THE SURVIVAL AND GROWTH OF <u>SPIRULINA</u> SPP. IN THE SALINE GROUNDWATERS OF NEW MEXICO

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ABSTRACT

New Mexico has some 15 billion acre-feet of saline groundwater. Much of this water has a salinity greater than 3,000 ppm Total Dissolved Solids (TDS) and thus cannot be used for potable water nor traditional agricultural activities.

The main objective of this project was to determine the technical feasibility of growing Spirulina in New Mexico using these saline groundwaters. Spirulina was chosen because it grows naturally in highly alkaline lakes in hot climates and is more readily harvested than other algae. Spirulina has many potential uses as a source of specialty chemicals, feed ingredient for livestock and aquatic organisms, and human health food.

Laboratory studies support empirical observations made at the pilot plant that several species of Spirulina will grow in full RTF saline water (14,000 ppm TDS), half RTF water (7,000 ppm TDS), New Mexico State University (NMSU) geothermal water (2,000 ppm TDS) and NMSU tap water (500 ppm TDS). Further, these species are capable of excellent growth at temperatures as high as 42°C. Growth appears to slow at 20°C and below.

The technical feasibility of Spirulina cultivation in New Mexico using saline groundwaters has been demonstrated. Market research should be performed to determine the size and location of potential markets and the price structure. If market research data is favorable, a small commercial size Spirulina culture operation should be established and operated for at least a year to determine actual operating and capital costs.

INTRODUCTION

Two-thirds of the contiguous United States is underlain by brackish water aquifers containing water with total dissolved solids (TDS) of at least 1,000 ppm (Feth 1965). All governmental agencies consider water of 1,500 ppm TDS and greater to be nonpotable. Water containing as much as 3,000 ppm TDS may be used for irrigation, although under most conditions it is unsatisfactory. Moderately saline water (3,000-10,000 ppm) is unsatisfactory for most uses and is seldom used for domestic supply (Hood and Kister 1962). Brackish water aquifers may contain waters with a TDS greater than that of seawater (35,000 ppm).

The vast areas of semiarid lands in the world account for 36 percent of the land availability, or the equivalent of a land area of 50 million square miles. The U.S. Southwest alone contains many millions of acres of arid and semiarid land as well as very high levels of solar insolation. The annual average is 6.0 kilowatt hours per square meter per day. During the summer, it averages 7.8 kilowatt hours per square meter per day.

It is evident that the Southwest has all the important criteria for high productivity algal farms: available water, land, and sunshine.

Spirulina is a particularly fine candidate for cultivation in high intensity algal farms in New Mexico because it grows naturally in many highly alkaline environments around the world. Various species of Spirulina are ubiquitous in aquatic environments ranging from freshwater to hypersaline. However, Spirulina seldom occurs at the dominant primary producer except under unusual environmental conditions such as exist in Lake Chad in Africa or Lake Texcoco in Mexico. The ability of Spirulina to tolerate the extreme alkaline conditions of these lakes to the exclusion of other competing or predator species is an important biological characteristic that can be utilized to maintain unialgal conditions in culture systems.

There are many potential uses for Spirulina. It lends itself particularly well to many of these applications because it grows in long filaments, which makes harvesting the algae considerably easier and less energy intensive than the harvest of single-celled microorganisms such as Chlorella.

Spirulina can be used as a feed for livestock and aquatic organisms (fish and shrimp), as a source of specialty chemicals for the pharmaceutical and cosmetics industry, and as a human nutritional supplementation (health food).

Specialty Chemicals

Spirulina culture can produce organic compounds of commercial value. Spirulina contains photosynthetic pigments at concentrations that can exceed 20 percent on a dry weight basis. Pigments common to Spirulina include some with significant commercial value, such as b-carotenes, phycocyanins, chlorophylls, and xanthophylls. The pigment content and concentration of Spirulina varies widely as a function of species, water chemistry, and a number of other environmental parameters.

Carotenoids (3.4 - 4.0 grams/kilogram). Carotenoids are generally responsible for the red and yellow hues seen in nature. More than 300 different carotenoids have been identified, including some that do not occur naturally. Carotenoids are produced in a wide variety of algae, bacteria, and plants, but not in animals. All animal species must procure their carotenoids from dietary sources. A large number of carotenoids have been synthesized and are currently utilized in a variety of industrial applications. They are now replacing, to an increasing extent, the yellow and red azo dyes being eliminated by food regulations. Carotenoids are broadly utilized as pigments in the food, pharmaceutical, and cosmetic industries as natural coloring agents. The carotenoid pigments of commercial importance found in Spirulina are b-carotenes and xanthophylls.

beta Carotenes (1.7 - 3.4 grams/kilogram). The primary significance of b-carotenes in nutrition and metabolism is as a vitamin A precursor of "provitamin A," because vitamin A is produced in animals by breaking a b-carotene molecule and attaching a water molecule in the end position. b-carotenes also are important as feed pigments. b-carotenes added to the feed for beef cattle produces a final meat product with a healthy red color. b-carotene can also be added to the rations of dairy cattle as a means of pigmenting the butter fat, but direct addition to butter during processing is more economical. In the food industry, b-carotenes are an important coloring agent for fat-based food, including margarine, butter, fats, oils, and shortenings.

b-carotenes are often added to the artificial diets supplied to birds so that they can maintain the brilliant hues characteristic of their plumage. Because the diets of these animals in captivity differ fundamentally from their natural foods, the addition of b-carotenes must be employed to maintain the natural red coloration. For similar reasons, the diets of tropical and/or exotic fish such as the Japanese "koi" are frequently fortified with b-carotenes.

During June 1982, a new potential health food market for b-carotenes was created by the nationwide publicity afforded a National Academy of Sciences report on "Diet-Sensitive Cancers." The study concluded that b-carotenes and ascorbic acid were the only two compounds found to be inhibitory to chemically induced cancers in laboratory tests and the consumption of either compound correlates with lower cancer rates in human populations. Thus, demand for b-carotene dietary supplements (stabilized with ascorbic acid as an antioxidant) may increase in the health food market.

b-carotene was first synthesized in 1950 and is now produced commercially by Hoffman-LaRoche Corporation.

Xanthophylls (1.00 grams/kilogram). The concentration of xanthophylls in Spirulina depends upon the species and the environmental conditions. Xanthophylls are an important source of orange-yellow pigments. They are used in the poultry industry as a feed additive to produce highly colored egg yolk as well as meat and skin pigmentation that is pleasing to consumers. Xanthophylls are also used to fortify the feeds of captive birds and exotic fish as discussed above to produce the strong yellow pigmentation common to these animals in the wild.

Phycocyanins (30 - 220 grams/kilogram). Some strains of Spirulina contain exceptional concentrations of phycocyanins. Phycocyanins are an important source of blue pigment for use in food colorings in Japan, although they are not apparently widely used for such purposes elsewhere.

Chlorophyll (6.8 - 11.0 grams/kilogram). Chlorophyll is an important natural green pigment used in food colorings.

Protein (600 - 700 grams/kilogram). One of the most striking characteristic of Spirulina is the unusually high concentration of protein. The protein matrix of Spirulina encompasses 18 amino acids, including the eight essential amino acids (isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine). A considerable market in the health food industry exists for protein concentrates. The wholesale value of protein concentrates depends on the mix of essential amino acids but should be favorable for Spirulina since it has an excellent ratio of amino acids. if necessary, the amino acid mix of Spirulina could be altered to more closely match the Food and Agriculture Organization/World Health Organization suggested pattern considered to be optimal for human nutrition by a combination of peptic hydrolysis and papain enzymatic treatments. This treatment results in increased concentrations of methionine, lysine, and tryptophan. Additional potential markets exist for the purified amino acids found in Spirulina as detailed below.

Tryptophan (11.3 grams/kilogram). Tryptophan is an important amino acid that often determines the nutritive value of protein because it is frequently limiting. Tryptophan has market potential as a natural sedative.

Phenylalanine (39.5 - 50.0 grams/kilogram). Phenylalanine is also an essential dietary amino acid for humans since it is not naturally produced in sufficient quantities. Phenylalanine has market potential as a natural stimulant.

Sterols (325 milligrams/kilogram). Sterols have market value either directly, as in the case of cholesterol, or as precursors for a variety of pharmaceuticals. Sterols are important biologically as hormones, components of membranes, and vitamin D. In humans, sterols are converted to cholesterol, which is then used as a precursor for other compounds. The economic value of sterols results from use either directly or as raw materials for synthetic reactions in nutrition pharmaceuticals or research.

Vitamins (variable concentrations). Spirulina contains a number of vitamins of potential commercial importance. These vitamins are as follows.

	milligrams/kilogram
Cyanocobalamin (B_{12})	2
d-Ca-Pantothenate	11
Inositol	350
Nicotinic Acid	118
Pyriodoxine (B_6)	3
Riboflavin (B_2)	40
Thiamine (B_1)	55

Tocopherol (E)	190
Niacin (B_3)	118

The economic viability of extracting, concentrating, and purifying these vitamins for the health food market is not clear, although the possibility of tapping this market exists.

Siderchromes (trace concentrations). Spirulina also contains a group of compounds called siderchromes that form stable complexes with metals. These metal-chelating agents have potential medicinal uses as detoxification and removal agents for heavy metals and radioactive plutonium. Presumably such compounds would be an attractive product for the health food market.

Food Additives. Spirulina is currently used as a useful food additive for humans and/or domestic animal rations and is supplied as a spray dried powder or tablets. Spirulina may be far more attractive to human consumers if the product were processed to remove the pigments before marketing. This approach is also useful because those pigments with important industrial applications can be marketed independently while increasing the marketability of the residual Spirulina product. The decolored residual would still retain its high protein and vitamin content. The depigmented product, then, could be sold through the health food industry or further processed to extract organically chelated metals, vitamins, amino acids, or protein concentrates as changing market conditions dictate.

Current Supply

Spirulina is currently harvested primarily from large aquatic ecosystems where it grows as a nearly unialgal culture as a result of extreme environmental conditions. The primary supplies of Spirulina are currently harvested from Lake Chad in Africa and Lake Texcoco in Mexico. In recent years, more intensive culture systems have become operational. Average production rates for Lake Texcoco are 10 metric tons per hectare per year. Laboratory experiments have yielded growth rates of 12 grams per square meter per day. This figure, when extrapolated, yields production rates of 43.8 metric tons per hectare per year. Highly controlled commercial production systems in a suitably warm climate could be expected to produce at the rate of up to 50 metric tons per hectare per year when carbon is added to the culture system in a usable form such as carbon dioxide gas or sodium bicarbonate.

If proposed marketing studies verify the demand for New Mexico-grown Spirulina at a reasonable price, significant profit potential may exist for a commercial Spirulina production facility.

Summary

In summary, finding uses for this underutilized resource--brackish groundwaters--could lead to expanded economic activity in the state. Those uses that make maximum benefit of the natural resources of New Mexico and yet are environmentally benign should be encouraged.

The growth of algae, such as Spirulina, on these brackish groundwaters make eminently good sense in New Mexico. The state has among the highest insolation levels in the United States; abundant, inexpensive flat land; and extensive geothermal reserves. These geothermal waters can be used to maintain optimum temperature levels of algal production ponds, as well as supply substantial amounts of carbon dioxide required by the algae.

Spirulina was the organism chosen for the project because of its ability to tolerate and grow in highly alkaline waters. Indeed, *Spirulina* blooms are often found in such environments to the exclusion of other plant life because of this ability. Thus, one of the major problems of outdoor microalgae culture--contamination by nondesirable competitive species--is ameliorated by the use of brackish groundwaters.

Additionally, *Spirulina's* larger size makes harvesting more efficient and economical. Finally, there is present and future profit potential in the commercial cultivation of *Spirulina*.

