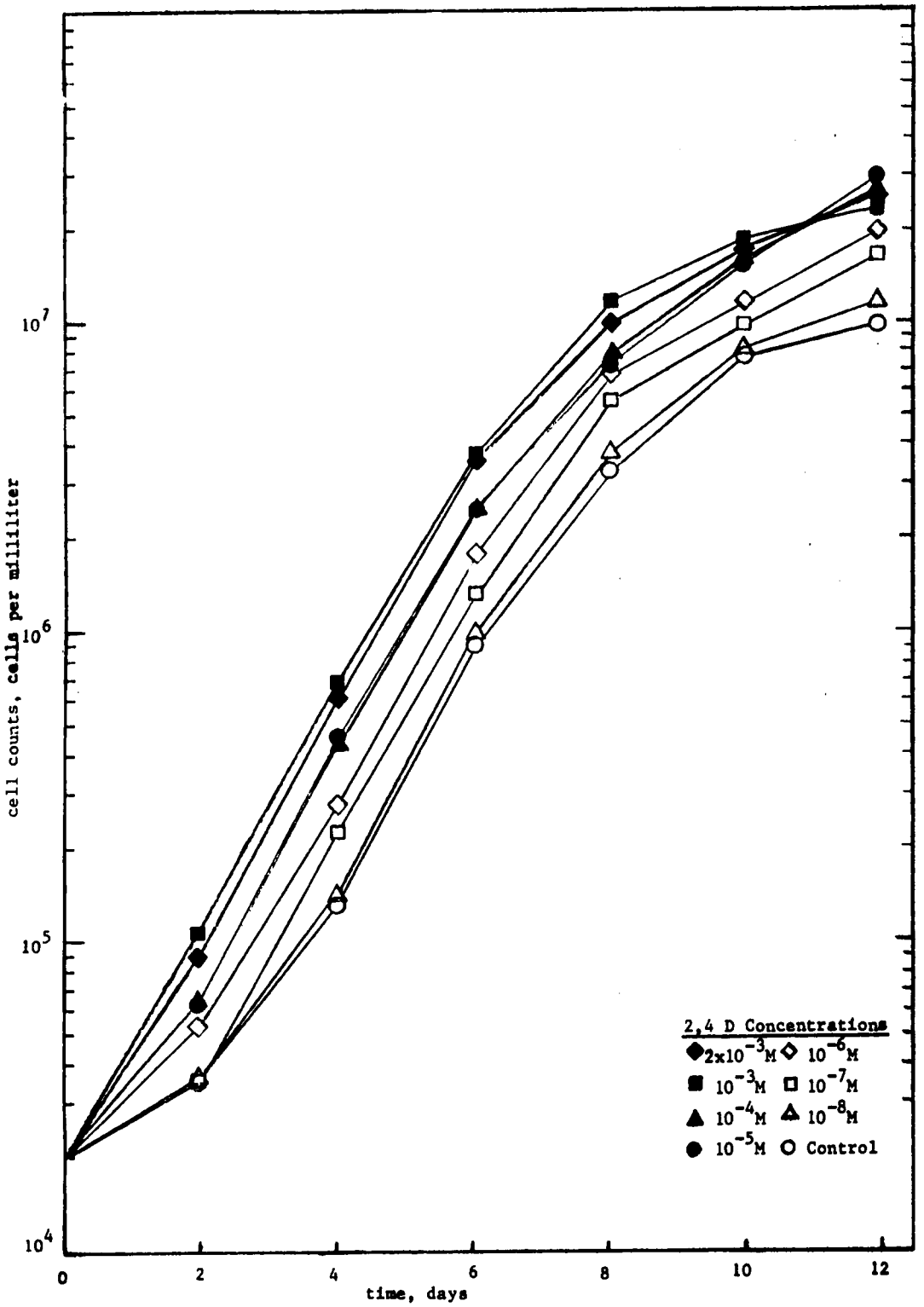

Figure 3. Effect of 2,4D on cell density at 220 ft-c and 20°C.

Table 1

Mean Values for Direct Cell Counts and

Fluorescence at 140 ft-c

Day	Cell count	Fluorescence	Cell count	Fluorescence
	Control		10^{-8} M	
2	3.13×10^4	3.50×10^1	3.23×10^4	4.27×10^1
4	8.50×10^4	5.37×10^1	8.57×10^4	5.67×10^1
6	1.28×10^5	1.13×10^2	1.91×10^5	1.13×10^2
8	4.73×10^5	3.03×10^2	5.72×10^5	3.95×10^2
10	1.12×10^6	7.17×10^2	1.48×10^6	8.50×10^2
12	1.81×10^6	1.07×10^3	2.92×10^6	1.28×10^3
	10^{-7} M		10^{-6} M	
2	3.80×10^4	6.17×10^1 *	3.83×10^4	7.03×10^1 *
4	9.10×10^4	7.47×10^1 *	1.20×10^5	8.23×10^1 *
6	3.80×10^5	2.45×10^2 *	4.39×10^5	3.23×10^2 *
8	9.07×10^5	6.10×10^2	1.57×10^6	8.37×10^2 *
10	2.26×10^6	1.00×10^3	3.34×10^6	1.92×10^3 *
12	3.43×10^6	2.14×10^3	6.39×10^6	3.23×10^3
	10^{-5} M		10^{-4} M	
2	4.40×10^4	7.77×10^1 *	5.10×10^4	8.60×10^1 *
4	1.52×10^5	9.40×10^1	1.83×10^5	1.23×10^2
6	5.13×10^5	3.70×10^2 *	8.07×10^5	4.97×10^2 *
8	1.65×10^6	9.00×10^2 *	3.05×10^6	1.29×10^3 *
10	3.62×10^6	2.13×10^3 *	6.00×10^6 *	3.69×10^3 *
12	6.94×10^6	3.68×10^3 *	9.95×10^6 *	7.36×10^3 *
	10^{-3} M		2×10^{-3} M	
2	9.17×10^4	1.07×10^2 *	9.60×10^4	1.01×10^2 *
4	3.95×10^5	4.53×10^2 *	4.73×10^5	3.80×10^2 *
6	2.19×10^6	1.31×10^3 *	2.39×10^6	1.21×10^3 *
8	5.78×10^6 *	2.84×10^3 *	5.42×10^6 *	2.17×10^3 *
10	1.14×10^7 *	7.80×10^3 *	9.03×10^6 *	6.18×10^3 *
12	1.43×10^7 *	1.03×10^4 *	1.71×10^7 *	1.11×10^4 *

*Statistically significant difference from control at 95% confidence level using Dunnett's test.

Table 2

Mean Values for Direct Cell Counts and

Fluorescence at 200 ft-c

Day	Cell count	Fluorescence	Cell count	Fluorescence
	Control		10^{-8} M	
2	3.20×10^{4}	4.53×10^{1}	3.23×10^{4}	5.83×10^{1}
4	1.09×10^{5}	7.10×10^{1}	1.27×10^{5}	1.17×10^{2}
6	5.16×10^{5}	4.97×10^{2}	5.69×10^{5}	6.33×10^{2}
8	1.67×10^{6}	1.29×10^{3}	2.99×10^{6}	1.47×10^{3}
10	4.69×10^{6}	3.94×10^{3}	6.48×10^{6}	4.03×10^{3}
12	8.95×10^{6}	6.18×10^{3}	1.03×10^{7}	8.35×10^{3}
	10^{-7} M		10^{-6} M	
2	3.70×10^{4}	6.90×10^{1}	4.17×10^{4}	7.43×10^{1} *
4	1.56×10^{5}	1.30×10^{2}	1.59×10^{5}	1.53×10^{2} *
6	6.99×10^{5}	7.13×10^{2}	9.15×10^{5}	7.77×10^{2}
8	3.78×10^{6}	1.71×10^{3}	3.71×10^{6}	1.77×10^{3}
10	7.59×10^{6}	4.40×10^{3}	8.42×10^{6}	3.17×10^{3}
12	1.18×10^{7}	8.52×10^{3}	1.39×10^{7}	9.40×10^{3}
	10^{-5} M		10^{-4} M	
2	5.73×10^{4}	7.80×10^{1}	5.87×10^{4}	8.67×10^{1} *
4	2.33×10^{5}	2.22×10^{2} *	3.23×10^{5} *	2.35×10^{2} *
6	1.33×10^{6}	1.23×10^{3} *	1.88×10^{6} *	1.39×10^{3} *
8	6.29×10^{6} *	2.35×10^{3} *	6.56×10^{6} *	2.49×10^{3}
10	1.02×10^{7}	5.11×10^{3}	1.18×10^{7} *	6.58×10^{3} *
12	1.64×10^{7}	1.13×10^{4} *	1.79×10^{7}	1.17×10^{4}
	10^{-3} M		2×10^{-3} M	
2	8.80×10^{4}	1.48×10^{2} *	9.77×10^{4} *	1.77×10^{2} *
4	4.28×10^{5} *	5.43×10^{2} *	5.73×10^{5} *	6.13×10^{2} *
6	3.06×10^{6} *	2.18×10^{3} *	3.32×10^{6} *	2.27×10^{3} *
8	8.94×10^{6} *	4.29×10^{3} *	9.40×10^{6} *	4.27×10^{3} *
10	1.46×10^{7} *	9.90×10^{3}	1.38×10^{7} *	1.01×10^{4}
12	2.08×10^{7}	1.43×10^{4} *	2.31×10^{7} *	2.08×10^{4} *

*Statistically significant difference from control at 95% confidence level using Dunnett's test.

Table 3

Mean Values for Direct Cell Counts and

Fluorescence at 220 ft-c

Day	Cell count	Fluorescence	Cell count	Fluorescence
	Control		10^{-8} M	
2	3.46×10^4	5.23×10^1	3.53×10^4	6.33×10^1
4	1.27×10^5	1.09×10^2	1.40×10^5	1.38×10^2
6	8.78×10^5	7.03×10^2	9.70×10^5	8.13×10^2
8	3.16×10^6	1.78×10^3	3.68×10^6	1.84×10^3
10	7.56×10^6	4.13×10^3	7.99×10^6	4.30×10^3
12	9.50×10^6	6.55×10^3	1.12×10^7	9.05×10^3
	10^{-7} M		10^{-6} M	
2	3.43×10^4	8.07×10^1	5.20×10^4	8.87×10^1
4	2.17×10^5	1.73×10^2	2.69×10^5	2.40×10^2 *
6	1.27×10^6	1.01×10^3	1.73×10^6	1.23×10^3 *
8	5.33×10^6	2.42×10^3	6.59×10^6 *	2.74×10^3 *
10	9.53×10^6	5.44×10^3	1.13×10^7	6.85×10^3
12	1.49×10^7	1.07×10^4	1.93×10^7	1.17×10^4
	10^{-5} M		10^{-4} M	
2	6.23×10^4	8.67×10^1	6.27×10^4	9.70×10^1
4	4.48×10^5	2.51×10^2	4.35×10^5	3.63×10^2
6	2.43×10^6	1.32×10^3 *	2.42×10^6	1.68×10^3 *
8	7.14×10^6 *	3.61×10^3 *	7.78×10^6 *	3.89×10^3 *
10	1.51×10^7	8.13×10^3 *	1.56×10^7 *	3.61×10^3 *
12	2.86×10^7	1.51×10^4 *	2.62×10^7	1.84×10^4 *
	10^{-3} M		2×10^{-3} M	
2	1.05×10^5 *	2.33×10^2 *	8.97×10^4	1.65×10^2
4	7.72×10^5 *	7.53×10^2 *	6.07×10^5	5.10×10^2 *
6	3.66×10^6 *	2.66×10^3 *	3.55×10^6 *	2.13×10^3 *
8	1.14×10^7 *	4.15×10^3 *	9.74×10^6 *	3.77×10^3 *
10	1.82×10^7 *	1.07×10^4 *	1.66×10^7 *	1.06×10^4 *
12	2.27×10^7	1.69×10^4 *	2.50×10^7	2.14×10^4 *

*Statistically significant difference from control at 95% confidence level using Dunnett's test.

Table 4

Linear Regression Analysis of Cell Count Data

Between Days 0 and 8*

2,4D concentration	Slope (log cells/days)	Ordinate Intercept (log cells)	Ordinate Intercept	Light intensity (ft-c)
Control	0.159	4.28	1.91×10^4	140
10^{-8} M	0.176	4.27	1.85×10^4	
10^{-7}	0.208	4.26	1.82×10^4	
10^{-6}	0.235	4.24	1.72×10^4	
10^{-5}	0.237	4.28	1.89×10^4	
10^{-4}	0.270	4.27	1.85×10^4	
10^{-3}	0.307	4.38	2.40×10^4	
2×10^{-3}	0.305	4.41	2.56×10^4	
Control	0.244	4.19	1.56×10^4	200
10^{-8} M	0.272	4.16	1.43×10^4	
10^{-7}	0.283	4.18	1.51×10^4	
10^{-6}	0.286	4.20	1.59×10^4	
10^{-5}	0.310	4.25	1.76×10^4	
10^{-4}	0.319	4.27	1.88×10^4	
10^{-3}	0.334	4.34	2.19×10^4	
2×10^{-3}	0.336	4.38	2.41×10^4	
Control	0.282	4.16	1.46×10^4	220
10^{-8} M	0.291	4.16	1.46×10^4	
10^{-7}	0.313	4.17	1.47×10^4	
10^{-6}	0.320	4.24	1.73×10^4	
10^{-5}	0.327	4.30	2.01×10^4	
10^{-4}	0.330	4.30	1.99×10^4	
10^{-3}	0.345	4.40	2.52×10^4	
2×10^{-3}	0.341	4.37	2.32×10^4	

*Basic equation:

$$\log y = \left(\frac{\log y_2 - \log y_1}{x_2 - x_1}\right) X + \log b$$

where

y = cell density

x = time, days

and 220 ft-c. Concentrations of increasing strength from 10^{-8} to 2×10^{-3} M increased the rate of growth of Chlorella. At 140 and 220 ft-c the rate of growth at 2×10^{-3} M 2,4D is less than 10^{-3} M depicting the inhibitory effect of higher 2,4D concentrations. This inhibition was not noticeable at 200 ft-c.

Through fluorometric analysis it was determined that chlorophyll and/or pheophytin productivity also increased exponentially with time. Pheophytin is known to cause positive interference in the detection of chlorophyll levels (152). These observations are depicted on semi-log plots in Figures 4, 5, and 6. Observations from these figures indicate that fluorescence levels are affected by increases in 2,4D concentration. Concentration from 10^{-8} to 2×10^{-3} M 2,4D have an increasing stimulatory effect on the levels of productivity. Mean values for the direct fluorometric units depicted in Figures 4, 5, and 6 can be found in Tables 1, 2, and 3, respectively. Although fluorometric determinations show more widely fluctuating rates of increase than direct cell counts, there is a continual increase until day 12 (Figures 4-6). For this reason, linear regression analysis for fluormetric units was constructed for days 0 through 12. Linear regression analyses results for fluorometric determinations are listed in Table 5 for 140, 200, and 220 foot-candles. At 140, 200, and 220 ft-c, increases in 2,4D concentration were followed by increased chlorophyll and/or pheophytin productivity, as indicated by increasing slopes. A concentration of 2×10^{-3} M at 140 and 220 ft-c appeared to be slightly inhibitory when compared to 10^{-3} M 2,4D.

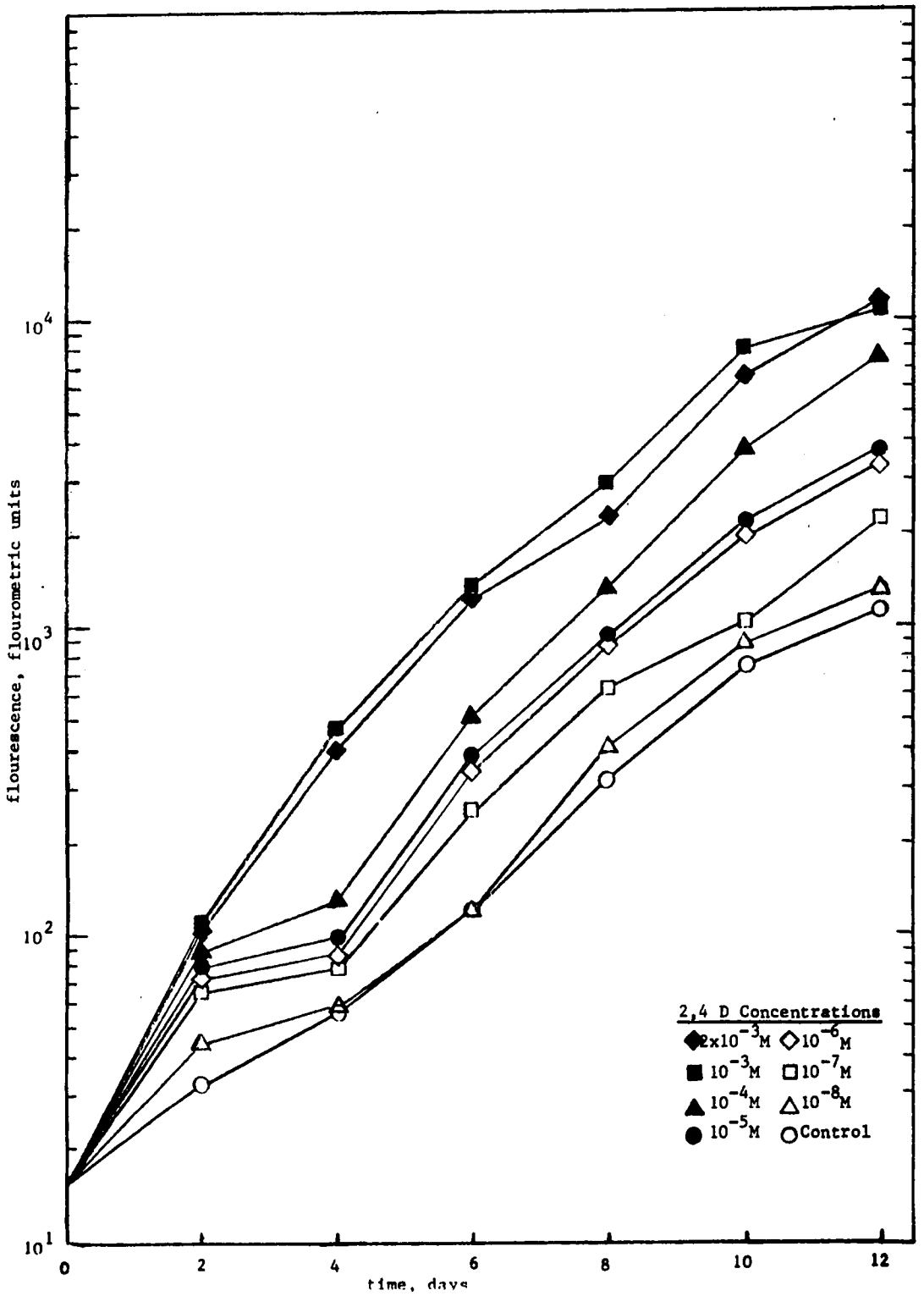

Figure 4. The effect of 2,4D on fluorescence of cell cultured under various 2,4D levels and 140 ft-c and 20°C.

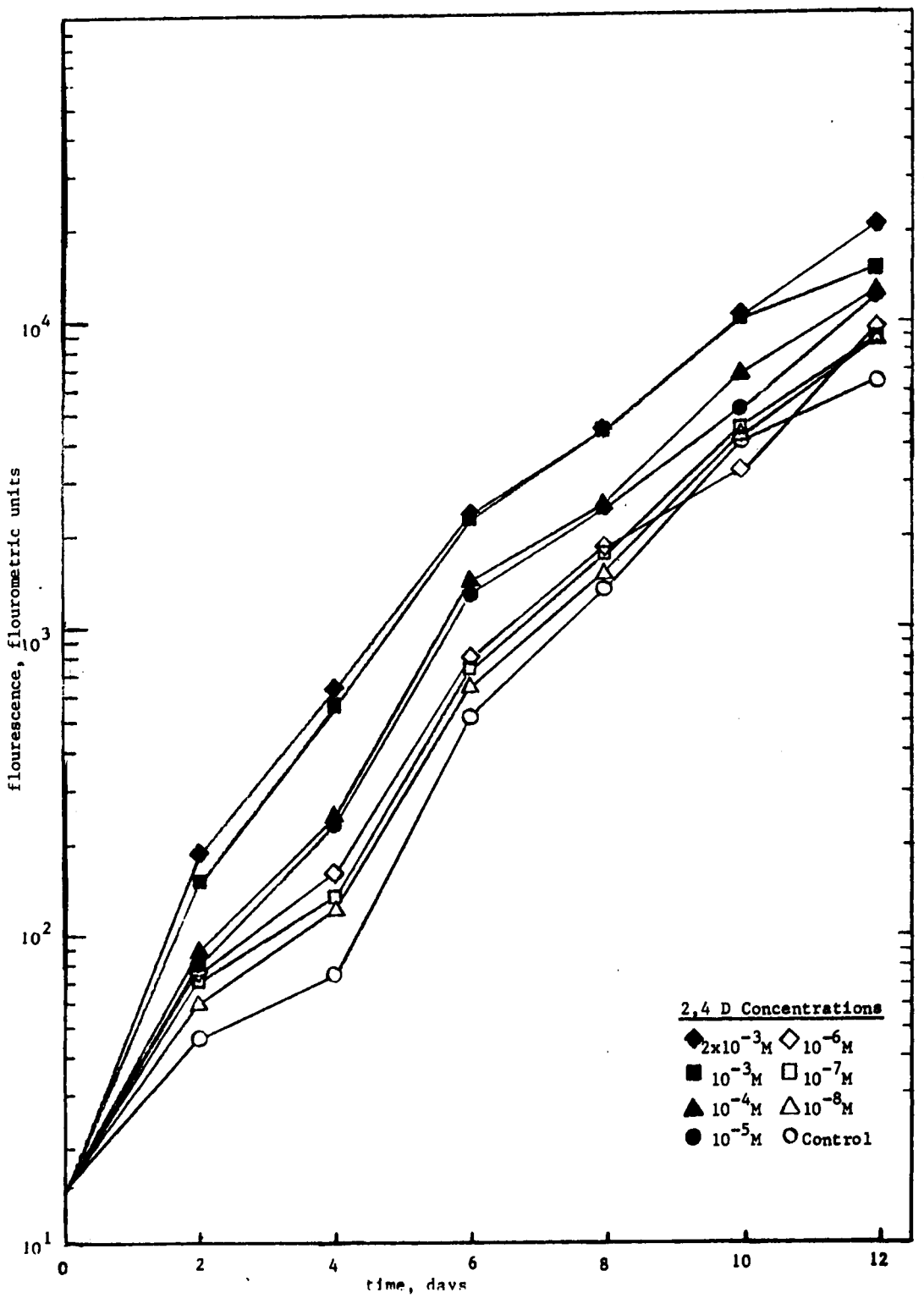

Figure 5. The effect of 2,4D on fluorescence of cells cultured under various 2,4D levels and 200 ft-c and 20°C.

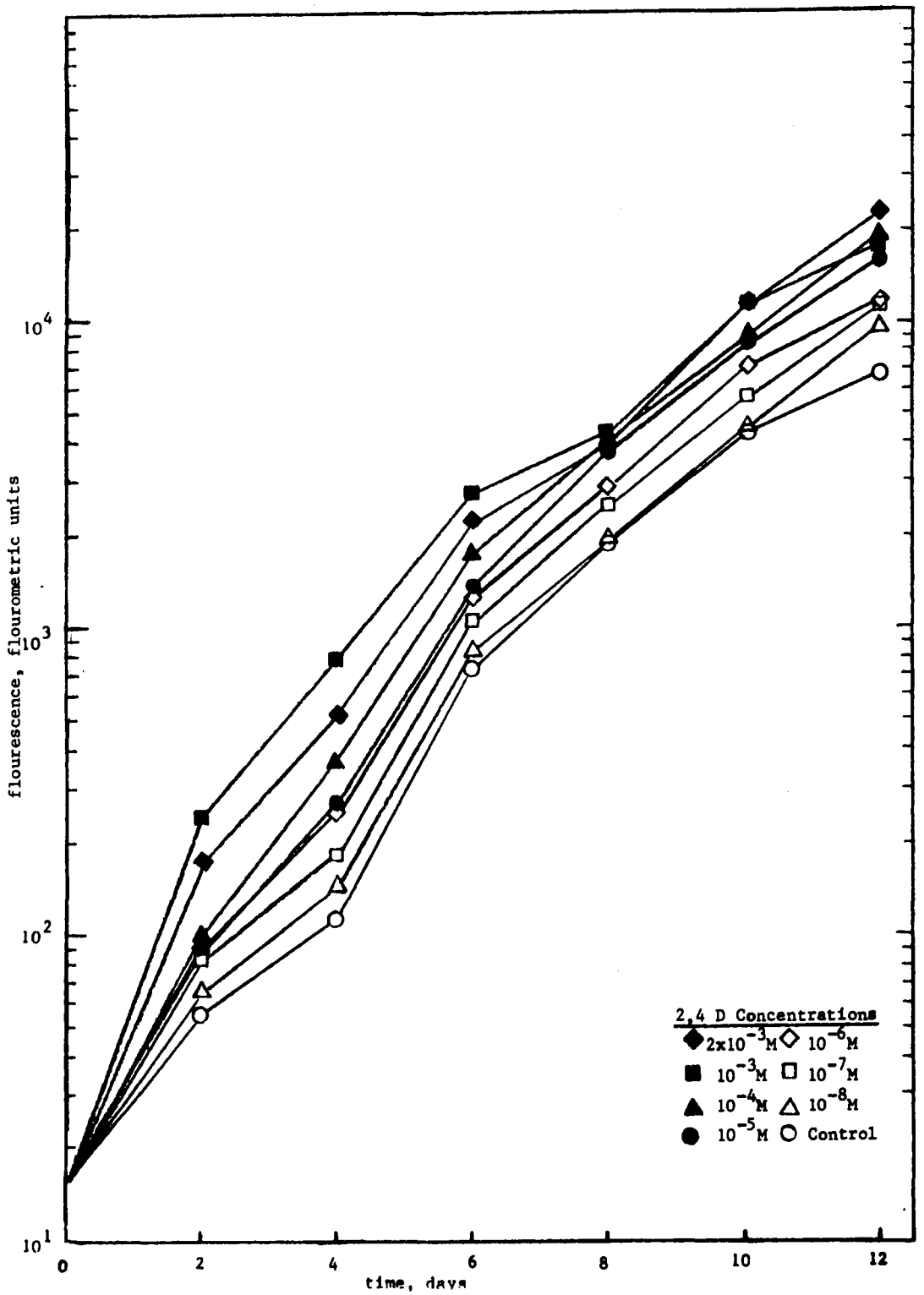

Figure 6. The effect of 2,4D on fluorescence of cells cultured under various 2,4D levels and 220 ft-c and 20°C.