RELATED RESEARCH

There is a relatively modest literature on the growth, biochemical composition, and potential nutritive value of *Spirulina* spp.

Leonhard and Compere (1967) described the natural production of *Spirulina* in Lake Chad and its use by natives while Clement et al. (1967) , Durand-Chastel and Silve (1977), and Durand-Chastel (1980) described the growth and use of *Spirulina* in Mexico at Lake Texcoco. Johnston (1970) and Switzer (1980) discuss the history and potential use of *Spirulina* in general terms.

The interest in *Spirulina* culture stems from its high nutritive value for man and economically valuable animals. Clement et al. (1967) and Clement (1975a) demonstrated the nutritional value of *Spirulina* for rats and chickens. Bourges et al. (1971), Pirie (1975), and National Academy of Sciences (1975) also discuss the great nutritional value of Spirulina. Arai et al. (1976) have even worked on technologies to further improve the nutritional qualities of protein extracted from Spirulina.

Sada (1975), Clement (1975b), Baron (1976), Hall (1978), Tel-Or et al. (1980), Aaronson et al. (1980), and others have discussed analytical methods for and use of various biochemical constituents of Spirulina.

Culture methods from laboratory scale to pilot scale have been discussed in the literature. Al'bitskaya et al. (1974) and Aiba and Ogawa (1977) have described effects of light levels and/or temperature on the growth of particular species of Spirulina on laboratory scale. A great deal of work on growing Spirulina on waste sources has been done: Kosaric et al. (1974), Oron et al. (1979), Benemann et al. (1978), Shelef et al. (1980), and Olguin et al. (1981). Soong (1980) describes the commercial production of Spirulina in Taiwan.

Bernend et al. (1980) has indicated that Spirulina is grown in Israel in brackish water somewhat similar to those found in New Mexico.

These studies actively support the thesis of this project: Spirulina has a very high nutritional value and its constituents can also be economically valuable. Extant lab studies on the growth of Spirulina indicate the general suitability of New Mexico's temperatures and sunlight levels for Spirulina growth.

None of these studies, however, has screened several species/strains of Spirulina for their ability to survive and grow on the major brackish water types found in the state. As a result, no studies have been performed on the effect of temperature, nutrient type, and concentration on the growth of those Spirulina species that grow best in New Mexico's brackish water. The research reported here is a major step in filling this gap in knowledge.

MATERIALS AND METHODS

The primary objective of this project was to determine the technical feasibility of cultivating Spirulina in the saline groundwaters of New Mexico.

Pilot Scale

In order to determine the technical feasibility of pilot-scale cultivation, it was necessary to design, construct, and debug an algal culture facility.

Such a facility was built on the grounds of the Roswell Test Facility (RTF) in Roswell, New Mexico (figure 1). It consists of four main systems: an outdoor, pilot-scale algal production system; an indoor water treatment/recycling system; an inoculation system; and a computerized measurement and control system. The outdoor algal production system consists of two greenhouses, each approximately 7.6 meters feet wide by 14.6 meters long. Each greenhouse has two layers of Monsanto 703 polyethylene film that are air inflated. Within each greenhouse is a concrete raceway, the walls of which are 0.3 meter thick and 0.3 meter high; the floor is 0.15 meter thick. A 0.3 meter high by 0.3 meter thick dividing wall runs down the center of most of the raceway (figure 2), which effectively creates a 2.1

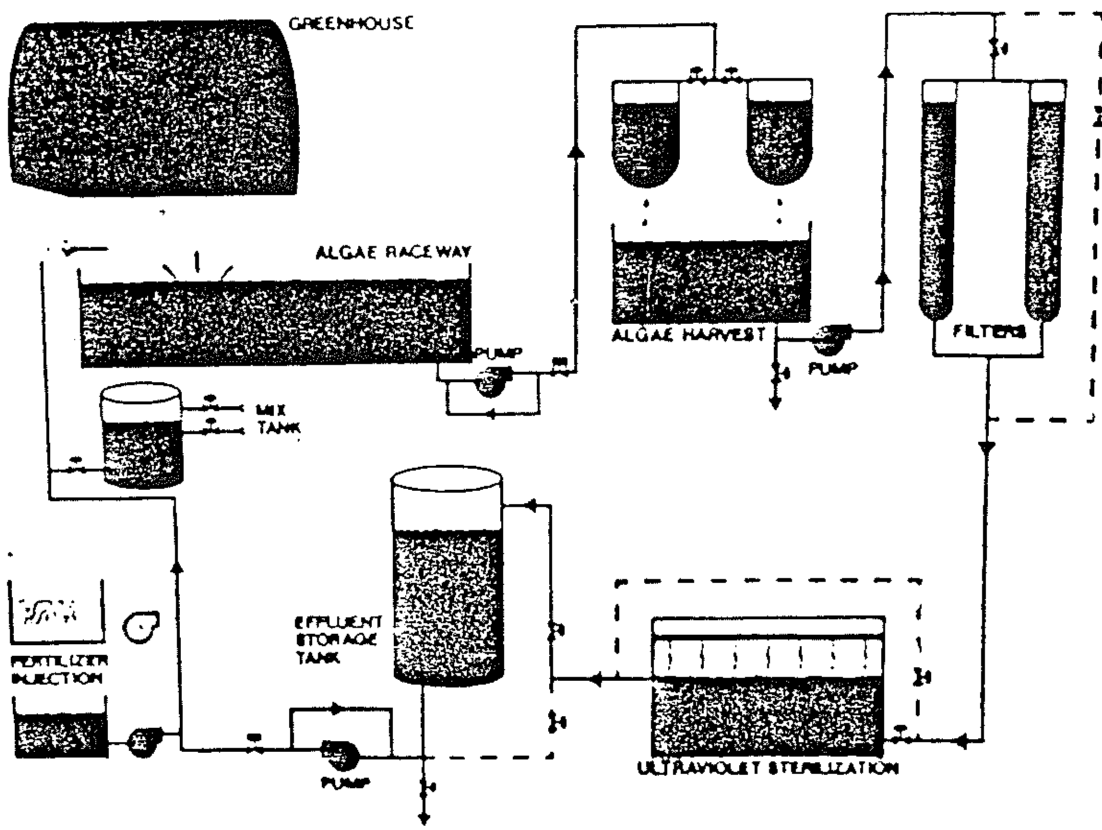

Figure 1. Flow Chart of Existing Algae Production System.

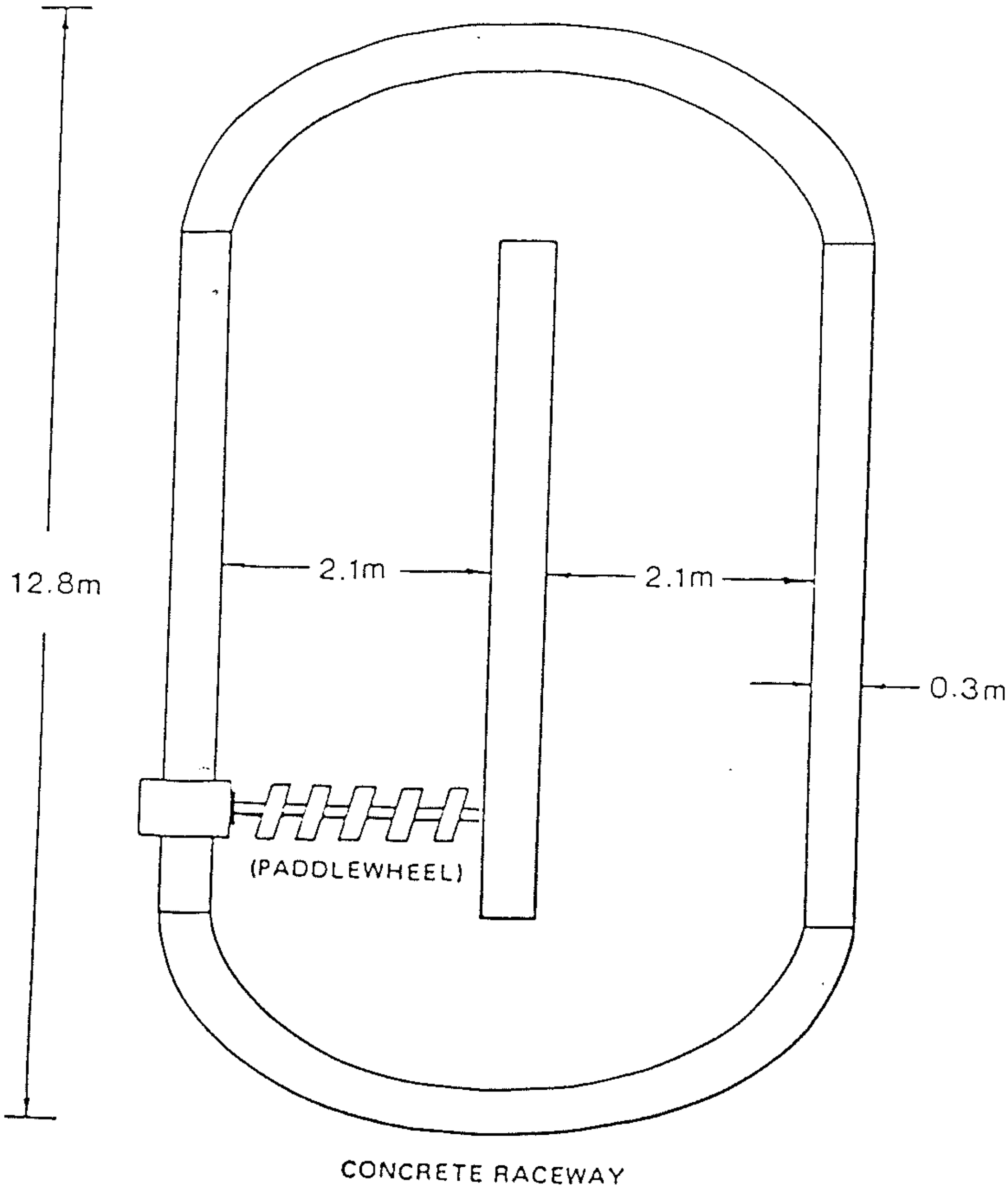

Figure 2. Concrete Raceway.

meter wide channel. Algae in the raceway is kept in homogeneous suspension by the movement of a paddlewheel driven by an electric motor. There are two large exhaust fans in each greenhouse for ventilation; these are effective in maintaining greenhouse temperatures near outside ambient air temperatures during the warmer months of the year. There is no active heating system in the greenhouses. Rather, the massive concrete walls and floor of the raceway store much of the solar energy falling on the greenhouse. As a result, air temperatures at night inside the greenhouse are warmer than outside ambient air temperatures. In general, when the fans are not used, greenhouse air temperatures tend to be almost 10°C higher in temperature than outside ambient air. Water temperatures in the raceways are more constant than air temperatures in the greenhouses.

Algal cultures depth can be maintained at 5 centimeters to 30 centimeters. At a 15 centimeter depth, the inflow and harvest systems can be operated such that a 100 percent daily turnover rate can be maintained. That is, 100 percent of the raceway volume can be exchanged daily.

The indoor inoculation system consists of a temperature-controlled inoculation clean room where pure cultures of Spirulina are grown in ever larger volumes from test tube to 570 liter vats. Artificial lighting is used. The contents of two vats (1,140 liters total) can be pumped to either or both greenhouses as an inoculum.

In the harvest system, algal culture is pumped from the greenhouses into the indoor pad to a harvest subsystem. This subsystem was initially 25 um cloth filter bags. It was found that a significant fraction of algae escaped. When 10 um bags were used, the insides became coated with the Spirulina and only a few hours of operation were possible before the bags needed to be recleaned. A better system, a SWECO vibrating screen or continuous centrifuge could not be purchased because of budgetary constraints.