Table 5

Linear Regression Analysis of Fluorometric Data

Between Days 0 and 12*

2,4D concentration	Slope (log fluorescence/ days)	Ordinate Intercept (log cells)	Ordinate Intercept	Light intensity (ft-c)
Control	0.160	1.17	14.7	
10^{-8}M	0.165	1.20	15.8	
10^{-7}	0.175	1.29	19.4	
10^{-6}	0.194	1.29	19.5	140
10^{-5}	0.197	1.31	20.5	
10^{-4}	0.221	1.31	20.4	
10^{-3}	0.241	1.43	26.7	
2 x 10^{-3}	0.231	1.47	29.8	
Control	0.232	1.17	14.7	
10^{-8}M	0.232	1.25	17.9	
10^{-7}	0.232	1.29	19.6	
10^{-6}	0.233	1.32	20.9	200
10^{-5}	0.237	1.38	24.2	
10^{-4}	0.240	1.40	25.3	
10^{-3}	0.241	1.59	38.6	
2 x 10^{-3}	0.246	1.60	39.9	
Control	0.231	1.26	18.0	
10^{-8}M	0.234	1.29	19.6	
10^{-7}	0.238	1.35	22.6	
10^{-6}	0.241	1.40	25.3	220
10^{-5}	0.252	1.39	24.4	
10^{-4}	0.253	1.45	27.9	
10^{-3}	0.236	1.69	48.9	
2 x 10^{-3}	0.249	1.56	36.4	

*Basic equation:

$$\log y = \left(\frac{\log y_2 - \log y_1}{x_2 - x_1}\right) x + \log b$$

where

y = fluorescence

x = time, days

Results obtained from using the Dunnett's test to statistically evaluate the effect of 2,4D acid on Chlorella pyrenoidosa are presented in Tables 1, 2, and 3. Concentrations which showed a significant difference from the control are denoted by a lower case letter next to the cell counts or fluorometric units.

Cell counts, generally, provided less values showing significant difference from the control than fluorometric units. Table 1 shows that concentrations of 10^{-4} to 2 x 10^{-3} M 2,4D significantly stimulated algal growth. The effect was noticeable on days 10 and 12. Table 2 illustrates that concentrations of 10^{-4} to 2 x 10^{-3} M 2,4D stimulated growth on days 6 through 10 at 200 ft-c. Stimulation was also noticeable on day 8 at 10^{-5} M 2,4D. Treatments with 2,4D were more effective at increasing light intensities as noted by the wider range of concentrations which caused stimulation. This can be seen in Table 3 which presents data obtained at 220 ft-c. On the eighth day significant differences were shown in growth using concentrations of 10^{-6} to 2 x 10^{-3} M 2,4D. Stimulation at 220 ft-c was also found on days 6 and 10 at 10^{-3} M and 10^{-4} to 2 x 10^{-3} M 2,4D, respectively.

Fluorometric determinations indicated that a greater range of concentrations caused stimulation at all three light intensities than did cell counts. Concentrations of 10^{-6} to 2 x 10^{-3} M 2,4D showed significant differences from the control on days 2 and 6 through 10 (Table 1). Fluorescence levels were also significantly different from the control at 10^{-7} M 2,4D on days 2 and 6 and at 10^{-3} to 2 x 10^{-3} M on day 4. Data presented in Table 2 illustrate that treated and control cultures were

significantly different on days 4, 6, 8, and 12 for concentrations ranging from 10^{-5} to 2 x 10^{-3} M 2,4D at 200 ft-c. On the second day, significant chlorophyll and/or pheophytin increases were also noted using concentrations of 10^{-4} to 2 x 10^{-3} M 2,4D. Fluorometric readings in Table 3 reveal that concentrations of 10^{-5} to 2 x 10^{-3} M 2,4D at days 6 through 12 were statistically different from the control. At a concentration of 10^{-6} M 2,4D there were significant differences on days 6 and 8 at 220 ft-c.

An alternative measure of cell counts is the maximum specific growth rate (148). This is a measure of the largest specific growth rate that occurs at any time during cell growth. The maximum specific growth rate is an average value determined for the largest specific growth rates of replicate flasks. Because the effect of herbicide treatments was not as dramatic when compared by maximum specific growth rates, it was not a chosen method of data interpretation in this study. Large standard deviations of values appeared to require more replicate observations. However, these values are included in the Appendix for the readers investigation.

Increases in light intensity from 140 to 220 foot candles had a marked effect on the growth rate of Chlorella pyrenoidosa. This is evident from Figure 7. The effect of varying light intensities on herbicide action are shown in Figures 8 through 14 for concentrations between 10^{-8} to 2 x 10^{-3} M 2,4D at 140, 200, and 220 foot candles. Utilization of 2,4D herbicide by the algae appeared to be affected little by increased light intensity. From inspection of Figures 1, 2, and 3 it can

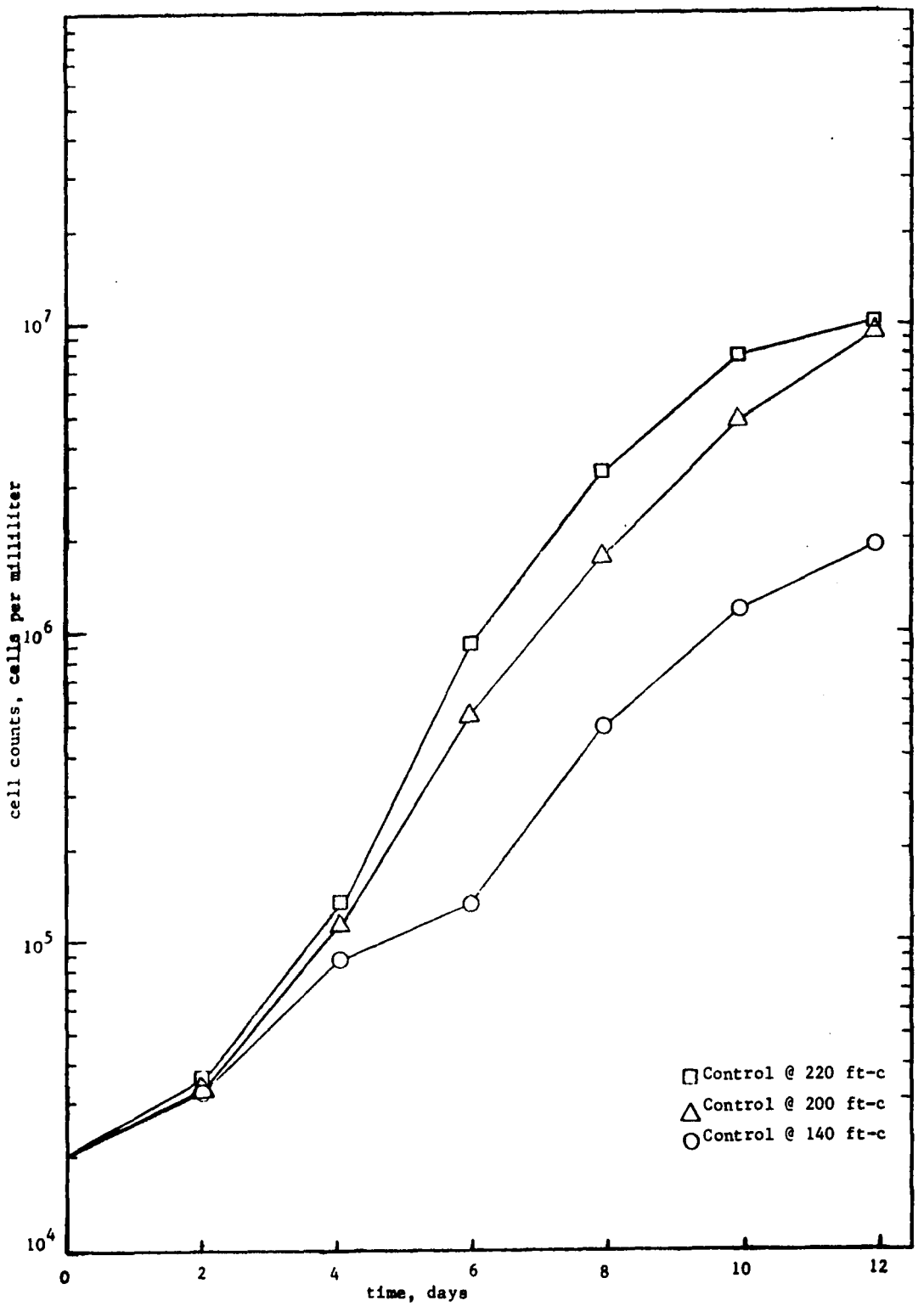

Figure 7. Effect of increasing light intensity on cell density.

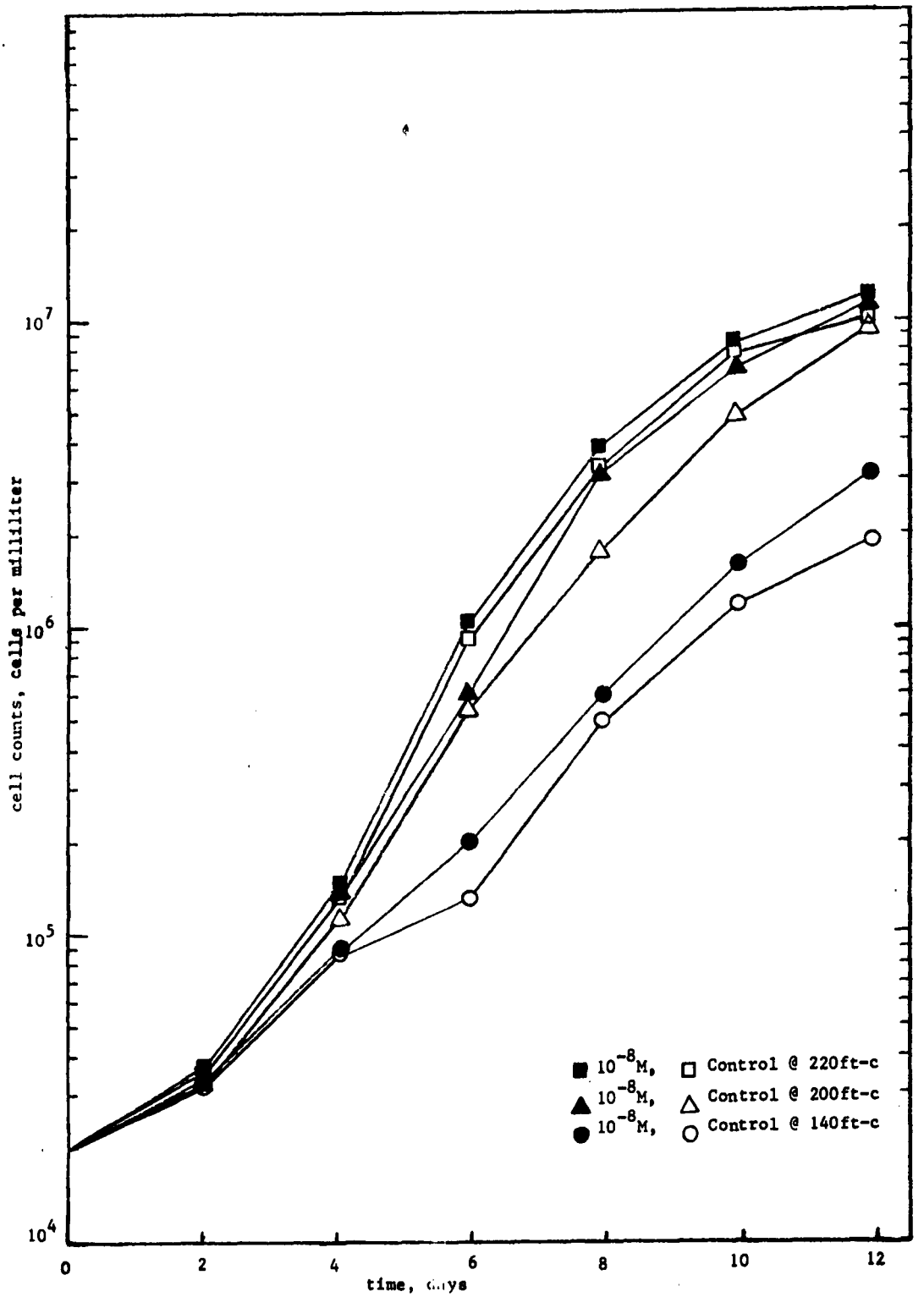

Figure 8. The effect of increasing light intensity on cell density at 10^{-8} M 2,4D

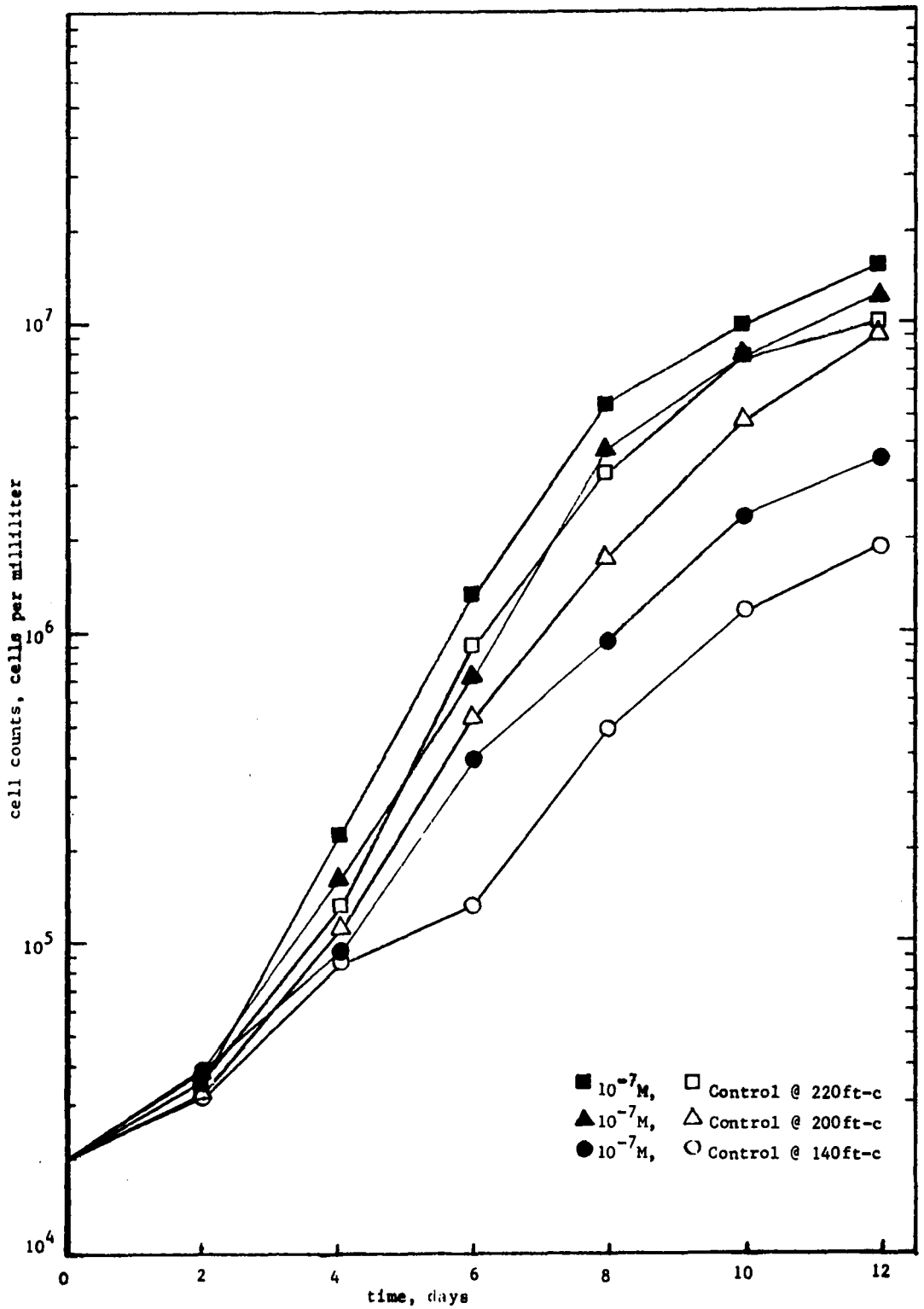

Figure 9. The effect of increasing light intensity on cell density at 10^{-7} M 2,4D.