After most of the algae has been removed from suspension, the medium is pumped through UV sterilizers and then through 1 um depth filters. A day's worth of this treated media is stored in tanks. Daily chemical analyses on this stored media are made and the necessary chemicals added as required. This reconstituted medium can be used as inflow water to the algal culture.

The measurement and control subsystem is based upon an Apple II and a Keithley DAS data acquisition and control system. Instead of installing all necessary sensors at each desired sampling point, it was decided to purchase only one set of sensors and bring the sample to the sensors. To this end, a test rack was designed and built. Five lines go to the test rack: each inflow to the two raceways; each outflow from the two raceways; and a purge line. A series of solenoid valves controlled by the computer diverts the desired sample stream to the test rack. In-line sensors measure pH, conductivity, and turbidity (a measure of algal biomass) of the sample stream. Each of the four sample streams are sequentially diverted and monitored and then the purge line is diverted to the test rack for cleaning the lines. The duration of monitoring any one sample stream can be changed as desired. The system, as initially used, obtains three data points per hour for each of four sampling points for three types of sensors. Additionally, the measurement and control system monitors temperatures at five points in each raceway: (1) 0.3 meter below slab; (2) interface of slab and ground; (3) mid-slab in center of channel (north side); (4) north wall; and (5) water temperature. These measurements can be used to construct heat budgets for the greenhouses and to indicate where and how much heat loss and gain is taking place. This data can be used to design a more effective passively heated/cooled greenhouse.

Finally, the measurement and control system monitors two light sensors: one that measured PAR (radiation in the photosynthetically active range--400 to 700 nm) incident to the surface of the raceway and one that measures PAR at the bottom of the algal culture. The above mentioned temperature sensors (thermocouples) and light sensors are monitored continuously to provide a very complete record of these important environmental parameters.

Laboratory Studies

Four *Spirulina* species (three strains of *S. platensis* (UTEX #1926, #1928, #2340), and *S. maxima* (#2342) were monitored for growth when grown in four different water types typical of New Mexico's saline groundwaters. These water types were: (1) full RTF well water (approximately 14,000 ppm TDS); (2) a 1:1 dilution of RTF well water and distilled water (7,000 ppm TDS); (3) NMSU geothermal water (2,000 ppm TDS); and (4) Las Cruces tap water (500 ppm TDS). Each species/water type was replicated three times. An experimental run consisted of 48 treatments: 4 species x 4 water types x 3 replicates. All treatments, unless otherwise noted, contained a nutritionally complete medium, Zarrouck's media. In addition, there were controls of each water type with full Zarrouck's media. Each run was conducted at one temperature in a plant growth chamber. Growth at five temperatures was evaluated: 10°C, 20°C, 27°C, 35°C, and 42°C.

Each 500 ml Nalgene Erlenmayer flask initially contained 400 milliliters of full Zarrouck's media made in one of the water types. All flasks' contents were kept in homogeneous suspension by bubbling humidified air into the flasks. Twenty milliliters of media were withdrawn daily from each flask. Ten milliliters were pipetted onto pre-dried, preweighed Gelman glass fiber filters for dry weight determinations. The filters, held in pre-tared aluminum weighing dishes, were placed into an oven at 60°C and dried to constant weight. The same procedure was used for the controls and the control's weight subtracted from the algal medium weight to give algal dry weight.

The other 10 milliliters were used for optical density measurements (Bausch and Laumb Spectronic 20) and fluorometric determination of in-vivo chlorophyll a (Arpco Fluoro-Tec). Again, the controls' readings were subtracted from the algal medium's readings.

This procedure was repeated daily for six to nine days at which point there was no additional net growth, or, in other words, when the "plateau stage" of the classical growth curve had been reached.

It should be pointed out that it is important to understand the limitations of extrapolating laboratory results to larger scale algae production. Whereas a pilot or commercial scale algae production system would most likely be operated in a continuous culture mode, these laboratory experiments were done in a batch mode. The assumption is that exponential growth will occur in a batch culture until some "nutrient" becomes limiting and that this exponential growth represents the potential performance of the algae in continuous culture. In our laboratory experiments, all nutrients were present in excess; the limiting "nutrient" that caused a slowing of the growth rate was light. Such high biomass levels were reached ($\geq$ 1 g dry weight/liter) that the average light level experienced by an individual algal cell was very low, limiting growth. Higher light levels would probably have resulted in higher final densities.

The positive side of these limitations, however, is that the low light levels and high standing biomass densities of the laboratory experiments are representative of conditions experienced by the algae even in outdoor, large-scale continuous culture. Actual PAR light levels at the bottom of 15 cm outdoor cultures were less than 1 percent of surface incident light.

In summary, the laboratory results should be regarded as indicative of comparative growth potential of the algae, not as absolute measurements of performance.

Finally, some comments on dry weight determinations are needed. The addition of Zarrouck's media to the various water types invariably resulted in turbidity via the formation of precipitates, despite the presence of chelators. Although the precipitates were not chemically analyzed, it is likely that many were relatively insoluble phosphate salts. Additionally, the large concentrations of added sodium bicarbonate added to the turbidity.

Thus, when an aliquot was filtered for algal dry weight determinations, a significant fraction of the dry weight was precipitate, making accurate measurements difficult. The availability of suitable instrumentation, especially a fluorometer, during experimental laboratory trials allowed the development of spectroscopic and fluorometric methods of algal biomass determinations that are appreciably less sensitive to the turbidity problems than dry weight measurements. These methods' reduced sensitivity to turbidity is attributable to using wavelengths of light that are preferentially absorbed (spectroscopy) or preferentially reemitted at a different wavelength (fluoroscopy) by the photosynthetic pigments of the Spirulina. The photosynthetic pigment concentration of the algal cell (as a percentage of weight) may change with different growth conditions, particularly light. However, temperature and incident light were kept constant during all experimental runs. It is probable that as the algal biomass increased that each algal cell experienced a decrease in average incident light. Photosynthetic pigment concentrations may have been increased by the cells as a response to decreased average light levels. Thus, algal biomass measurements based on pigment concentration can overestimate actual algal biomass towards the end of experimental runs. However, all species tested were taxonomically similar and probably respond to decreased light levels in a similar manner. Further, the purpose of the laboratory experimentation was to compare species. Thus, a uniform overestimation of algal biomass does not decrease the value of such experimentation.

A fluorometer was not available for the pilot scale trials. An improved method of dry weight determinations involving rinsing of the filters with acidified water to dissolve precipitates allowed the use of a dry weight as the measure of algae biomass, instead, in these trials.

Statistical Analysis

Because algae reproduce by dividing, their numbers will increase approximately exponentially. The statistical analysis used here, covariance in a cross-classified data structure, assumes additivity of the contributions of the several factors. Thus, the growth-related responses were analyzed on natural logarithm scale. The initial cultures (day 0) could not be started with the same density of culture because they were started at various times. Yet the initial density influenced the final density, on both the density and log density scales. The analysis of covariance of log of final density, using log of initial density as the covariate, removes the effect of variations in initial density. The "adjusted treatment means" of a standard analysis of covariance estimate what the response would have been if the initial densities had all been the same.

The study reported here has an unreplicated three factor factorial treatment strucutre: Water Sources x Temperature x Algae Species. The associated anlaysis of variance paritioned out all three main effects, the three two-factor interactions, and variation accounted for by the covariate; the remainder was used to estimate residual variation.

The analysis was executed using SAS (the Statistical Analysis System), on an IBM 3081D at New Mexico State University. The analysis was managed by the University Statistics Center of the same institution.

RESULTS AND DISCUSSION

Sixteen combinations of species type and water type were evaluated: four species and four water types (table 1). Each of these sixteen combinations were evaluated at five temperatures: 10°C, 20°C, 27°C, 35°C, and 42°C. Thus, there were 80 distinct treatments evaluated, each with a unique combination of species, water type, and temperature. The amount of growth of each treatment after six days was compared and statistically analyzed. Because the final biomass density at day 6, determined fluorometrically, is corrected for initial biomass density, the final corrected biomass density represents the amount of growth that took place in six days. This can be used as an index of net growth rate.

1.0 Species/Water Type/Temperature Interactions

The major source of significant ($P<0.001$) variation in growth rate for all species/temperature/water types tested is temperature (F value of 207.86) followed by water/ temperature interaction (F value of 12.65) and temperature/species interaction (F value of 9.51) (figures 3 to 6). When all water types and species combinations are

TABLE 1
The combinations of species and water type evaluated for growth potential at five temperatures

Species: / Water Type	1926	1928	2340	2342
Las Cruces				
Geothermal				
Half RTF				
RTF				

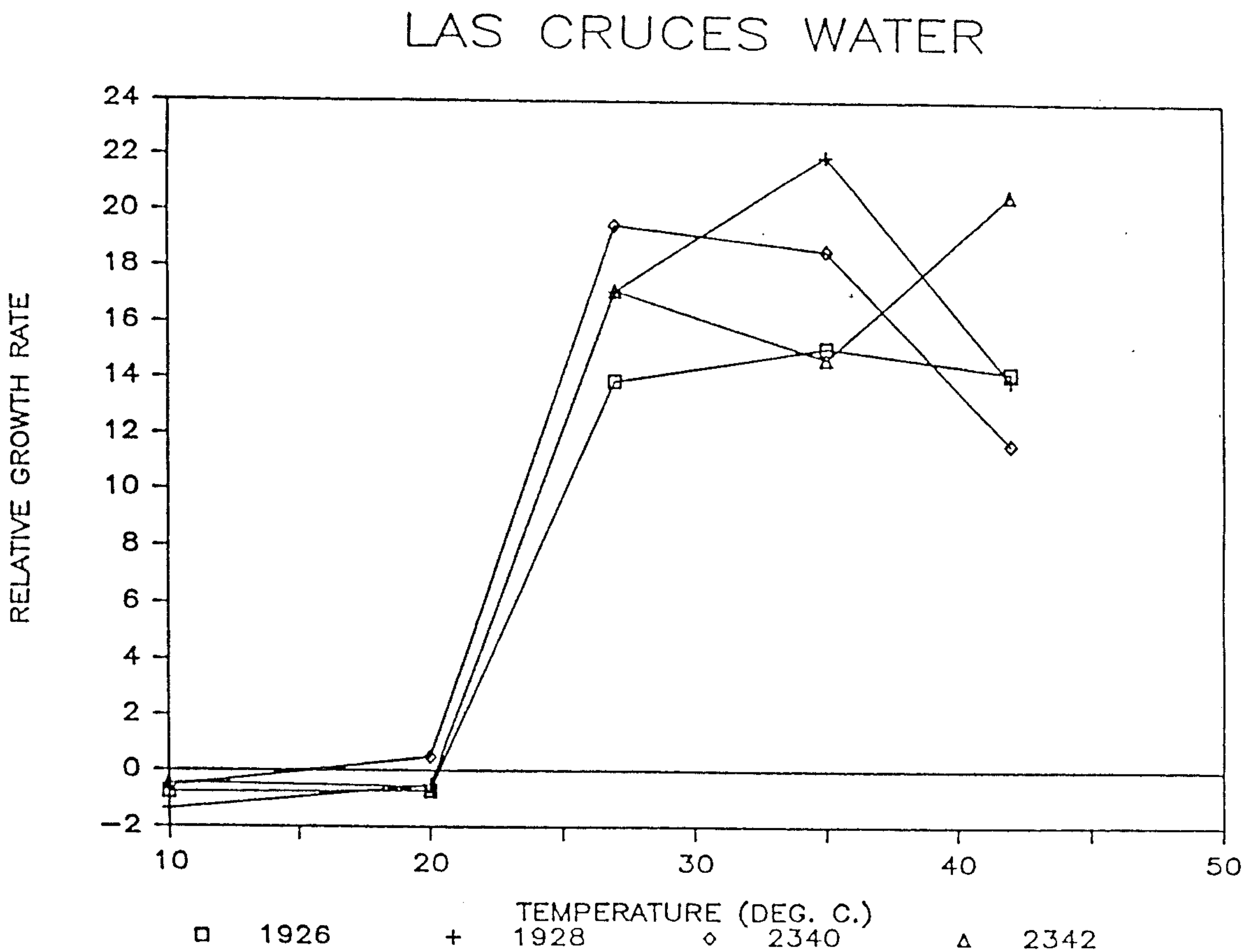

Figure 3. The effect of temperature on the growth rate of four species of Spirulina grown in Las Cruces water.

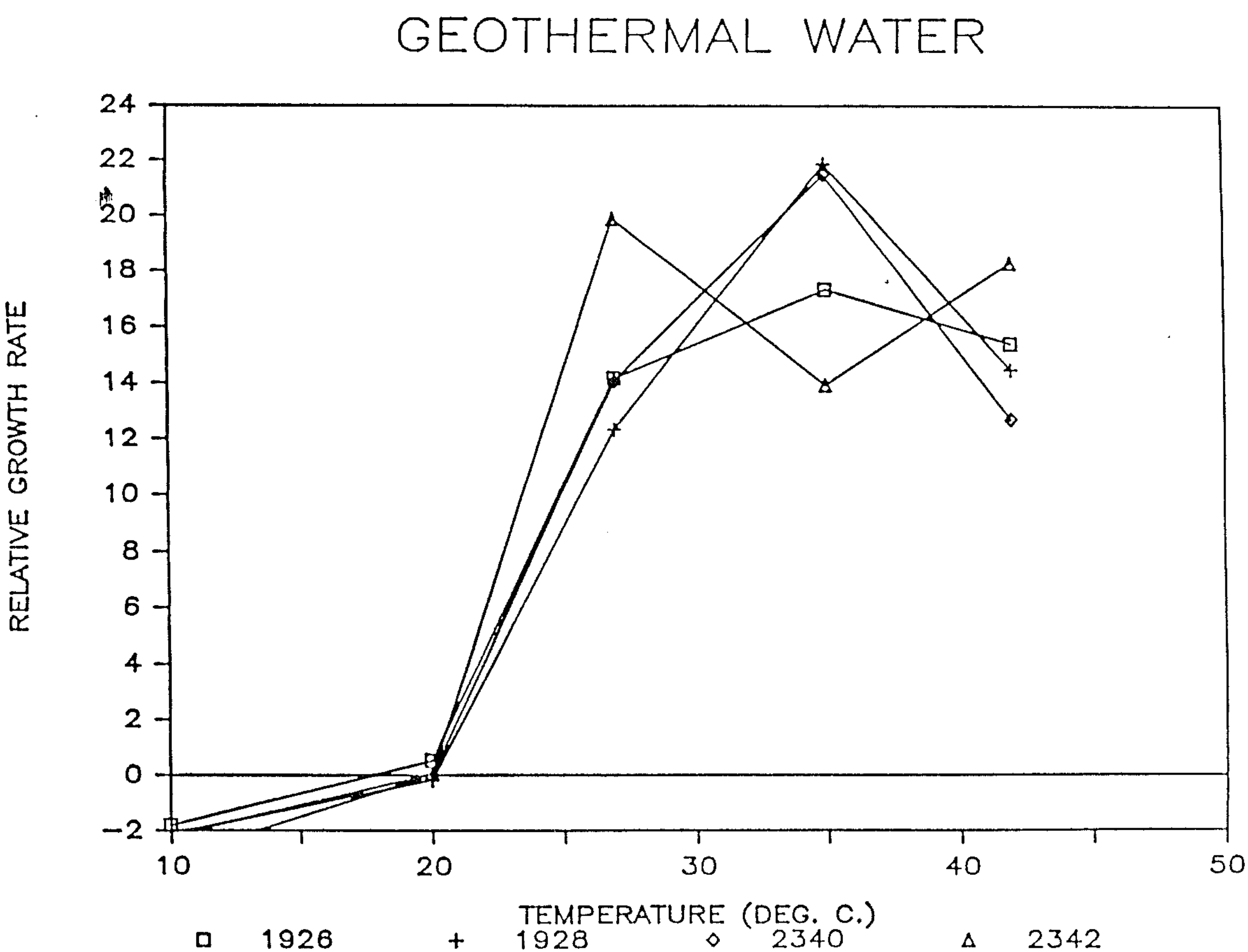

Figure 4. The effect of temperature on the growth rate of four species of _Spirulina_ grown in geothermal water.

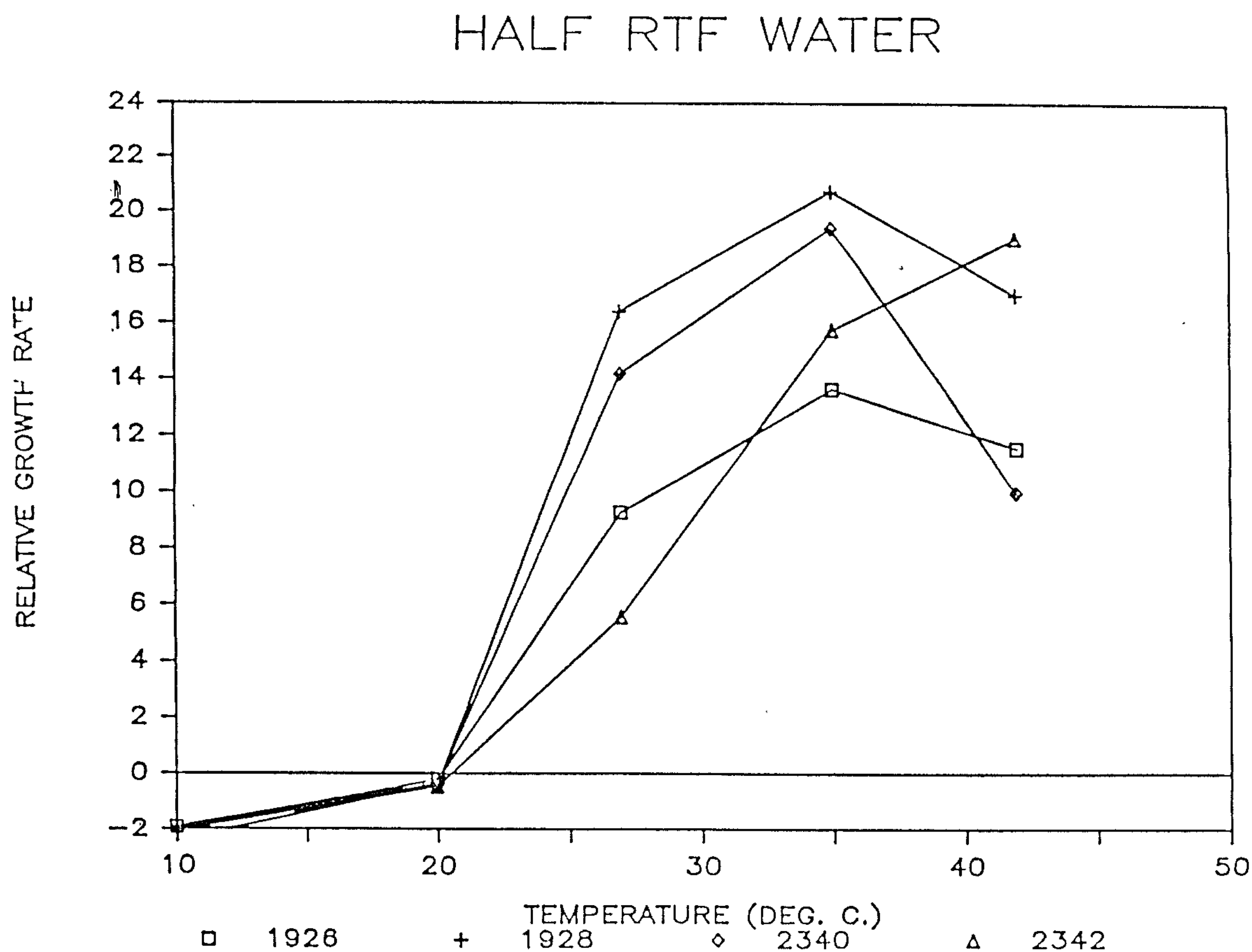

Figure 5. The effect of temperature on the growth rate of four species of Spirulina grown in half RTF water.

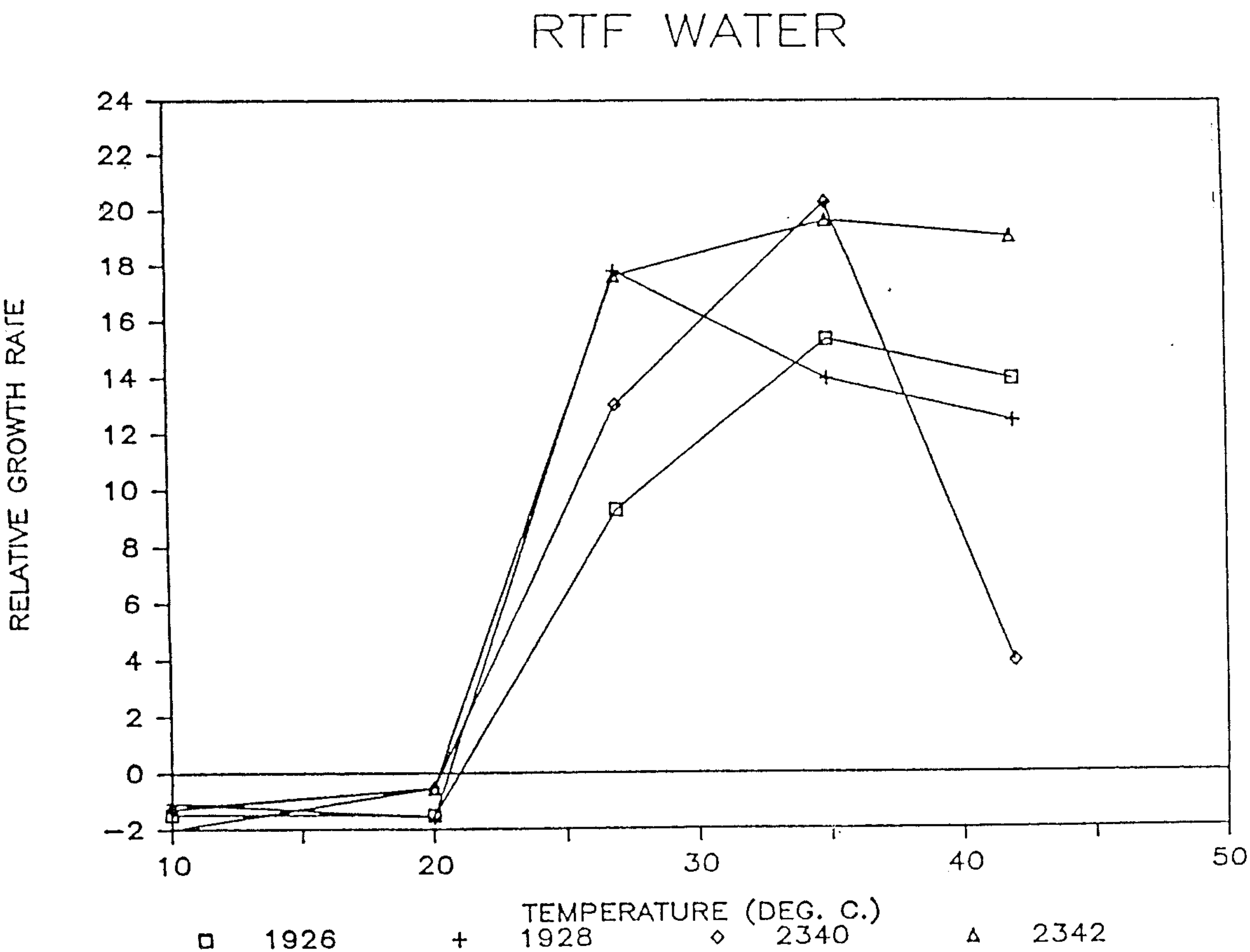

Figure 6. The effect of temperature on the growth rate of four species of Spirulina grown in RTF water.

considered, it was found that overall growth was signficantly better at temperatures of 27°C, 35°C, and 42°C, than at 20°C and 10°C. Although there was no significant difference in growth at temperatures of 27°, 35°, and 42°C, they may be ranked 27°C> 35°C> 42°C > 20°C> 10°C.