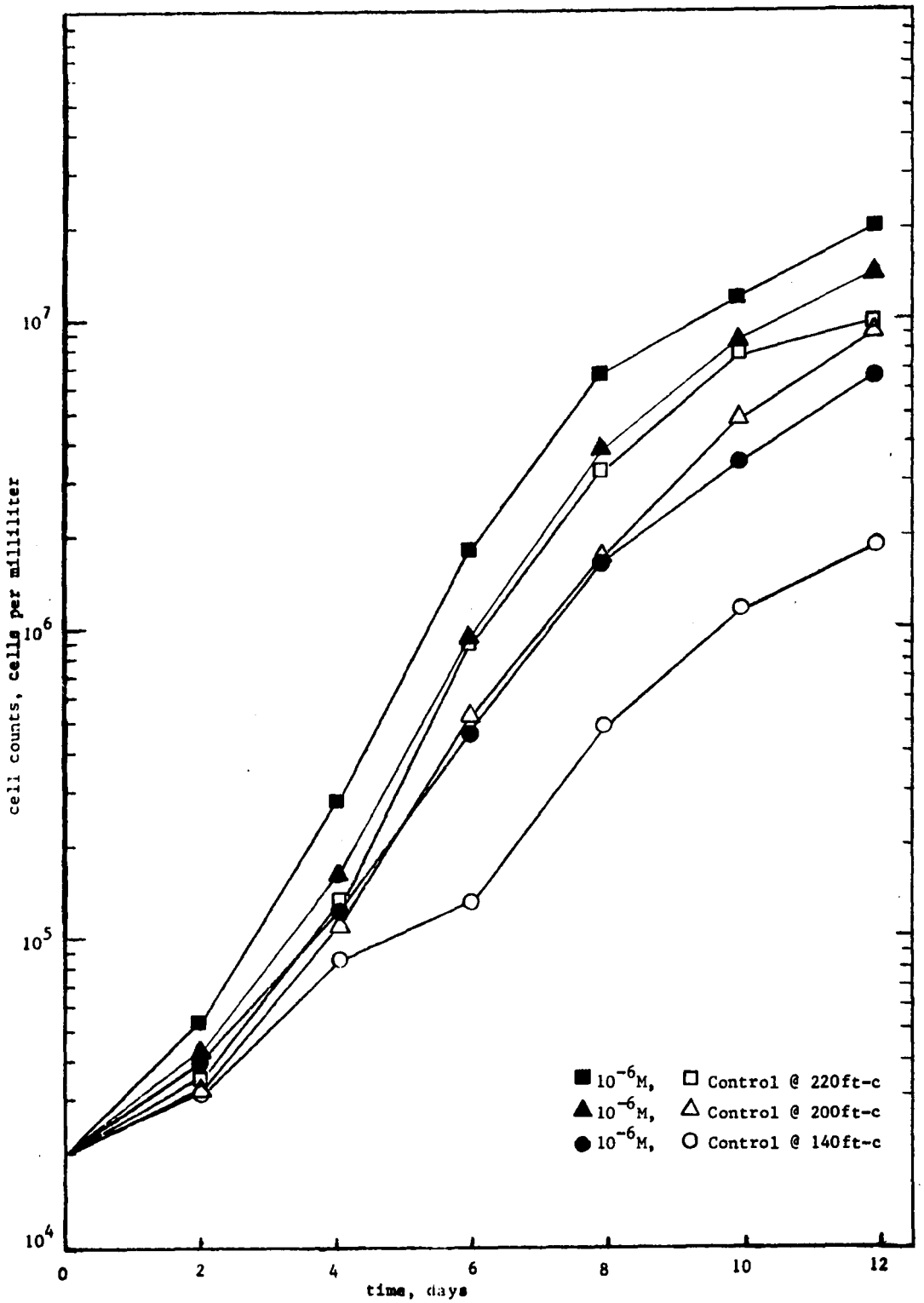

Figure 10. The effect of increasing light intensity on cell density at 10^{-6} M 2,4D.

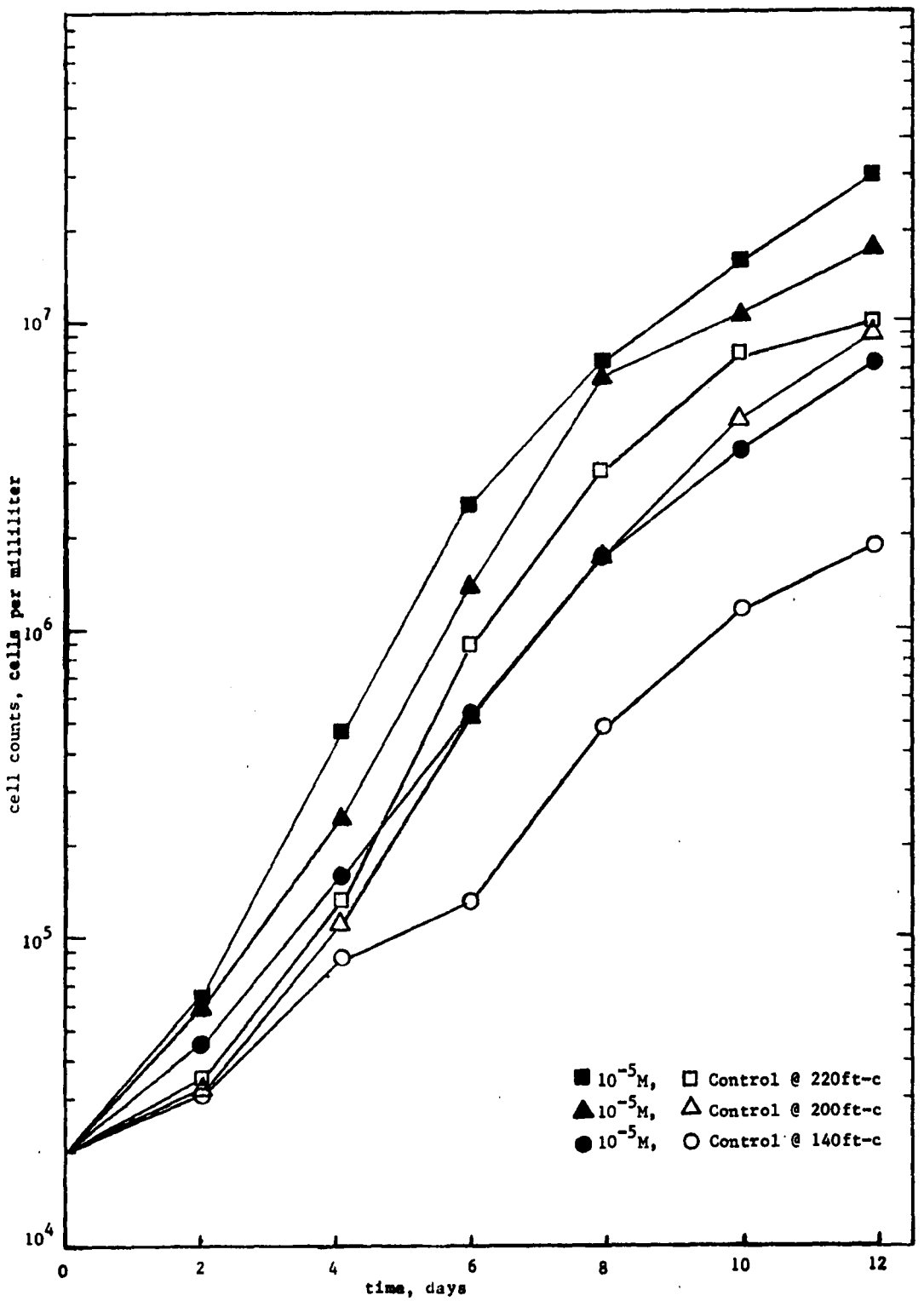

Figure 11. The effect of increasing light intensity on cell density at 10^{-5} M 2,4D.

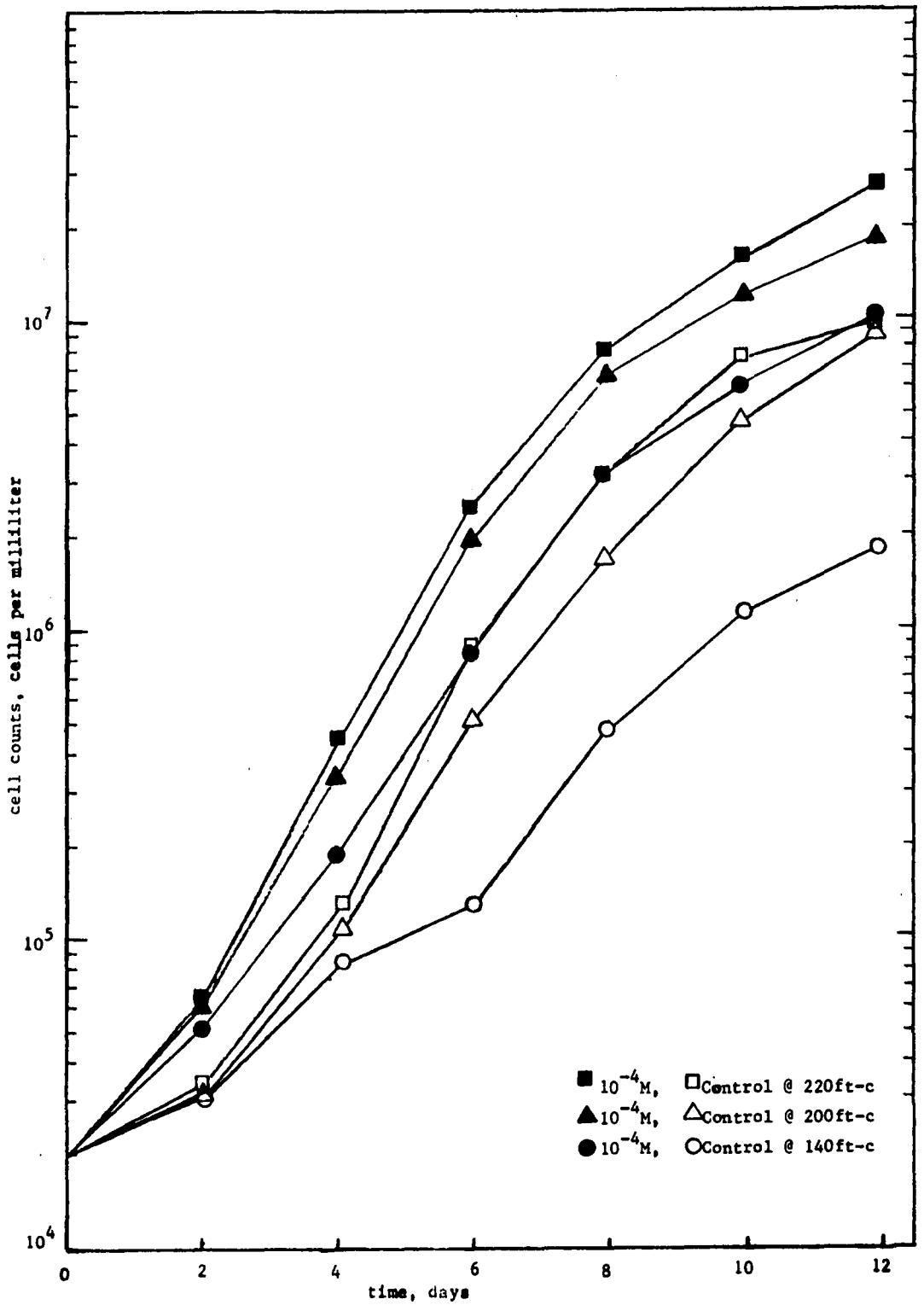

Figure 12. The effect of increasing light intensity on cell density at 10^{-4} M 2,4D.