Although there was no significant difference among water types (for all species and temperature conditions tested), they may be ranked as half RTF water > RTF > geothermal > Las Cruces water. There was also no significant difference between species for all temperatures and water types combinations tested. However, the species' performance may be ranked as #1926 > #1928 > #2340 > #2342.

When examining water/temperature interactions more closely, several combinations of water/temperature were found to be superior for growth when all Spirulina strains tested are grouped together. Half RTF water at 35°C is significantly better than RTF water at 42°C; RTF water at 20°C is significantly better than geothermal water at 10°C; and Las Cruces water at 10°C and 20°C was significantly worse than all other waters at 10°C and 20°C. Half RTF water at 35°C was most optimal of all water/temperature combinations tested for growth when all species are considered. This water temperature and type was subsequently chosen for further lab studies and outdoor pilot scale work at the RTF.

Table 2 ranks the various water/temperature combinations for all species tested by decreasing final corrected biomass densities.

Group 1 is significantly better than group 2 which is significantly better than group 3. Within each group, there is no

TABLE 2
Water/Temperature Interactions for all Species Tested
(Fluorometric Values)

Rank	Overall Performance: Water	Water/Temperature Temp (°C)	Final Biomass Density (Relative)
		GROUP 1	
1	Half RTF	35	4.66
2	LC	27	4.51
3	RTF	27	4.49
4	Half RTF	42	4.35
5	RTF	35	4.34
6	Geo	35	4.29
7	Half RTF	27	4.27
8	Geo	42	4.21
9	Geo	27	4.20
10	LC	35	4.17
11	LC	42	4.11
12	RTF	42	4.08
		GROUP 2	
13	RTF	20	3.32
14	Half RTF	20	3.24
15	Half RTF	10	3.22
16	RTF	10	3.13
17	Geo	20	2.94
18	Geo	10	2.81
		GROUP 3	
19	LC	20	2.23
20	LC	10	2.19

significant difference except the following: in group 1, half RTF/35°C is significantly better than RTF/42°C; in group 2, RTF/20°C is significantly better than Geo/10°C; and LC/10°C and LC/20°C are significantly worse than all other water types at 10° and 20°C.

Table 3 ranks the various water/species combinations for all temperatures tested by decreasing final corrected biomass density. Overall, there was no significant difference among members of the group. However, species 2342 grown in geothermal water did signficantly worse than species 1928 in half RTF water, species 1926 in half RTF water, species 1926 in RTF water, and species 2342 in RTF water. It should be noted that no other combination of species and water type was significantly better than species 2342 in half RTF water, the species and water type used for outdoor pilot scale trials at the RTF. Half RTF/1928 and half RTF/2342 were chosen for additional lab studies, as well.

Temperature/species interactions are presented in table 4. Within each group, there is no significant difference between members of the group. However, there are significant difference between groups 1, 2, and 3. It should be noted that, for all water types tested, species 2342 is the best species to use at 42°C, a temperature often encountered in the summer months of the American Southwest.

Summary of results based on fluorometric readings:

- No one species/strain of Sprirulina grew significantly better than any other species in a particular water type.

- Species 1926, 1928, and 2340 demonstrated no significant difference in growth in any of the water types tested.

TABLE 3
Water/Species Interactions for All Temperatures Tested
(Fluorometric Values)

Overall Performance: Water/Species

Rank	Water	Species	Final Biomass Density (Relative)
1	Half RTF	1928	4.03
2	Half RTF	1926	4.00
3	RTF	1926	3.95
4	RTF	2342	3.94
5	Half RTF	2340	3.92
6	RTF	1926	3.86
7	Half RTF	2342	3.84
8	Geo	1926	3.78
9	Geo	2340	3.76
10	RTF	2340	3.74
11	Geo	1928	3.70
12	Geo	2342	3.74
13	LC	1928	3.48
14	LC	2340	3.46
15	LC	1926	3.44
16	LC	2342	3.38

TABLE 4
Temperature/Species Interactions for All Water Types Tested
(Fluorometric Values)

	Overall Performance:	Temperature/Species	
Rank	Temperature (°C)	Species	Final Biomass Density
		Group 1	
1	35	1926	4.56
2	35	1928	4.55
3	42	2342	4.49
4	27	2340	4.46
5	27	1928	4.46
6	35	2340	4.45
7	42	1926	4.38
8	27	1926	4.34
9	27	2342	4.18
10	42	1928	4.10
		Group 2	
11	35	2342	3.89
12	42	2340	3.78
		Group 3	
13	20	2340	3.09
14	20	2342	2.91
15	20	1928	2.88
16	10	2342	2.87
17	20	1926	2.85
18	10	1926	2.85
19	10	1928	2.85
20	10	2340	2.80

- Species 2342 grew significantly better in RTF water than it did in geothermal water.

- In all water types, growth was significantly different (better) at 27°, 35°, and 42°C than at 10°C and 20°C.

- There was no significant difference among water types at 27°, 35°, and 42°C except that half RTF/35°C is significantly better than RTF/42°C.

- All species grew signifcantly better at 27, 35, and 42°C than at 10°C and 20°C.

Table 5 summarizes these results by ranking the best combinations of species, temperature, and water type.

Using these results, one may select the best species to be used for a particular water type and expected temperature regime. However, because there is little growth for any species/strain at 20°C and below, commercial Spirulina production in New Mexico will be either seasonal (late spring, summer, and early autumn) or carried out using geothermal energy. Thus, we have several "best" species for a particular set of environmental parameters:

- The "best" overall species for each water type (at 27°, 35°, and 42°C).

- The best species and temperature combination for geothermal water (theoretically, desired culture temperature can be readily maintained at 35°C).

TABLE 5
Best Species in a Given Water Type at a Given Temperature
Analysis by Ranking: 1 = Highest, 4 = Lowest

	1926	1928	2340	2342	Best
Geo 10	4	3	1.5	1.5	2340 & 2342
20	3.5	2	1	3.5	2340
27	2	3	1	4	2340
35	3	2	1	4	2340
42	1	3	4	2	1926
Rank	2.7	2.6	1.7	3	
LC 10	3.5	2	3.5	1	2342
20	4	2	1	3	2340
27	4.	3	1	2	2340
35	1	2	3	4	1926
42	2	3	4	1	2342
Rank	2.9	2.4	2.5	2.2	
Half RTF					
10	3	2	4	1	2342
20	2	3	1		2340
27	3	1	2	4	1928
35	2	1	3	4	1928
42	2	3	4	1	2342
Rank	2.5	2	2.8	2.5	
RTF 10	1	2.5	4	2.5	1926
20	4	3	1	2	2340
27	3	1	4	2	1928
35	1	3	2	4	1926
42	2	3	4	1	2342
Rank	2.2	2.5	3	2.3	
Overall Rank	2.55	2.375	2.5	2.5	
Frequency of					
1's	4	3	7	5	
4's	3	0	7	6	

When comparing all water/temperature combinations for all species tested, there is no significant difference in species performance in any water type (except as noted for Geo/2342 above) and no significant difference in species performance at 27°C. At 35°C, #2342 grew significantly worse than all other species and at 42°C, #2340 grew significantly worse than all other species. Thus, on the basis of water type alone, no species may be preferentially recommended for use in a particular water type or in any water type at 27°C. However, some water/temperature combinations are better than others (although not necessarily significantly so) for a particular water type.

The "best" species to employ when growing Spirulina in southern New Mexico's geothermal waters is #2340 because it grows best at 35°C of all species tested in geothermal waters (table 5). The best water temperature to use is 35°C because when all water/temperature combinations are evaluated for all species tested (Table 2), 35°C in geothermal water ranked highest for any combinations of geothermal water and temperature. However, if higher culture media temperatures are anticipated (e.g., 42°C) #2340 will not perform well; any of the other species/strains will do better. It may be wise to use mixed cultures of #2340 and #2342 (does very well at 42°C) during the summer months so that one or the other species will be favored as the culture temperature varies during the day.

When Las Cruces water is to be used for Spirulina growth, 27°C is the best growth temperature followed by 35°C and 42°C (Table 2). At 27°C in LC water, species #2340 and 2342 are recommended (Table 5). At 42°C, species #2342 is recommended. However, at

35°C, neither of these species are superior. This may be an anamolous result. However, although there is no one best species for use in LC water, because culture temperatures will vary during the day (especially in summer) from below 27°C to 42°C, species #2342 will probably perform best overall.

When using half RTF water for the commercial production of Spirulina, water temperatures of 35°C are best (Table 2) and species #1928 does best at 35°C in half RTF water (Table 5). However, as culture temperatures climb, species #2342 will do better than #1928. At lower temperatures of 27°C, #1928 is again preferred. Thus, if culture temperatures can be kept from going above 35°C by suitable culture management strategies, species #1928 is the preferred species for Spirulina production in half RTF water. If temperatures cannot be suitably controlled, species #2342 may be co-inoculated into the #1928 cultures in summer to produce Spirulina biomass at higher temperatures.

The use of RTF water for commercial Spirulina production requires species that do well at 27°C and above (Table 2). Species #2342 does best in RTF water of all species tested at 42°C and second best at 27°C. It does quite poorly at 35°C, which may be an anomalous result. However, the culture temperature will vary diurnally, and species #2342 will probably perform best overall in RTF water. Species #2340 may be used as a co-inoculant in summer months.

Thus, a mixed culture of #2340 and #2342 may be the best overall combination to use in all water types tested when culture temperatures are expected to range from 27°C to 42°C (regular growing season). If specific temperatures can be economically and technically maintained, more specific species recommendations may be made for a particular water type.

2.0 Nutrient Types and Concentrations

Two species/strains of Sprulina were selected for further investigation into nutrient requirements: #1928 and #2342. All experiments were run at 35°C in half RTF water.

2.1 Fertilizer Concentrations

A series of experiments were run to determine the effect of total fertilizer concentration on the growth of species #1928 and #2342. Full, 3/4, 1/2, and 1/10 of full Zarrouck's concentration were tested (figures 7, 8). There is no significant difference in growth rate among the treatments receiving different fertilizer concentrations.

Thus, even one-tenth of full Zarrouck's level of fertilization was sufficient to provide all needed nutrients. Higher fertilizer concentrations would not and did not increase the growth rate. However, at higher light levels, the resulting faster growth rates may require higher fertilizer concentrations.

The only significant ($P < 0.01$) source of variation is species. Species #1928 demonstrated significantly better growth for all fertilizer concentrations tested than did species #2342. Species #1928 has already demonstrated better growth in half RTF water than #2342; further, #1928 is a better grower at 35°C than #2342. Thus, it should not be surprising that #1928 grew better than #2342 in all treatments, because all experiments were run at 35°C using

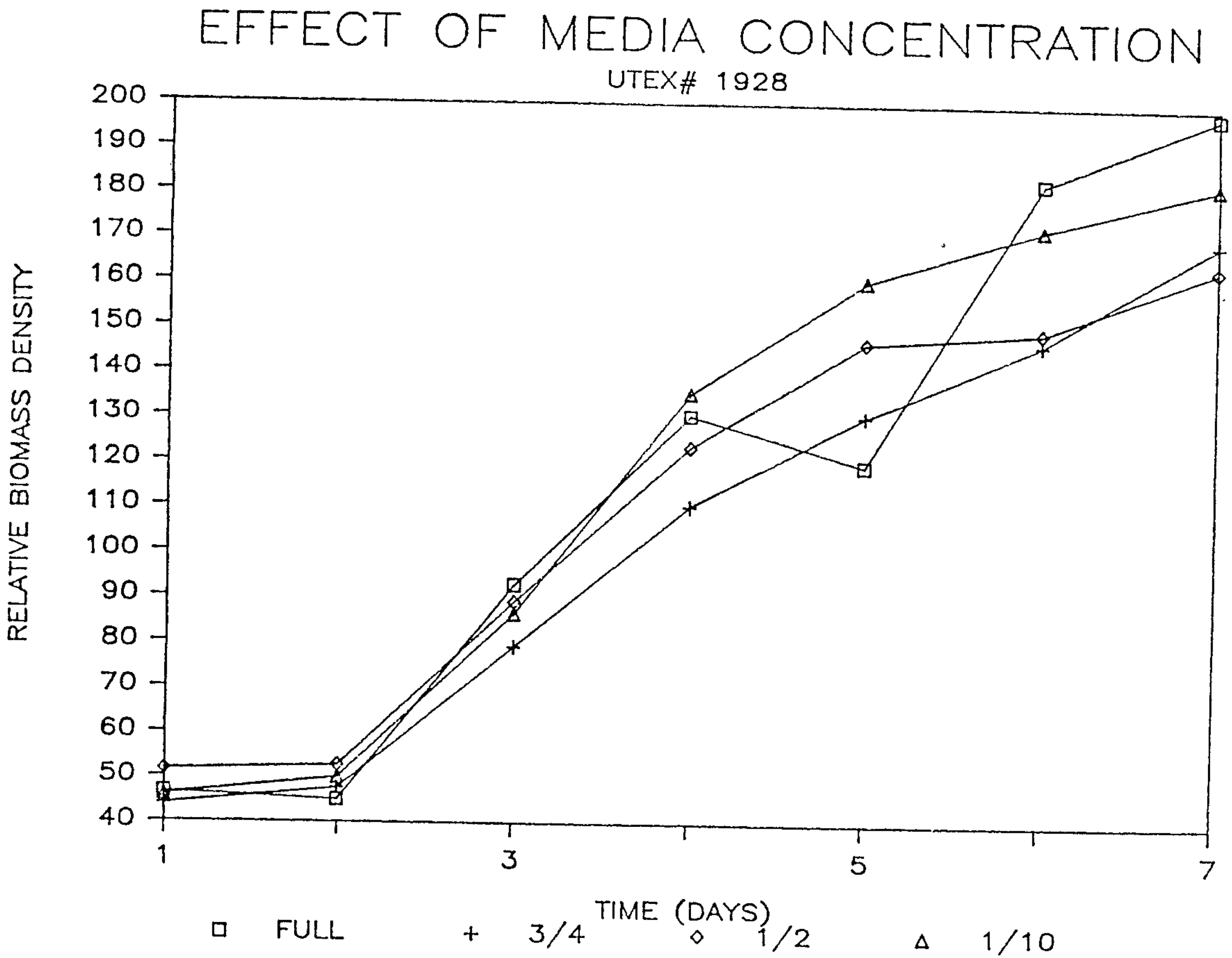

Figure 7. The effect of media concentration on the growth of UTEX #1928.

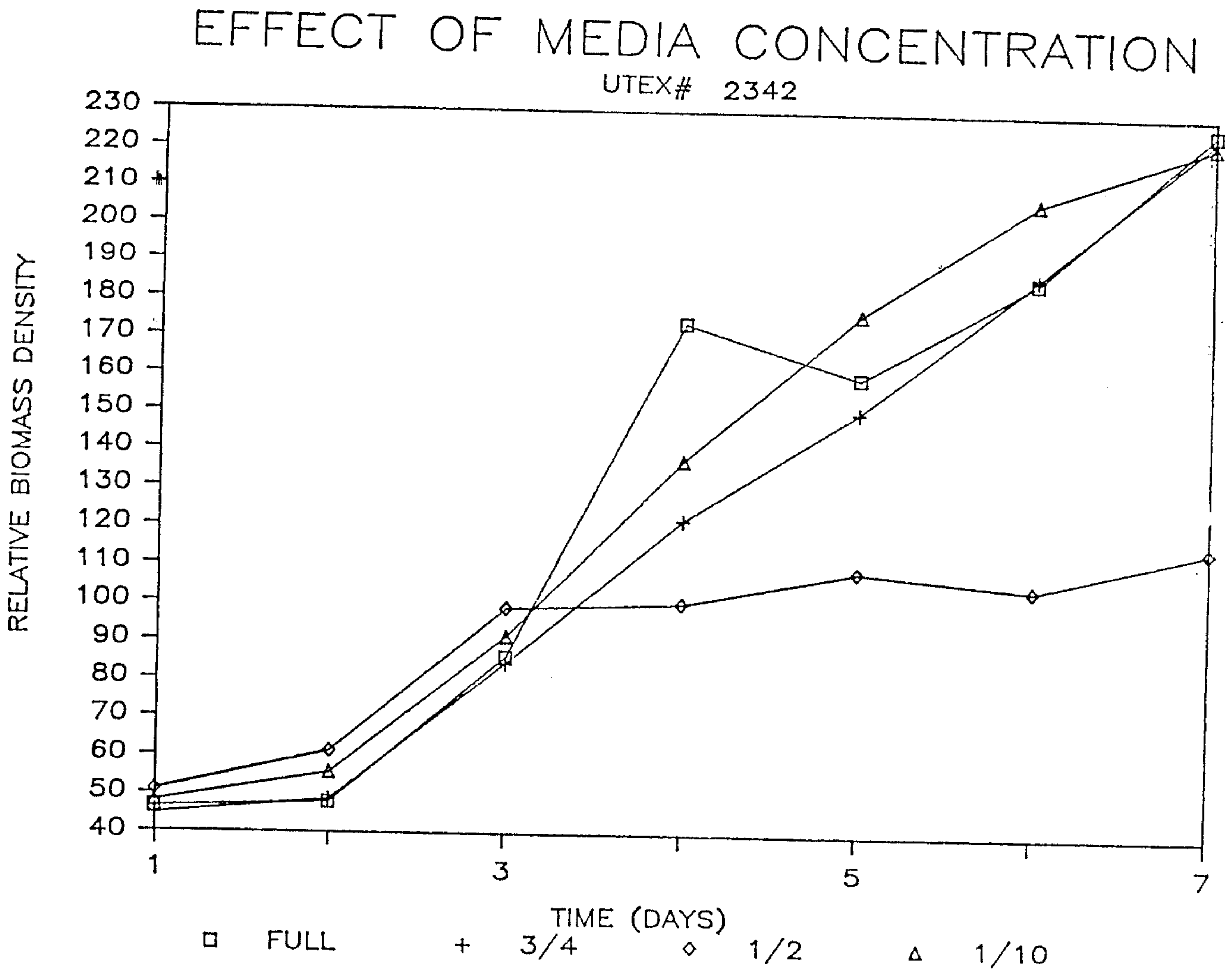

Figure 8. The effect of media concentration on the growth of UTEX #2342.

half RTF water. However, these results do not mitigate the fact that all concentrations were equally good for growth of both species.

2.2 Nitrogen Source

Three sources of nitrogen (urea, ammonium sulfate, and sodium nitrate) were evaluated for their effect on growth of species #1928 and #2342. The absolute nitrogen concentration was the same in all trials: full Zarrouck's level of nitrogen (0.4g "N"/liter) (figure 9, 10). There is no significant difference in growth rate among any of the species/nitrogen source combinations tested. However, growth rates were highest when sodium nitrate and ammonium sulfate were used, as compared to urea. Similarly, species #2342 had a greater rate of growth (for all nitrogen sources tested) than did species #1928; the difference was not significant, however.

It appears that Spirulina can utilize both reduced and oxidized forms of nitrogen. This might be expected of an aquatic plant species that is almost always subdominant ecologically and must be ready to exploit any source of nutrients as they become available. Because there was no significant difference in growth between nitrogen sources tested, the most cost-effective nitrogen source should be chosen for commercial production purposes.

2.3 Carbon Type and Concentration

In this series of experiments, the effect of two carbon sources (sodium bicarbonate and sodium carbonate) at four different concentrations of each carbon source (on an equal carbon basis) on the growth of species #1928 and #2342 was evaluated (figures 11 to 14).

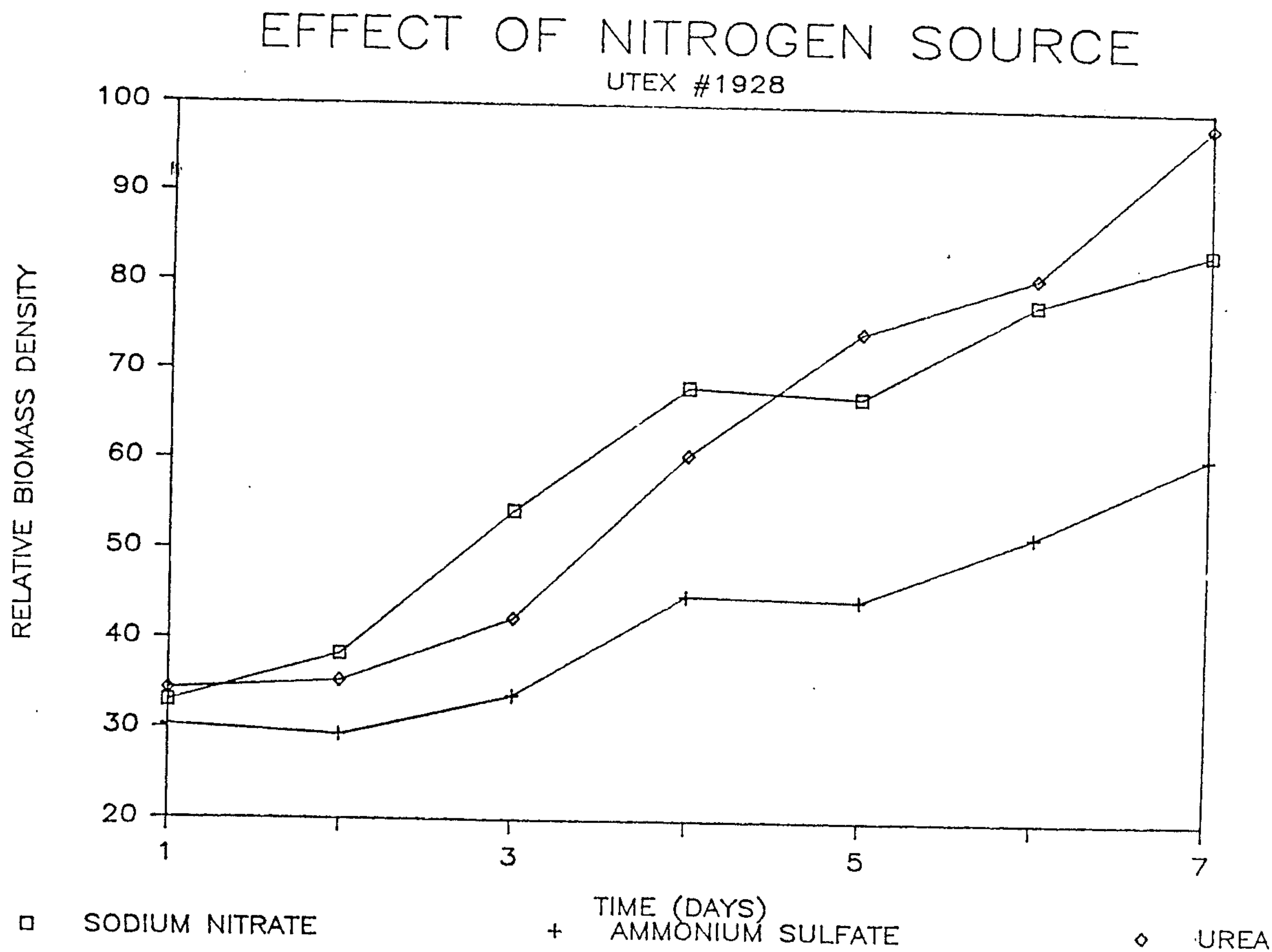

Figure 9. The effect of nitrogen type on the growth of UTEX #1928.