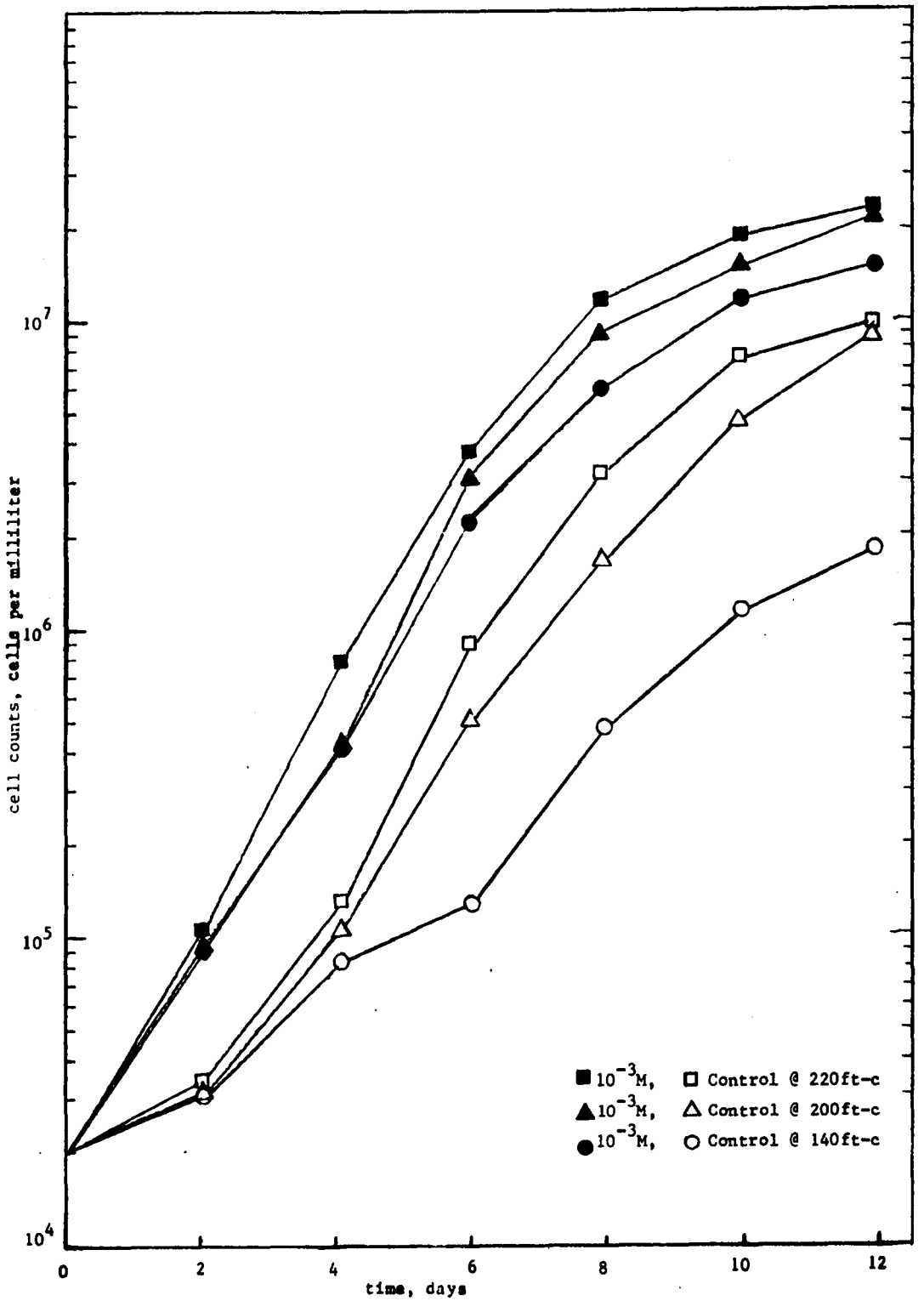

Figure 13. The effect of increasing light intensity on cell density at 10^{-3} M 2,4D.

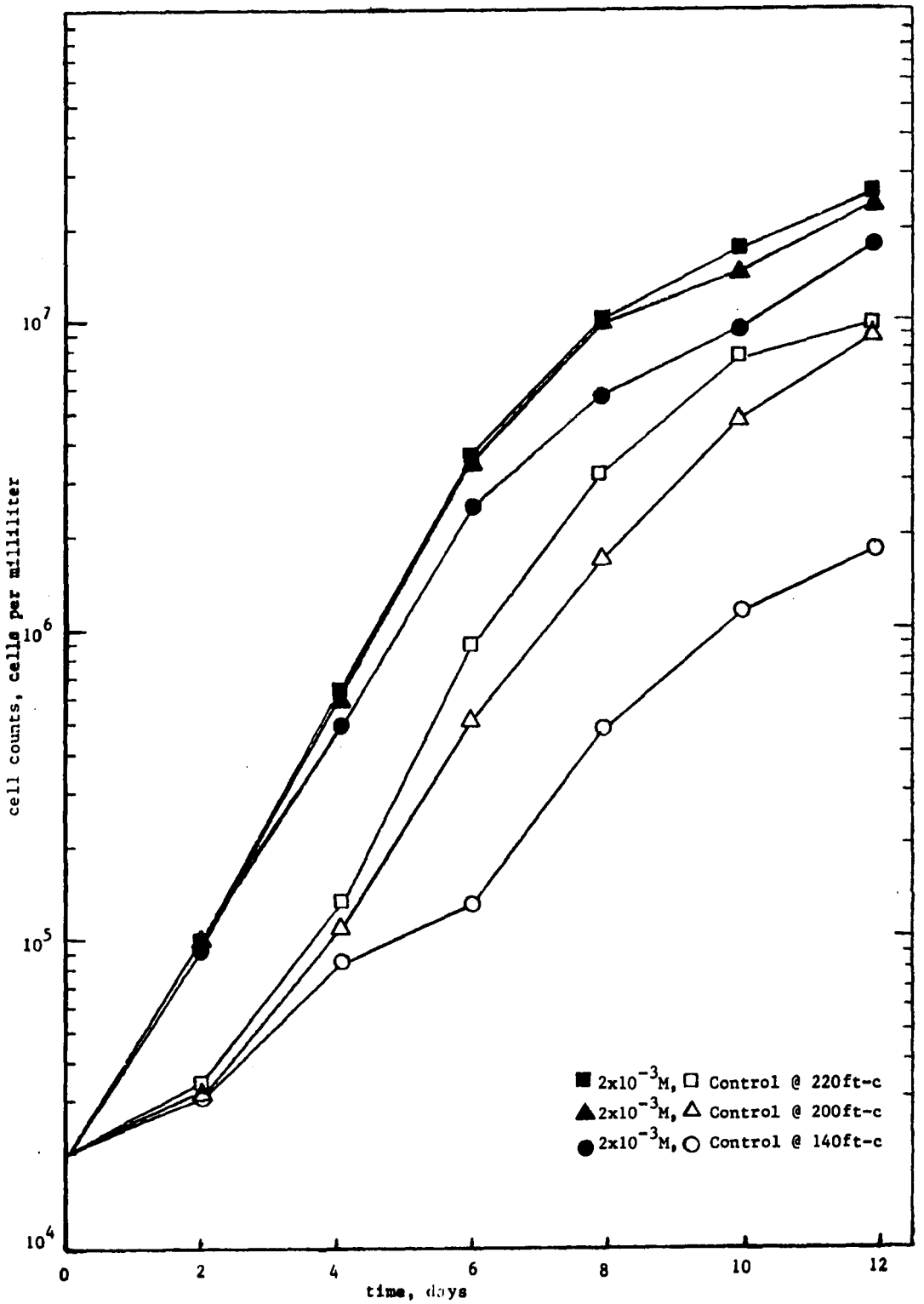

Figure 14. The effect of increasing light intensity on cell density at 2 x 10^{-3} M 2,4D.

be seen that differences between density of cells in the control and 2,4D treatment systems decreased with increasing light intensity. For example, although the yield of cells at 10^{-3} M 2,4D did not change significantly with the differing light intensities, cell counts in the control were appreciably greater at 140 ft-c than at 200 and 220 ft-c. This is most clearly evident on days 4 to 8.

From preliminary experiments it was determined that Chlorella pyrenoidosa reached the beginning of their stationary phase of growth at about 12 days. After this time, little net growth occurred. From Figs. 1, 2 and 3 it is evident that the growth of Chlorella began to stabilize after 8 days. Cell counts were compared on day 12 and the results, along with final fluorescence levels, are tabulated in Table 6. Increasing cell counts and increasing fluorescence can be noted at each light intensity. At 220 ft-c, concentrations of 10^{-4}, 10^{-3} and 2×10^{-3} M 2,4D had a lower growth rate than 10^{-5} M 2,4D. It appears that 10^{-4} M 2,4D is on the border between stimulation and inhibition for Chlorella at 220 ft-c.

If herbicides significantly contribute to the formation of algal blooms the importance of studies such as this is considerable. Increased costs of treating water means an increased cost to the consumer. Recreation sites may be left unused due to odor and color in lakes or reservoirs. However, Winton and Ritty (153) concluded that the phenoxy-aliphatic herbicides are decomposed into CO_2, inorganic chloride ions and H_2O and will not create a water pollution problem when properly applied to watershed areas. Perhaps fish kills resulted from poor

Table 6

Mean Cell Count and Fluorescence at Day 12

Light intensity (ft-c)	2,4D Conc.	Cell counts	Fluorescence
140	Control	1.81×10^6	1.07×10^3
	10^{-8} M	2.92×10^6	1.28×10^3
	10^{-7} M	3.43×10^6	2.14×10^3
	10^{-6} M	6.39×10^6	3.23×10^3
	10^{-5} M	6.94×10^6	3.68×10^3
	10^{-4} M	9.95×10^6	7.36×10^3
	10^{-3} M	1.43×10^7	1.03×10^4
	2×10^{-3} M	1.71×10^7	1.11×10^4
200	Control	8.95×10^6	6.18×10^3
	10^{-8} M	1.03×10^7	8.35×10^3
	10^{-7} M	1.18×10^7	8.52×10^3
	10^{-6} M	1.39×10^7	9.40×10^3
	10^{-5} M	1.64×10^7	1.13×10^4
	10^{-4} M	1.79×10^7	1.17×10^4
	10^{-3} M	2.08×10^7	1.43×10^4
	2×10^{-3} M	2.31×10^7	2.08×10^4
220	Control	9.50×10^6	6.55×10^3
	10^{-8} M	1.12×10^7	9.05×10^3
	10^{-7} M	1.49×10^7	1.07×10^4
	10^{-6} M	1.93×10^7	1.17×10^4
	10^{-5} M	2.86×10^7	1.51×10^4
	10^{-4} M	2.62×10^7	1.84×10^4
	10^{-3} M	2.27×10^7	1.69×10^4
	2×10^{-3} M	2.50×10^7	2.14×10^4

planning and use of 2,4D and that education regarding the use of the chemical is necessary. Further investigations of the effect of 2,4D on the growth of algae are necessary and important.

V. CONCLUSIONS

2,4-dichlorophenoxyacetic acid was tested in concentrations ranging from 10^{-8} to 2 x 10^{-3} M on the green alga, Chlorella pyrenoidosa. Results were analyzed to determine if low level concentrations of 2,4D were stimulatory to growth when grown at three light intensities. The following conclusions were reached based on the data obtained from this study:

1. A linear regression analyses of direct cell counts revealed that growth rate was proportional to 2,4D concentrations ranging from 10^{-8} to 2 x 10^{-3} M. However, at 140 and 220 foot candles, the highest 2,4D concentration, 2 x 10^{-3} M, produced a lower growth rate than 10^{-3} M 2,4D.
2. A linear regression analyses of fluorometric determinations revealed that increases in 2,4D concentrations from 10^{-8} to 2 x 10^{-3} M increased chlorophyll and/or pheophytin production at 140, 200 and 220 foot candles. At 140 and 220 ft-c, 2 x 10^{-3} M 2,4D produced a lower growth rate than 10^{-3} M 2,4D.
3. Using the Dunnett's analysis, concentrations of 10^{-4} to 2 x 10^{-3} M 2,4D were found to stimulate growth on days 10 and 12 at 140 ft-c and at days 6 through 10 at 200 ft-c. At 220 ft-c significant differences in treated cultures from the control were observed at day 8 using 10^{-6} to 2 x 10^{-3} M 2,4D and at days 6 and 10 at 10^{-3} M and 10^{-4} to 2 x 10^{-3} M 2,4D, respectively.

4. Use of the Dunnett's test to analyze fluorometric determinations indicated that concentrations of 10^{-6} to 2 x 10^{-3} M 2,4D showed significant differences from the control on days 2 and 6 through 10 at 140 foot candles. At 200 ft-c concentrations ranging from 10^{-5} to 2 x 10^{-3} M 2,4D were discovered to increase fluorometric measurements on days 4, 6, 8 and 12. At 220 ft-c concentrations were significantly different from the controls when measured at days 6 through 12 using concentrations of 2,4D ranging from 10^{-5} to 2 x 10^{-3} M. Hence, more herbicide levels appeared to evoke stimulation when growth was monitored with fluorometry than by cell counts.
5. Increases in light intensity were observed to increase the growth rate of the test alga. Light intensities over the range studied did not appear to affect the rate at which 2,4D herbicide was utilized by the algae for growth.