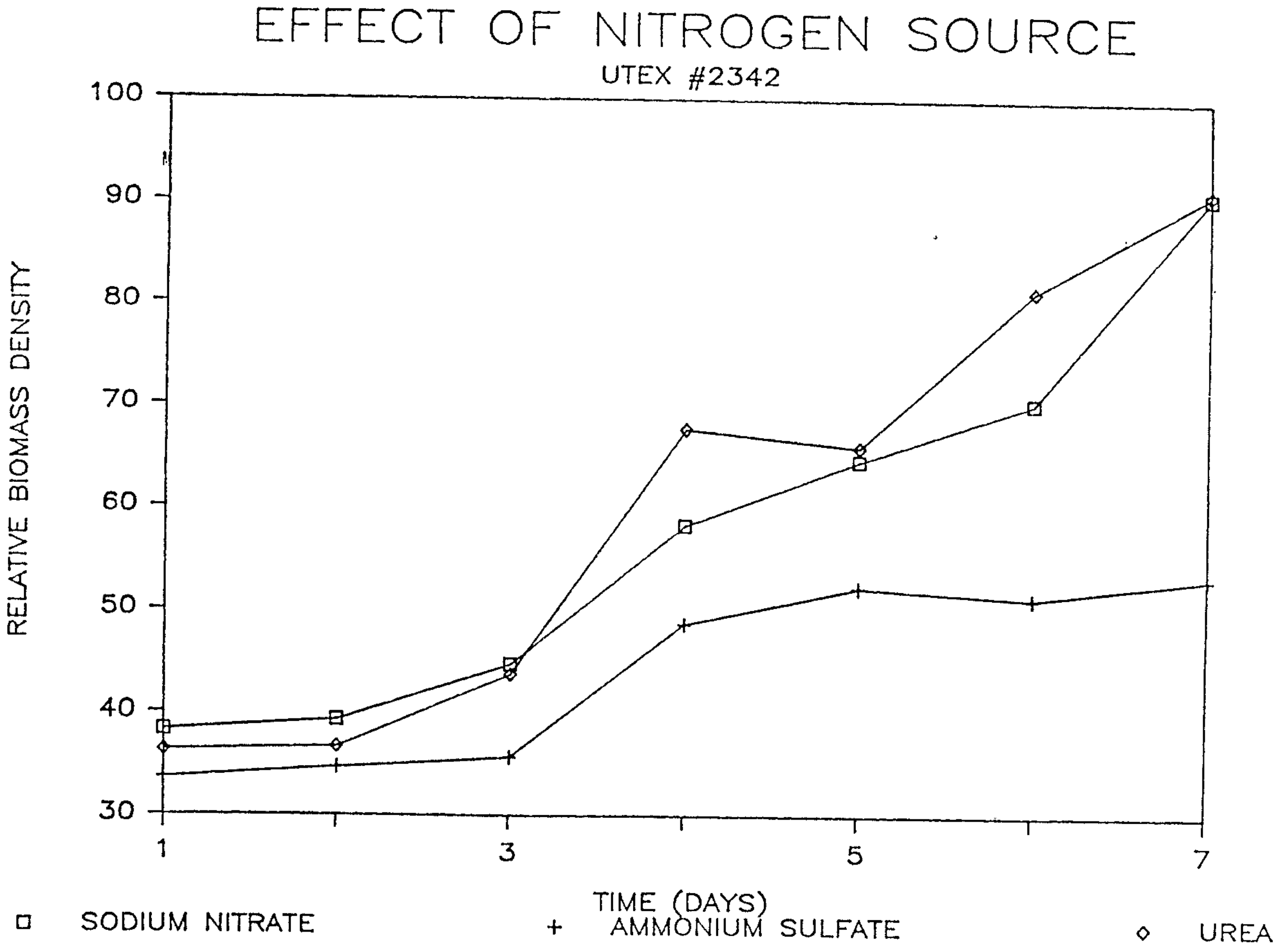

Figure 10. The effect of nitrogen type on the growth of UTEX #2342.

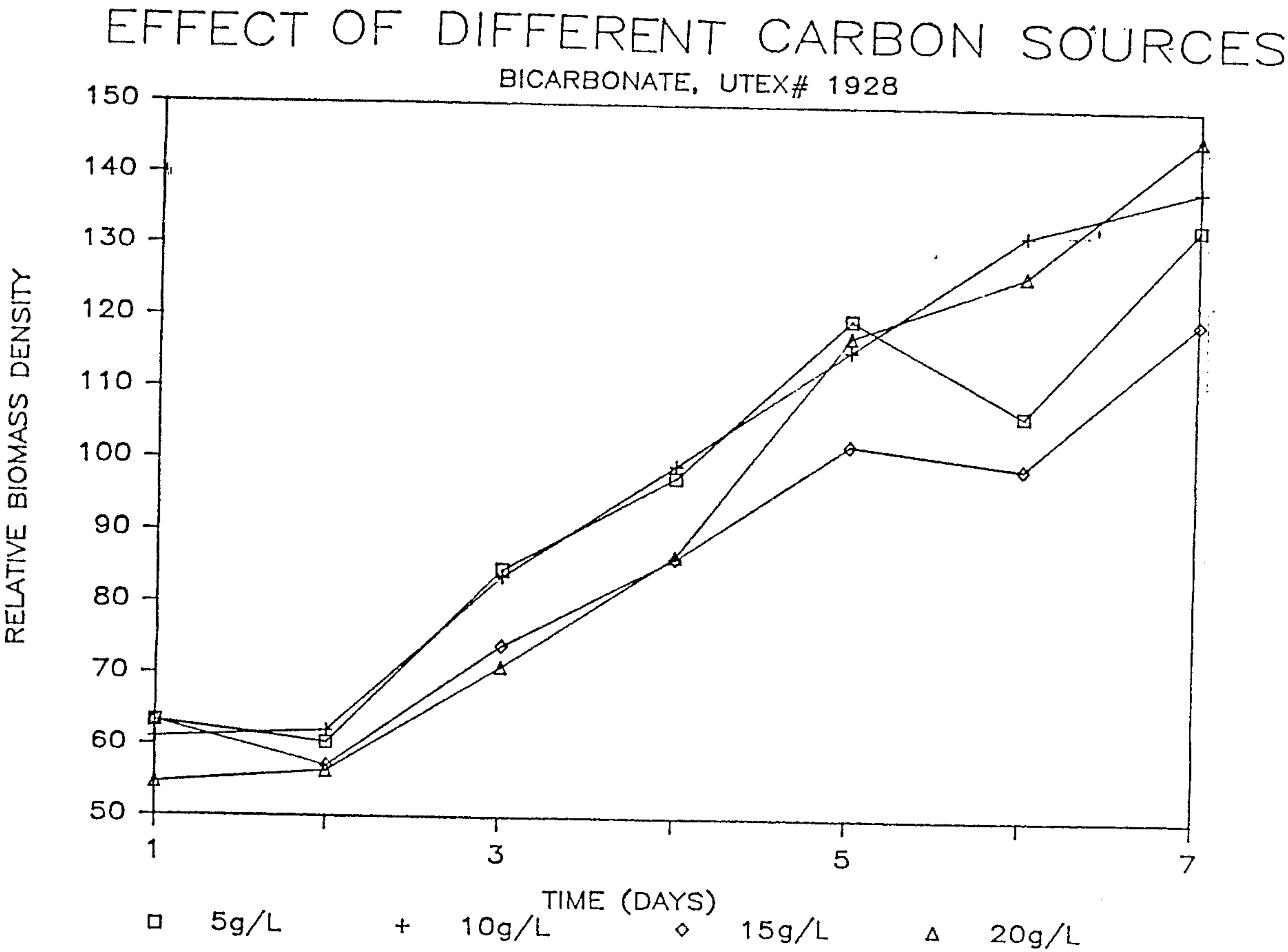

Figure 11. The effect of bicarbonate concentration on the growth of UTEX #1928.

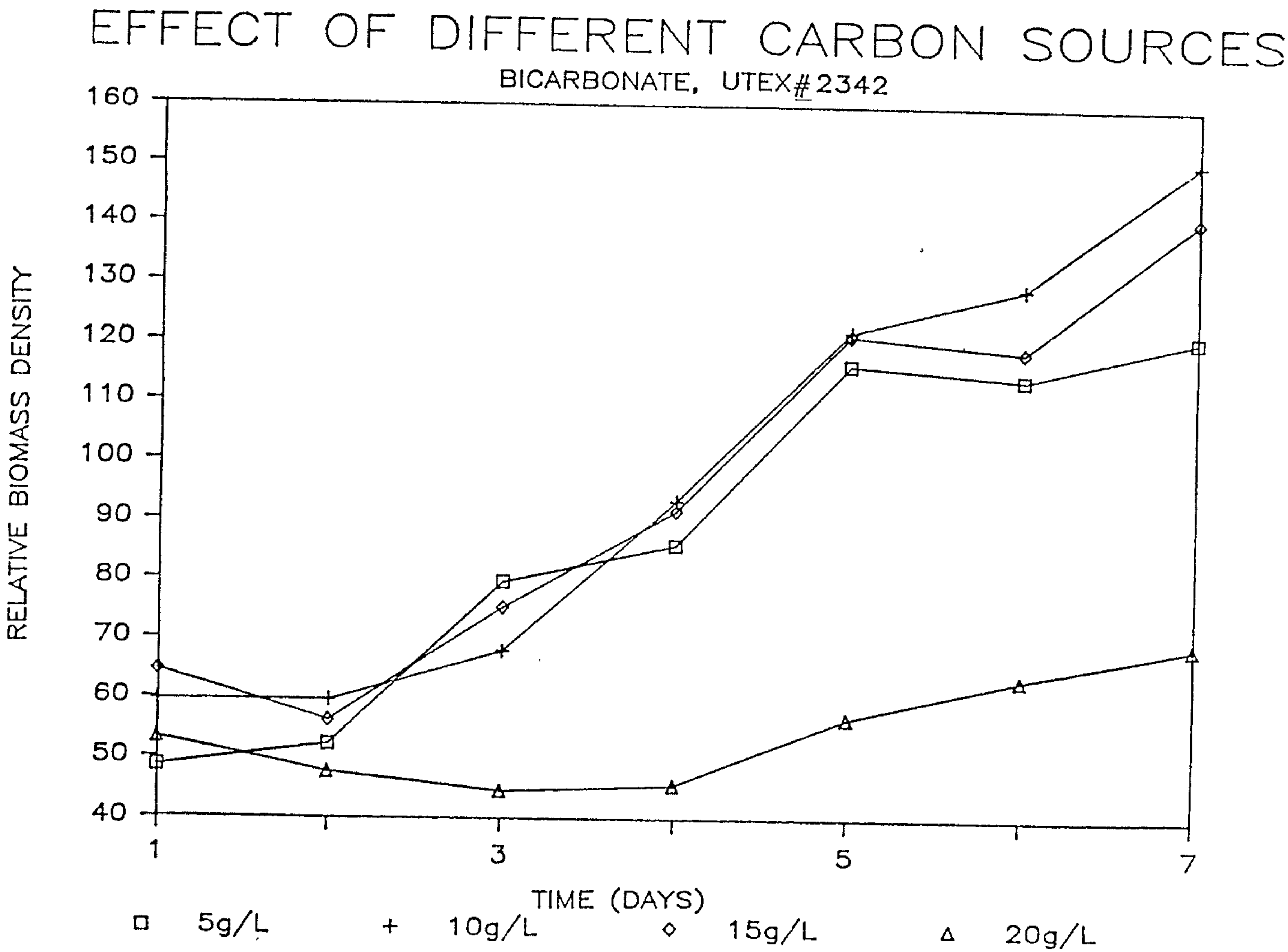

Figure 12. The effect of bicarbonate concentrations on the growth of UTEX #2342.