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APPENDICES

APPENDIX A

Cell Count Data at 140 ft-c

2,4D concentration	Days	Replicates 1	2	3	Mean	Std. Dev.	Log mean
Control	2	3.20×10^4	3.00×10^4	3.20×10^4	3.13×10^4	1.20×10^3	4.50
	4	6.50×10^4	1.00×10^4	9.00×10^4	8.50×10^4	1.80×10^4	4.93
	6	1.35×10^5	1.20×10^5	1.30×10^5	1.28×10^5	8.00×10^3	5.11
	8	4.10×10^5	5.00×10^5	5.10×10^5	4.73×10^5	5.50×10^4	5.67
	10	1.14×10^5	1.23×10^6	1.00×10^6	1.12×10^6	1.20×10^5	6.05
	12	1.87×10^5	1.77×10^6	1.79×10^6	1.81×10^6	5.00×10^4	6.26
10^{-8} M	2	3.30×10^4	3.20×10^4	3.20×10^4	3.23×10^4	5.80×10^3	4.51
	4	6.20×10^4	1.40×10^5	5.50×10^4	8.57×10^4	4.72×10^4	4.93
	6	1.87×10^5	1.73×10^5	2.13×10^5	1.91×10^5	2.00×10^4	5.28
	8	5.10×10^5	6.25×10^5	5.80×10^5	5.72×10^5	5.80×10^4	5.76
	10	1.42×10^6	1.63×10^6	1.40×10^6	1.48×10^6	1.30×10^5	6.17
	12	3.18×10^6	2.68×10^6	2.89×10^6	2.92×10^6	2.50×10^5	6.47
10^{-7} M	2	3.80×10^4	3.70×10^4	3.90×10^4	3.80×10^4	1.00×10^3	4.58
	4	9.80×10^4	9.52×10^4	8.00×10^4	9.10×10^4	8.70×10^3	4.46
	6	3.85×10^5	3.62×10^5	3.93×10^5	3.80×10^5	1.60×10^4	5.58
	8	9.00×10^5	9.30×10^5	8.90×10^5	9.07×10^5	2.10×10^4	5.96
	10	2.06×10^6	2.45×10^6	2.27×10^6	2.26×10^6	2.00×10^5	6.35
	12	3.50×10^6	3.42×10^6	3.37×10^6	3.43×10^6	1.20×10^5	6.54
10^{-6} M	2	3.80×10^4	4.00×10^4	3.70×10^4	3.83×10^4	1.50×10^4	4.58
	4	1.12×10^5	1.50×10^5	9.80×10^4	1.20×10^5	2.70×10^5	5.08
	6	4.50×10^5	4.20×10^5	4.47×10^5	4.39×10^5	1.70×10^5	5.64
	8	1.50×10^6	1.73×10^6	1.48×10^6	1.57×10^6	1.40×10^5	6.20
	10	3.38×10^6	3.23×10^6	3.40×10^6	3.34×10^6	9.00×10^4	6.52
	12	7.45×10^6	5.63×10^6	6.10×10^6	6.39×10^6	9.50×10^5	6.81
10^{-5} M	2	3.10×10^4	5.30×10^4	4.80×10^4	4.40×10^4	1.15×10^4	4.64
	4	1.13×10^5	2.22×10^5	1.20×10^5	1.52×10^5	6.10×10^4	5.18
	6	4.95×10^5	5.23×10^5	5.20×10^5	5.13×10^5	1.50×10^4	5.71
	8	1.41×10^6	2.01×10^6	1.53×10^6	1.65×10^6	3.20×10^5	6.22
	10	3.28×10^6	3.83×10^6	3.75×10^6	3.62×10^6	3.00×10^5	6.56
	12	6.23×10^6	7.78×10^6	6.81×10^6	6.94×10^6	7.80×10^5	6.84
10^{-4} M	2	4.30×10^4	5.80×10^4	5.20×10^4	5.10×10^4	7.60×10^3	4.71
	4	1.54×10^5	2.80×10^5	1.14×10^5	1.83×10^5	8.70×10^4	5.26
	6	7.75×10^5	8.47×10^5	8.00×10^5	8.07×10^5	3.70×10^4	5.91
	8	2.63×10^6	3.48×10^6	3.04×10^6	3.05×10^6	4.30×10^5	6.48
	10	6.48×10^6	5.48×10^6	6.05×10^6	6.00×10^6	5.00×10^6	6.78
	12	1.11×10^7	9.87×10^6	8.89×10^6	9.95×10^6	1.11×10^6	7.00
10^{-3} M	2	1.20×10^5	7.20×10^4	8.30×10^4	9.17×10^4	2.50×10^4	4.96
	4	3.60×10^5	4.05×10^5	4.20×10^5	3.95×10^5	3.10×10^4	5.60
	6	2.50×10^6	1.87×10^6	2.21×10^6	2.14×10^6	3.20×10^5	6.34
	8	6.38×10^6	5.43×10^6	5.53×10^6	5.78×10^6	5.20×10^6	6.76
	10	1.11×10^7	1.08×10^7	1.23×10^7	1.14×10^7	8.00×10^5	7.06
	12	1.56×10^7	1.32×10^7	1.42×10^7	1.43×10^7	1.20×10^6	7.16
2×10^{-3} M	2	1.30×10^5	7.00×10^4	8.80×10^4	9.60×10^4	3.08×10^4	4.98
	4	3.95×10^5	5.23×10^5	5.00×10^5	4.73×10^5	6.80×10^4	5.67
	6	1.54×10^6	3.23×10^6	2.40×10^6	2.39×10^6	8.50×10^5	6.38
	8	4.88×10^6	5.80×10^6	5.58×10^6	5.42×10^6	4.80×10^5	6.73
	10	9.60×10^6	8.59×10^6	8.91×10^6	9.03×10^6	5.20×10^5	6.96
	12	1.66×10^7	1.80×10^7	1.68×10^7	1.71×10^7	8.00×10^5	7.23

APPENDIX B

Cell Count Data at 200 ft-c

2,4D concentration	Days	Replicates 1	2	3	Mean	Std. Dev.	Log Mean
Control	2	3.30×10^4	3.10×10^4	3.20×10^4	3.20×10^4	1.00×10^3	4.51
	4	1.17×10^5	1.00×10^5	1.10×10^5	1.09×10^5	9.00×10^3	5.03
	6	5.35×10^5	4.87×10^5	5.25×10^5	5.16×10^5	2.50×10^4	5.71
	8	2.24×10^6	1.08×10^6	1.68×10^6	1.67×10^6	5.80×10^5	6.22
	10	4.80×10^6	4.56×10^6	4.72×10^6	4.69×10^6	1.20×10^5	6.67
	12	9.20×10^6	8.83×10^6	8.82×10^6	8.95×10^6	2.20×10^5	6.95
10^{-8} M	2	3.10×10^4	3.30×10^4	3.30×10^4	3.23×10^4	1.20×10^3	4.51
	4	1.31×10^5	1.40×10^5	1.10×10^5	1.27×10^5	1.50×10^4	5.10
	6	5.75×10^5	5.60×10^5	5.72×10^5	5.69×10^5	8.00×10^3	5.76
	8	3.22×10^6	2.73×10^6	3.02×10^6	2.99×10^6	2.50×10^5	6.48
	10	7.80×10^6	5.32×10^6	6.32×10^6	6.48×10^6	1.25×10^6	6.81
	12	1.09×10^7	1.02×10^7	9.80×10^6	1.03×10^7	6.00×10^5	7.01
10^{-7} M	2	3.70×10^4	3.80×10^4	3.60×10^4	3.70×10^4	1.00×10^3	4.57
	4	1.67×10^5	1.52×10^5	1.50×10^5	1.56×10^5	9.00×10^3	5.19
	6	8.95×10^5	4.98×10^5	7.05×10^5	6.99×10^5	1.99×10^5	5.84
	8	3.90×10^6	3.62×10^6	3.81×10^6	3.78×10^6	1.40×10^5	6.58
	10	8.10×10^6	7.45×10^6	7.23×10^6	7.59×10^6	4.50×10^5	6.88
	12	1.12×10^7	1.23×10^7	1.20×10^7	1.18×10^7	6.00×10^5	7.07
10^{-6} M	2	4.00×10^4	4.30×10^4	4.20×10^4	4.17×10^4	1.50×10^3	4.62
	4	1.68×10^5	1.50×10^5	1.60×10^5	1.59×10^5	9.00×10^3	5.20
	6	1.14×10^6	7.84×10^5	8.20×10^5	9.15×10^5	1.96×10^5	5.96
	8	3.75×10^6	3.57×10^6	3.82×10^6	3.71×10^6	1.30×10^5	6.57
	10	8.50×10^6	8.37×10^6	8.40×10^6	8.42×10^6	7.00×10^4	6.93
	12	1.37×10^7	1.41×10^7	1.39×10^7	1.39×10^7	2.00×10^5	7.14
10^{-5} M	2	6.40×10^4	5.30×10^4	5.50×10^4	5.73×10^4	5.90×10^3	4.76
	4	2.43×10^5	2.22×10^5	2.35×10^5	2.33×10^5	1.10×10^4	5.37
	6	1.43×10^6	1.30×10^6	1.27×10^6	1.33×10^6	9.00×10^4	6.12
	8	5.91×10^6	6.71×10^6	6.25×10^6	6.29×10^6	4.00×10^5	6.80
	10	1.11×10^7	9.27×10^6	1.03×10^7	1.02×10^7	9.00×10^5	7.01
	12	1.67×10^7	1.62×10^7	1.63×10^7	1.64×10^7	3.00×10^5	7.21
10^{-4} M	2	6.10×10^4	5.80×10^4	5.70×10^4	5.87×10^4	2.10×10^3	4.77
	4	3.60×10^5	2.80×10^5	3.27×10^5	3.23×10^5	4.00×10^4	5.51
	6	1.99×10^6	1.87×10^6	1.79×10^6	1.88×10^6	1.00×10^5	6.27
	8	5.74×10^6	7.14×10^6	6.81×10^6	6.56×10^6	7.30×10^5	6.82
	10	1.08×10^7	1.35×10^7	1.12×10^7	1.18×10^7	1.50×10^6	7.07
	12	1.86×10^7	1.70×10^7	1.81×10^7	1.79×10^7	8.00×10^5	7.25
10^{-3} M	2	1.10×10^5	7.20×10^4	8.20×10^4	8.80×10^4	1.97×10^4	4.94
	4	4.55×10^5	4.05×10^5	4.25×10^5	4.28×10^5	2.50×10^4	5.63
	6	3.12×10^6	3.08×10^6	2.98×10^6	3.06×10^6	7.00×10^4	6.49
	8	9.12×10^6	8.70×10^6	9.00×10^6	8.94×10^6	2.20×10^5	6.95
	10	1.48×10^7	1.45×10^7	1.46×10^7	1.46×10^7	2.00×10^5	7.16
	12	2.12×10^7	1.91×10^7	2.20×10^7	2.08×10^7	1.50×10^6	7.32
2×10^{-3} M	2	1.30×10^5	7.00×10^4	9.30×10^4	9.77×10^4	3.03×10^4	4.99
	4	6.30×10^5	5.23×10^5	5.68×10^5	5.73×10^5	5.40×10^4	5.76
	6	3.54×10^6	3.23×10^6	3.19×10^6	3.32×10^6	1.90×10^5	6.52
	8	9.93×10^6	9.03×10^6	9.25×10^6	9.40×10^6	4.70×10^5	6.87
	10	1.33×10^7	1.43×10^7	1.39×10^7	1.38×10^7	5.00×10^5	7.14
	12	2.71×10^7	1.97×10^7	2.25×10^7	2.31×10^7	3.70×10^6	7.36

APPENDIX C

Cell Count Data at 220 ft-c

2,4D concentration	Days	Replicates 1	2	3	Mean	Std. Dev.	Log Mean
Control	2	3.70×10^4	3.50×10^4	3.20×10^4	3.46×10^4	2.50×10^3	4.54
	4	1.31×10^5	1.20×10^5	1.30×10^5	1.27×10^5	6.00×10^3	5.10
	6	9.05×10^5	8.50×10^5	8.80×10^5	8.78×10^5	2.80×10^4	5.94
	8	3.32×10^6	3.00×10^6	3.15×10^6	3.16×10^6	1.60×10^5	6.50
	10	8.55×10^6	6.57×10^6	7.55×10^6	7.56×10^6	9.90×10^5	6.88
	12	9.90×10^6	9.20×10^6	9.40×10^6	9.50×10^6	3.60×10^5	6.98
10^{-8} M	2	3.50×10^4	3.70×10^4	3.40×10^4	3.53×10^4	1.50×10^3	4.55
	4	1.44×10^5	1.35×10^5	1.41×10^5	1.40×10^5	5.00×10^3	5.15
	6	1.04×10^6	9.10×10^5	9.60×10^5	9.70×10^5	6.60×10^4	5.99
	8	3.88×10^6	3.45×10^6	3.72×10^6	3.68×10^6	2.20×10^5	6.57
	10	8.63×10^6	7.23×10^6	8.00×10^6	7.99×10^6	7.10×10^5	6.90
	12	1.18×10^7	1.10×10^7	1.08×10^7	1.12×10^7	5.00×10^5	7.05
10^{-7} M	2	3.30×10^4	3.40×10^4	3.60×10^4	3.43×10^4	1.50×10^3	4.54
	4	2.30×10^5	2.00×10^5	2.20×10^5	2.17×10^5	1.50×10^4	5.34
	6	1.26×10^6	1.24×10^6	1.18×10^6	1.27×10^6	4.00×10^4	6.10
	8	5.55×10^6	5.12×10^6	5.32×10^6	5.33×10^6	2.20×10^5	6.73
	10	1.04×10^7	1.00×10^7	8.20×10^6	9.53×10^6	1.17×10^6	6.98
	12	1.57×10^7	1.43×10^7	1.48×10^7	1.49×10^7	7.00×10^5	7.17
10^{-6} M	2	5.80×10^4	5.00×10^4	4.80×10^4	5.20×10^4	5.30×10^3	4.72
	4	2.65×10^5	2.73×10^5	2.68×10^5	2.69×10^5	4.00×10^3	5.43
	6	1.68×10^6	1.80×10^6	1.70×10^6	1.73×10^6	6.00×10^4	6.24
	8	6.40×10^6	6.78×10^6	6.60×10^6	6.59×10^6	1.90×10^5	6.82
	10	1.13×10^7	1.25×10^7	1.00×10^7	1.13×10^7	1.30×10^6	7.05
	12	2.00×10^7	1.88×10^7	1.92×10^7	1.93×10^7	6.00×10^5	7.29
10^{-5} M	2	6.50×10^4	6.20×10^4	6.00×10^4	6.23×10^4	2.50×10^3	4.79
	4	5.15×10^5	4.10×10^5	4.20×10^5	4.48×10^5	5.80×10^4	5.65
	6	2.64×10^6	2.25×10^6	2.40×10^6	2.43×10^6	2.00×10^5	6.39
	8	7.05×10^6	7.17×10^6	7.19×10^6	7.14×10^6	1.00×10^5	6.85
	10	1.07×10^7	1.46×10^7	1.40×10^7	1.51×10^7	1.40×10^6	7.18
	12	2.95×10^7	3.23×10^7	2.39×10^7	2.86×10^7	4.30×10^6	7.46
10^{-4} M	2	6.10×10^4	6.30×10^4	6.40×10^4	6.27×10^4	1.50×10^3	4.80
	4	4.05×10^5	4.37×10^5	4.62×10^5	4.35×10^5	2.90×10^4	5.64
	6	2.38×10^6	2.43×10^6	2.45×10^6	2.42×10^6	4.00×10^4	6.38
	8	7.75×10^6	7.79×10^6	7.80×10^6	7.78×10^6	3.00×10^4	6.89
	10	1.51×10^7	1.60×10^7	1.56×10^7	1.56×10^7	5.00×10^5	7.19
	12	2.48×10^7	2.30×10^7	3.09×10^7	2.62×10^7	4.10×10^6	7.42
10^{-3} M	2	1.40×10^5	7.60×10^4	9.80×10^4	1.05×10^5	3.30×10^4	5.02
	4	9.35×10^5	6.30×10^5	7.50×10^5	7.72×10^5	1.54×10^5	5.89
	6	4.20×10^6	3.08×10^6	3.70×10^6	3.66×10^6	5.60×10^5	6.56
	8	1.13×10^7	1.10×10^7	1.20×10^7	1.14×10^7	5.00×10^5	7.06
	10	1.86×10^7	1.83×10^7	1.78×10^7	1.82×10^7	4.00×10^5	7.26
	12	1.90×10^7	2.22×10^7	2.70×10^7	2.27×10^7	4.00×10^6	7.36
2×10^{-3} M	2	1.10×10^5	7.20×10^4	8.70×10^4	8.97×10^4	1.91×10^4	4.95
	4	6.30×10^5	5.70×10^5	6.20×10^5	6.07×10^5	3.20×10^5	5.78
	6	3.79×10^6	3.23×10^6	3.62×10^6	3.55×10^6	2.90×10^5	6.55
	8	8.83×10^6	1.08×10^7	9.60×10^6	9.74×10^6	9.90×10^5	6.99
	10	1.62×10^7	1.71×10^7	1.65×10^7	1.66×10^7	5.00×10^5	7.22
	12	2.80×10^7	2.63×10^7	2.06×10^7	2.50×10^7	3.90×10^6	7.40

APPENDIX D

Fluorometric Data at 140 ft-c

2,4D concentration	Days	Replicates 1	2	3	Mean	Std. Dev.	Log mean
Control	2	3.50×10^1	3.10×10^1	3.90×10^1	3.50×10^1	4.00	1.54
	4	5.40×10^1	5.30×10^1	5.40×10^1	5.37×10^1	0.58	1.73
	6	1.14×10^2	1.11×10^2	1.15×10^2	1.13×10^2	2.08	2.05
	8	2.90×10^2	3.20×10^2	3.00×10^2	3.03×10^2	1.53×10^1	2.48
	10	6.90×10^2	7.50×10^2	7.10×10^2	7.17×10^2	3.05×10^1	2.86
	12	1.02×10^3	1.11×10^3	1.08×10^3	1.07×10^3	4.58×10^1	3.03
10^{-8} M	2	4.30×10^1	4.50×10^1	4.00×10^1	4.27×10^1	2.52	1.63
	4	5.70×10^1	5.50×10^1	5.80×10^1	5.67×10^1	1.32	1.75
	6	1.38×10^2	1.29×10^2	1.33×10^2	1.13×10^2	4.51	2.12
	8	3.80×10^2	4.00×10^2	4.05×10^2	3.95×10^2	1.18×10^2	2.60
	10	8.10×10^2	8.70×10^2	8.70×10^2	8.50×10^2	3.46×10^1	2.93
	12	1.26×10^3	1.29×10^3	1.29×10^3	1.28×10^3	1.73×10^1	3.11
10^{-7} M	2	6.00×10^1	6.40×10^1	6.10×10^1	6.17×10^1	2.08	1.79
	4	7.50×10^1	7.20×10^1	7.70×10^1	7.47×10^1	2.52	1.87
	6	2.46×10^2	2.46×10^2	2.42×10^2	2.44×10^2	2.31	2.39
	8	6.20×10^2	6.00×10^2	6.10×10^2	6.10×10^2	9.99	2.79
	10	9.60×10^2	1.14×10^3	9.00×10^2	1.00×10^3	1.24×10^2	3.00
	12	2.07×10^3	2.16×10^3	2.20×10^3	2.14×10^3	6.66×10^1	3.33
10^{-6} M	2	7.10×10^1	6.80×10^1	7.20×10^1	7.03×10^1	2.08	1.85
	4	8.50×10^1	8.20×10^1	8.00×10^1	8.23×10^1	2.52	1.92
	6	3.00×10^2	3.50×10^2	3.20×10^2	3.23×10^2	2.52×10^1	2.51
	8	7.90×10^2	8.70×10^2	8.50×10^2	8.37×10^2	2.52×10^1	2.92
	10	1.89×10^3	1.95×10^3	1.92×10^3	1.92×10^3	3.00×10^1	3.28
	12	3.00×10^3	3.40×10^3	3.30×10^3	3.23×10^3	2.08×10^2	3.51
10^{-5} M	2	7.70×10^1	7.50×10^1	8.10×10^1	7.70×10^1	3.06	1.89
	4	9.50×10^1	9.20×10^1	9.40×10^1	9.40×10^1	1.99	1.97
	6	3.70×10^2	3.80×10^2	3.60×10^2	3.70×10^2	9.99	2.57
	8	9.00×10^2	9.00×10^2	9.20×10^2	9.00×10^2	2.00×10^1	2.95
	10	2.10×10^2	2.08×10^3	2.20×10^3	2.13×10^3	6.43×10^1	3.33
	12	3.66×10^3	3.72×10^3	3.65×10^3	3.68×10^3	3.79×10^1	3.57
10^{-4} M	2	8.50×10^1	8.90×10^1	8.40×10^1	8.60×10^1	2.65	1.93
	4	1.26×10^2	1.24×10^2	1.20×10^2	1.23×10^2	3.06	2.09
	6	4.90×10^2	5.00×10^2	5.00×10^2	4.96×10^2	5.77	2.70
	8	1.32×10^3	1.26×10^3	1.29×10^3	1.29×10^3	3.00×10^1	3.11
	10	3.60×10^3	3.78×10^3	3.68×10^3	3.69×10^3	9.01×10^1	3.57
	12	7.20×10^3	7.50×10^3	7.38×10^3	7.36×10^3	1.51×10^2	3.87
10^{-3} M	2	1.11×10^2	1.06×10^2	1.05×10^2	1.07×10^2	3.21	2.03
	4	5.00×10^2	4.20×10^2	4.40×10^2	4.53×10^2	4.16×10^1	2.66
	6	1.31×10^3	1.30×10^3	1.32×10^3	1.31×10^3	5.86×10^1	3.11
	8	2.73×10^3	2.89×10^3	2.90×10^3	2.84×10^3	9.54×10^1	3.45
	10	7.65×10^3	7.95×10^3	7.80×10^3	7.80×10^3	1.50×10^2	3.89
	12	1.02×10^4	1.05×10^4	1.03×10^4	1.03×10^4	1.53×10^2	4.01
2×10^{-3} M	2	1.05×10^2	1.00×10^2	9.80×10^1	1.01×10^2	3.61	2.00
	4	3.60×10^2	4.00×10^2	3.80×10^2	3.80×10^2	1.99×10^1	2.50
	6	1.21×10^3	1.22×10^3	1.20×10^3	1.21×10^3	9.99	3.08
	8	2.20×10^3	2.14×10^3	2.13×10^3	2.17×10^3	3.79×10^1	3.34
	10	6.30×10^3	6.00×10^3	6.25×10^3	6.18×10^3	1.61×10^2	3.79
	12	1.04×10^4	1.14×10^4	1.14×10^4	1.11×10^4	6.12×10^2	4.04

APPENDIX E

Fluorometric Data at 200 ft-c

2,4D concentration	Days	Replicates 1	2	3	Mean	Std. Dev.	Log mean
Control	2	4.80×10^1	4.30×10^1	4.50×10^1	4.53×10^1	2.52	1.66
	4	7.00×10^1	7.20×10^1	7.10×10^1	7.10×10^1	0.99	1.85
	6	5.00×10^2	4.80×10^2	5.00×10^2	4.97×10^2	1.53×10^1	2.70
	8	1.26×10^3	1.32×10^3	1.28×10^3	1.29×10^3	3.06×10^1	3.11
	10	3.90×10^3	4.02×10^3	3.90×10^3	3.94×10^3	6.93×10^1	3.60
	12	6.30×10^3	6.00×10^3	6.25×10^3	6.18×10^3	1.61×10^2	3.79
10^{-8} M	2	6.70×10^1	5.80×10^1	5.00×10^1	5.83×10^1	8.50	1.77
	4	1.14×10^2	1.20×10^2	1.18×10^2	1.17×10^2	3.06	2.07
	6	6.40×10^2	6.30×10^2	6.30×10^2	6.33×10^2	5.77	2.80
	8	1.50×10^3	1.44×10^3	1.46×10^3	1.47×10^3	3.06×10^1	3.17
	10	4.20×10^3	3.90×10^3	4.00×10^3	4.03×10^3	1.53×10^2	3.61
	12	8.25×10^3	8.55×10^3	8.25×10^3	8.35×10^3	1.73×10^2	3.92
10^{-7} M	2	7.00×10^1	6.90×10^1	6.80×10^1	6.90×10^1	0.99	1.84
	4	1.35×10^2	1.25×10^2	1.30×10^2	1.30×10^2	4.99	2.11
	6	7.40×10^2	7.00×10^2	7.00×10^2	7.13×10^2	2.31×10^1	2.85
	8	1.74×10^3	1.68×10^3	1.70×10^3	1.71×10^3	3.06×10^1	3.23
	10	4.50×10^3	4.30×10^3	4.40×10^3	4.40×10^3	9.99×10^1	3.64
	12	8.40×10^3	8.60×10^3	8.55×10^3	8.52×10^3	1.05×10^2	3.93
10^{-6} M	2	7.70×10^1	7.20×10^1	7.40×10^1	7.43×10^1	2.52	1.87
	4	1.59×10^2	1.48×10^2	1.53×10^2	1.53×10^2	5.50	2.19
	6	7.60×10^2	7.80×10^2	7.90×10^2	7.76×10^2	1.53×10^1	2.89
	8	1.74×10^3	1.76×10^3	1.80×10^3	1.77×10^3	3.06×10^1	3.25
	10	4.62×10^3	4.80×10^3	4.70×10^3	3.17×10^3	9.02×10^1	3.67
	12	8.55×10^3	1.12×10^4	8.40×10^3	9.40×10^3	1.60×10^3	3.97
10^{-5} M	2	7.90×10^1	7.50×10^1	8.00×10^1	7.80×10^1	2.65	1.89
	4	2.22×10^2	2.20×10^2	2.24×10^2	2.22×10^2	1.99	2.35
	6	1.26×10^3	1.20×10^3	1.22×10^3	1.23×10^3	3.06×10^1	3.09
	8	2.43×10^3	2.19×10^3	2.42×10^3	2.35×10^3	1.36×10^2	3.37
	10	5.16×10^3	4.98×10^3	5.20×10^3	5.11×10^3	1.17×10^2	3.71
	12	1.15×10^4	1.10×10^4	1.13×10^4	1.13×10^4	3.00×10^2	4.05
10^{-4} M	2	8.70×10^1	8.90×10^1	8.40×10^1	8.67×10^1	2.52	1.94
	4	2.40×10^2	2.30×10^2	2.35×10^2	2.35×10^2	4.99	2.37
	6	1.44×10^3	1.36×10^3	1.38×10^3	1.39×10^3	4.16×10^1	3.14
	8	2.49×10^3	2.48×10^3	2.50×10^3	2.49×10^3	9.99	3.40
	10	6.45×10^3	6.80×10^3	6.50×10^3	6.58×10^3	1.89×10^2	3.82
	12	1.16×10^4	1.20×10^4	1.16×10^4	1.17×10^4	2.50×10^2	4.07
10^{-3} M	2	1.53×10^2	1.40×10^2	1.50×10^2	1.48×10^2	6.81	2.17
	4	5.60×10^2	5.30×10^2	5.40×10^2	5.43×10^2	1.53×10^1	2.74
	6	2.25×10^3	2.10×10^3	2.20×10^3	2.18×10^3	7.64×10^1	3.34
	8	4.26×10^3	4.20×10^3	4.40×10^3	4.29×10^3	1.03×10^2	3.63
	10	9.75×10^3	1.01×10^4	9.90×10^3	9.90×10^3	1.50×10^2	4.00
	12	1.46×10^4	1.38×10^4	1.46×10^4	1.43×10^4	4.33×10^2	4.16
2×10^{-3} M	2	1.80×10^2	1.82×10^2	1.70×10^2	1.77×10^2	6.43	2.25
	4	6.20×10^2	6.00×10^2	6.20×10^2	6.13×10^2	1.16×10^1	2.79
	6	2.28×10^3	2.28×10^3	2.26×10^3	2.27×10^3	1.16×10^1	3.36
	8	4.38×10^3	4.14×10^3	4.30×10^3	4.27×10^3	1.22×10^2	3.63
	10	1.06×10^4	9.75×10^3	1.03×10^4	1.01×10^4	3.21×10^2	4.01
	12	2.07×10^4	2.10×10^4	2.07×10^4	2.08×10^4	1.73×10^2	4.32

APPENDIX F

Fluorometric Data at 220 ft-c

2,4D concentration	Days	Replicates 1	2	3	Mean	Std. Dev.	Log mean
Control	2	5.40×10^1	5.00×10^1	5.30×10^1	5.20×10^1	2.08	1.72
	4	1.17×10^2	1.00×10^2	1.10×10^2	1.09×10^2	2.54	2.04
	6	7.10×10^2	7.00×10^2	7.00×10^2	7.03×10^2	5.77	2.85
	8	1.86×10^3	1.71×10^3	1.78×10^3	1.78×10^3	7.51×10^1	3.25
	10	4.26×10^3	4.14×10^3	4.00×10^3	4.13×10^3	1.30×10^2	3.62
	12	6.75×10^3	6.45×10^3	6.45×10^3	6.55×10^3	1.73×10^2	3.82
10^{-8} M	2	6.80×10^1	5.80×10^1	6.40×10^1	6.33×10^1	5.03	1.80
	4	1.38×10^2	1.39×10^2	1.36×10^2	1.38×10^2	1.53	2.14
	6	8.30×10^2	8.00×10^2	8.10×10^2	8.13×10^2	1.53×10^1	2.91
	8	1.83×10^3	1.83×10^3	1.85×10^3	1.84×10^3	8.66	3.26
	10	4.40×10^3	4.26×10^3	4.20×10^3	4.30×10^3	1.24×10^2	3.63
	12	9.15×10^3	9.45×10^3	8.55×10^3	9.05×10^3	4.58×10^2	3.96
10^{-7} M	2	8.20×10^1	7.80×10^1	8.20×10^1	8.07×10^1	2.31	1.91
	4	1.92×10^2	1.48×10^2	1.80×10^2	1.73×10^2	2.27×10^1	2.24
	6	1.08×10^3	9.60×10^2	1.00×10^3	1.01×10^3	6.11×10^1	3.01
	8	2.23×10^3	2.64×10^3	2.40×10^3	2.42×10^3	2.06×10^2	3.38
	10	5.70×10^3	5.46×10^3	5.16×10^3	5.44×10^3	2.70×10^2	3.74
	12	1.14×10^4	9.75×10^3	1.10×10^4	1.07×10^4	8.52×10^2	4.03
10^{-6} M	2	8.80×10^1	8.80×10^1	9.00×10^1	8.87×10^1	4.16	1.45
	4	2.22×10^2	2.58×10^2	2.40×10^2	2.40×10^2	1.80×10^1	2.38
	6	1.26×10^3	1.20×10^3	1.22×10^3	1.23×10^3	3.06×10^1	3.09
	8	2.63×10^3	2.79×10^3	2.80×10^3	2.74×10^3	9.54×10^1	3.44
	10	6.90×10^3	6.75×10^3	6.90×10^3	6.85×10^3	8.66×10^1	3.84
	12	1.20×10^4	1.11×10^4	1.20×10^4	1.17×10^4	5.20×10^2	4.07
10^{-5} M	2	8.40×10^1	8.60×10^1	9.00×10^1	8.67×10^1	3.06	1.94
	4	2.35×10^2	2.70×10^2	2.50×10^2	2.52×10^2	1.76×10^1	2.40
	6	1.30×10^3	1.36×10^3	1.31×10^3	1.32×10^3	3.22×10^1	3.12
	8	3.54×10^3	3.60×10^3	3.68×10^3	3.61×10^3	7.02×10^1	3.56
	10	8.20×10^3	7.95×10^3	8.25×10^3	8.13×10^3	1.61×10^2	3.91
	12	1.56×10^4	1.46×10^4	1.50×10^4	1.50×10^4	5.27×10^2	4.18
10^{-4} M	2	9.90×10^1	9.70×10^1	9.50×10^1	9.70×10^1	1.99	1.99
	4	3.90×10^2	3.20×10^2	3.80×10^2	3.63×10^2	3.79×10^1	2.56
	6	1.65×10^3	1.71×10^3	1.68×10^3	1.68×10^3	3.00×10^1	3.23
	8	3.76×10^3	3.82×10^3	4.09×10^3	3.89×10^3	1.76×10^2	3.59
	10	8.75×10^3	8.50×10^3	8.60×10^3	8.62×10^3	1.25×10^2	3.94
	12	1.80×10^4	1.65×10^4	2.07×10^4	1.84×10^4	2.13×10^3	4.26
10^{-3} M	2	2.25×10^2	2.75×10^2	2.00×10^2	2.33×10^2	3.82×10^1	2.37
	4	8.70×10^2	6.40×10^2	7.50×10^2	7.53×10^2	1.15×10^2	2.88
	6	2.73×10^3	2.64×10^3	2.60×10^3	2.66×10^3	7.11×10^2	3.42
	8	4.26×10^3	3.90×10^3	4.30×10^3	4.15×10^3	2.20×10^2	3.62
	10	1.05×10^4	1.11×10^4	1.05×10^4	1.07×10^4	3.46×10^2	4.03
	12	1.71×10^4	1.64×10^4	1.71×10^4	1.69×10^4	4.04×10^2	4.23
2×10^{-3} M	2	1.59×10^2	1.75×10^2	1.60×10^2	1.65×10^2	8.96	2.22
	4	5.30×10^2	5.20×10^2	4.80×10^2	5.10×10^2	2.65×10^1	2.71
	6	2.16×10^3	2.13×10^3	2.10×10^3	2.13×10^3	3.00×10^1	3.33
	8	3.72×10^3	3.84×10^3	3.76×10^3	3.77×10^3	4.93×10^1	3.58
	10	1.08×10^4	1.05×10^4	1.05×10^4	1.06×10^4	1.73×10^2	4.03
	12	2.13×10^4	2.19×10^4	2.10×10^4	2.14×10^4	4.58×10^2	4.33

APPENDIX G

Specific Growth Rates at 140 ft-c

Day	Replicates 1	2	3	Replicates 1	2	3
	Control			10^{-8} M conc		
2	0.35	0.60	0.52	0.32	0.74	0.27
4	0.37	0.09	0.18	0.55	0.11	0.68
6	0.56	0.71	0.68	0.50	0.64	0.50
8	0.51	0.45	0.34	0.51	0.48	0.44
10	0.25	0.18	0.29	0.40	0.25	0.36
12						
	10^{-7} M conc			10^{-6} M conc		
2	0.47	0.47	0.36	0.54	0.66	0.49
4	0.68	0.67	0.80	0.70	0.52	0.75
6	0.43	0.47	0.41	0.60	0.71	0.60
8	0.41	0.48	0.47	0.41	0.31	0.42
10	0.27	0.17	0.20	0.40	0.28	0.29
12						
	10^{-5} M conc			10^{-4} M conc		
2	0.65	0.72	0.46	0.64	0.79	0.39
4	0.74	0.43	0.73	0.81	0.55	0.97
6	0.52	0.67	0.54	0.61	0.71	0.67
8	0.42	0.32	0.45	0.45	0.23	0.34
10	0.32	0.35	0.30	0.27	0.29	0.19
12						
	10^{-3} M conc			2×10^{-3} M conc		
2	0.55	0.86	0.81	0.56	1.01	0.87
4	0.97	0.77	0.83	0.68	0.91	0.78
6	0.47	0.53	0.46	0.58	0.29	0.42
8	0.28	0.34	0.40	0.34	0.49	0.23
10	0.17	0.10	0.07	0.27	0.37	0.32
12						

APPENDIX H

Specific Growth Rates at 200 ft-c

Day	Replicates 1	2	3	Replicates 1	2	3
	Control			10^{-8} M conc		
2	0.63	0.59	0.63	0.72	0.72	0.60
4	0.76	0.79	0.78	0.74	0.69	0.82
6	0.72	0.40	0.58	0.86	0.79	0.83
8	0.38	0.72	0.52	0.44	0.33	0.37
10	0.33	0.33	0.31	0.17	0.33	0.22
12						
	10^{-7} M conc			10^{-6} M conc		
2	0.75	0.69	0.71	0.72	0.63	0.67
4	0.84	0.59	0.77	0.96	0.83	0.82
6	0.74	0.99	0.84	0.60	0.76	0.77
8	0.36	0.36	0.32	0.40	0.43	0.39
10	0.16	0.25	0.25	0.24	0.26	0.25
12						
	10^{-5} M conc			10^{-4} M conc		
2	0.67	0.72	0.73	0.89	0.79	0.87
4	0.89	0.88	0.84	0.86	0.95	0.85
6	0.71	0.82	0.80	0.53	0.67	0.67
8	0.32	0.16	0.25	0.32	0.32	0.25
10	0.20	0.28	0.23	0.27	0.12	0.24
12						
	10^{-3} M conc			2×10^{-3} M conc		
2	0.71	0.86	0.82	0.79	1.01	0.91
4	0.96	1.01	0.97	0.86	0.91	0.86
6	0.54	0.52	0.55	0.52	0.51	0.53
8	0.24	0.26	0.24	0.15	0.23	0.20
10	0.18	0.14	0.21	0.36	0.16	0.24
12						

APPENDIX I

Specific Growth Rates at 220 ft-c

Day	Replicates 1	2	3	Replicates 1	2	3
	Control			10^{-8} M conc		
2						
4	0.63	0.62	0.70	0.71	0.65	0.71
6	0.97	0.98	0.96	1.11	0.95	0.96
8	0.65	0.68	0.64	0.66	0.67	0.68
10	0.47	0.39	0.44	0.40	0.37	0.39
12	0.07	0.17	0.11	0.16	0.21	0.14
	10^{-7} M conc			10^{-6} M conc		
2						
4	0.97	0.89	0.91	0.76	0.85	0.86
6	0.85	0.91	0.84	0.92	0.94	0.92
8	0.74	0.71	0.75	0.67	0.66	0.68
10	0.31	0.33	0.22	0.28	0.31	0.21
12	0.21	0.18	0.30	0.29	0.20	0.33
	10^{-5} M conc			10^{-4} M conc		
2						
4	1.04	0.95	0.97	0.95	0.97	0.99
6	0.82	0.85	0.87	0.89	0.86	0.83
8	0.49	0.58	0.55	0.59	0.58	0.58
10	0.43	0.36	0.33	0.33	0.36	0.35
12	0.28	0.27	0.27	0.25	0.18	0.34
	10^{-3} M conc			2×10^{-3} M conc		
2						
4	0.95	1.06	1.02	0.87	1.03	0.98
6	0.75	0.79	0.80	0.90	0.87	0.88
8	0.45	0.64	0.59	0.42	0.60	0.49
10	0.25	0.26	0.20	0.30	0.23	0.27
12	0.01	0.10	0.21	0.27	0.22	0.11

APPENDIX J

Maximum Specific Growth Rates

Replicates	Concentration							
	Control	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}	2×10^{-3}
---------- 140 ft-c: Maximum Specific Growth Rate (μ max) ----------								
1	0.56	0.55	0.68	0.70	0.74	0.81	0.91	0.68
2	0.71	0.74	0.67	0.71	0.72	0.79	0.86	1.01
3	0.68	0.68	0.80	0.75	0.73	0.97	0.83	0.87
Avg. (μ max)	0.65 ± .08	0.66 ± .10	0.72 ± .07	0.72 ± .03	0.73 ± .01	0.86 ± .10	0.89 ± .07	0.85 ± .16
---------- 200 ft-c: Maximum Specific Growth Rate (μ max) ----------								
1	0.76	0.86	0.84	0.96	0.89	0.89	0.96	0.86
2	0.79	0.79	0.99	0.83	0.88	0.95	1.01	1.01
3	0.78	0.83	0.84	0.82	0.84	0.87	0.97	0.91
Avg. (μ max)	0.78 ± .02	0.83 ± .04	0.89 ± .09	0.87 ± .08	0.87 ± .02	0.90 ± .04	0.98 ± .03	0.93 ± .07
---------- 220 ft-c: Maximum Specific Growth Rate (μ max) ----------								
1	0.97	1.11	0.97	0.92	1.04	0.95	0.95	0.90
2	0.93	0.95	0.91	0.94	0.95	0.97	1.06	1.03
3	0.96	0.96	0.91	0.92	0.97	0.99	1.02	0.98
Avg. (μ max)	0.92 ± .01	1.01 ± .09	0.93 ± .04	0.93 ± .01	0.98 ± .05	0.97 ± .03	1.01 ± .06	0.97 ± .07

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THE EFFECT OF LOW LEVEL CONCENTRATIONS OF 2,4 DICHLOROPHENOXYACETIC ACID ON CHLORELLA PYRENDIDOSA

by

Kenneth Paul Parr

(ABSTRACT)

Algal blooms in our streams, lakes and reservoirs have been known to cause nuisance problems. Water treatment problems include clogging of filtration units, taste and odor, turbidity and green coloration of water. Dense algal blooms reduce the recreational value of water and, recently, have been reported to kill fish. Algal blooms have been attributed to increased nutrients in waterways from wastewater discharges and runoff from agricultural land which contains fertilizers and herbicides. A widely used herbicide, 2,4 dichlorophenoxyacetic acid (2,4D), has been found in waters adjacent to land applications in concentrations known to be stimulatory to many plants.

The purpose of this study was to evaluate the effect of 2,4D on the growth of Chlorella pyrenoidosa in batch cultures. Cultures were grown over a 12 day period in 2,4D concentrations of 10^{-8} to 2×10^{-3} M. The culture medium was inoculated to provide 2.0×10^{4} cells/ml and incubated under continuous fluorescent lighting at 140, 200 and 220 foot candles and $20 \pm 1.0°C$. Cell counts and fluorometric readings

were made on even days and analyzed by least squares regression and analysis of variance.

The results indicate that 2,4D promoted growth of Chlorella pyrenoidosa. Increased growth was discovered with increases in 2,4D concentration from 10^{-8} to 2×10^{-3} M 2,4D. A concentration of 2×10^{-3} M 2,4D appeared to cause slight inhibition when compared to 10^{-3} M 2,4D. Light intensity had little effect on 2,4D utilization by Chlorella.

The results of this study indicate that herbicides used currently for weed control may be correlated to increase algal growth and thereby influence water quality.