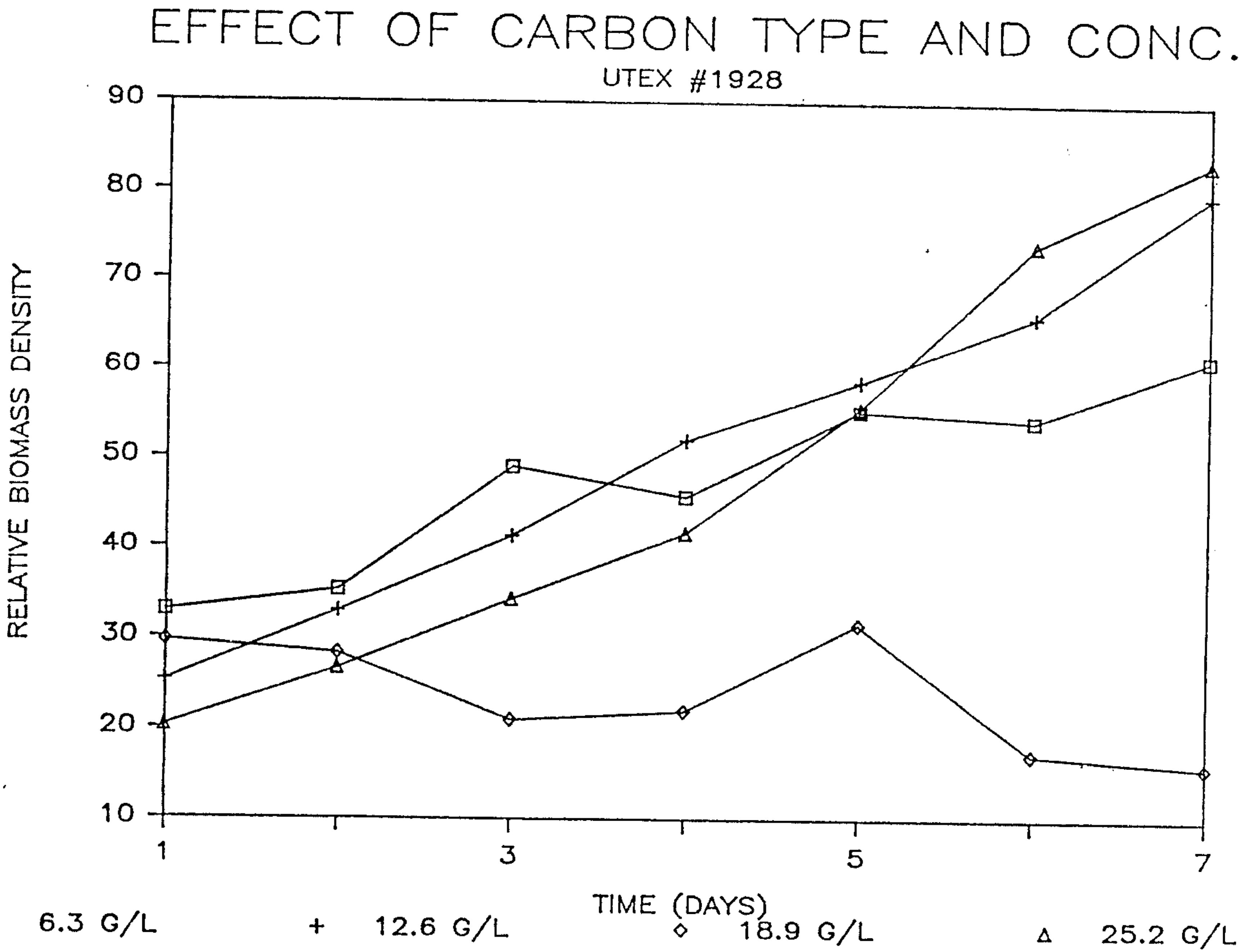

Figure 13. The effect of carbonate concentration on the growth of UTEX #1928.

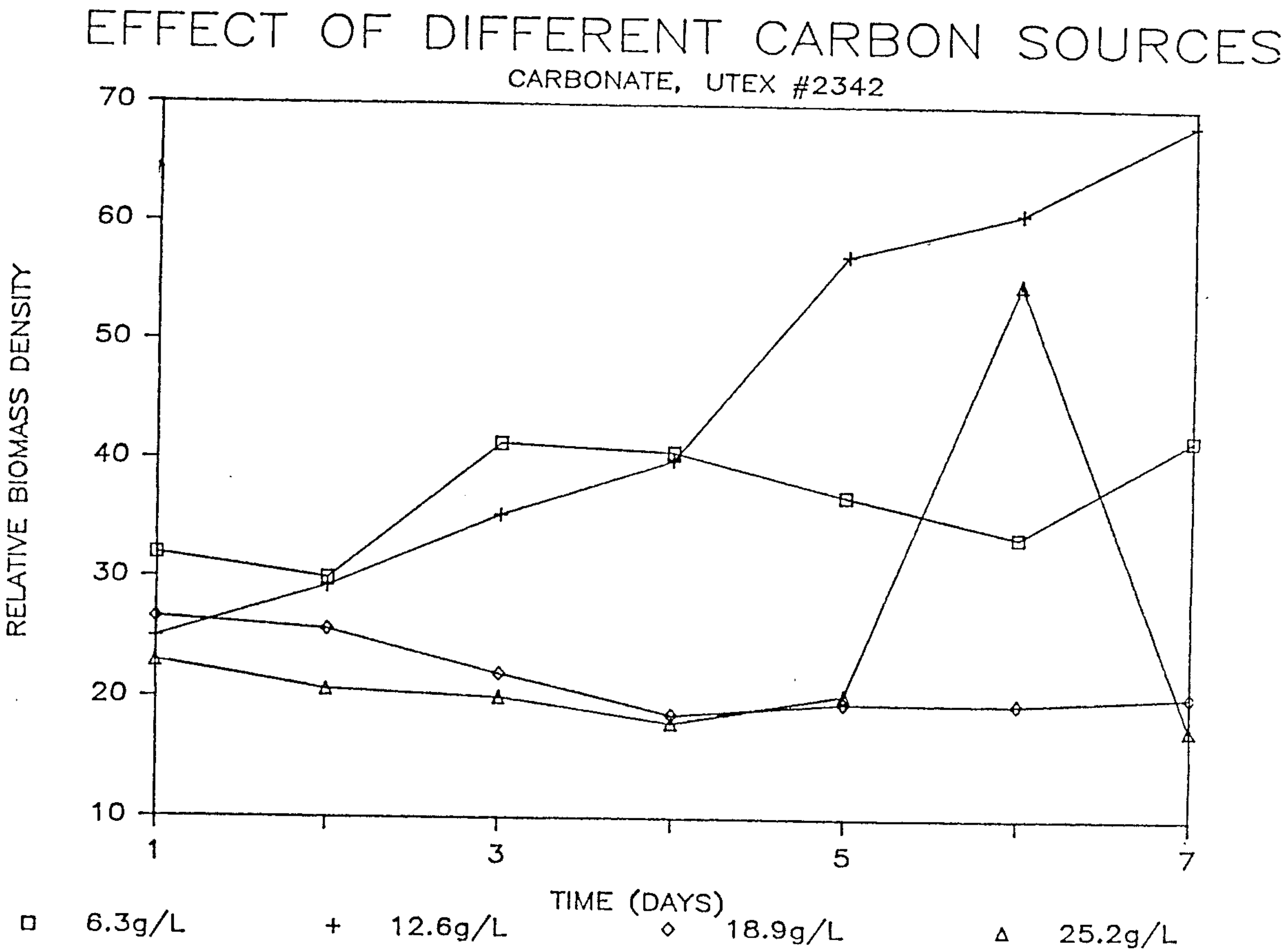

Figure 14. The effect of carbonate concentration on the growth of UTEX #2342.

There was no significant difference in growth of the two species tested at all carbon types and concentrations used. Because of light limitation on the growth rate in these experiments, little carbon was actually required for growth. This carbon was probably sufficiently supplied by either the added bicarbonate or the added carbonate ions at all concentrations tested. Of course, the dynamic equilibrium of the carbon buffer system would result in some usable dissolved CO_2 and bicarbonate ion even in those cultures just receiving carbonate ion, as long as the pH was below pH 8.0. Unfortunately, pH was not measured. We must assume that sufficient usable carbon was available to all cultures.

There was significant difference in the growth of the two species; species #1928 grew better than species #2342. Although this may be due to the experimental conditions of half RTF water and 35°C, such results do not lessen the fact that there is no advantage to using bicarbonate rather than carbonate for either species. However, at commercial scale, growth rates may be so much greater than in these laboratory studies that bicarbonate may supply needed carbon easier and faster than can the carbonate ion. In fact, if the relatively high pH preferred by Spirulina could be maintained, supplying carbon as CO_2 may be preferable and more cost-effective. However, the Israelis have demonstrated the use of bicarbonate as a means of controlling species dominance in outdoor Spirulina cultures. Thus, the choice of carbon sources must take into account more than cost effectiveness. Perhaps, both carbon sources (bicarbonate and CO_2) should be integrated into an overall culture management strategy.

3.0 Turbidity Effects

Some of the most important information gathered during this project concerns the practical problems of working with RTF water, a moderately saline water with an ionic composition that is different from seawater: it is relatively low in magnesium and potassium and high in calcium. When Zarrouck's medium was added to this water, appreciable turbidity resulted. Turbidity was a real problem for several reasons. First, the turbidity reduced the amount of light that can reach the algae, ultimately reducing growth. Additionally, a percentage of the concentration of one or more of the nutrients was being reduced by an unknown amount by being tied up in precipitates. This nutrient reduction could cause growth limitation. Finally, it made accurate dry weight determinations extremely difficult. Therefore, an investigation into the causes of the turbidity was launched. A very revealing experiment (figure 15) involved adding different levels of $CaCl_2 \cdot 2H_2O$ to (normally used in Zarrouck's media) 1 liter of RO (Reverse Osmosis) water to which had been added 0.5 g KH_2PO_4, the normal concentration of KH_2PO_4 found in Zarrouck's media. After addition of the $CaCl_2$, the contents of the flask were well mixed and filtered. Both KH_2PO_4 and $CaCl_2 \cdot 2H_2O$, at the concentrations and temperatures used, should be completely soluble and thus leave no residue on the filters. However, there was a clear increase in residue weight with an increase in the amount of $CaCl_2$ added. We interpret this to mean that calcium phosphates, which are relatively insoluble, were being formed. This finding confirms that necessary nutrients, such as phosphate, are being reduced in concentration and are less available to the algae,

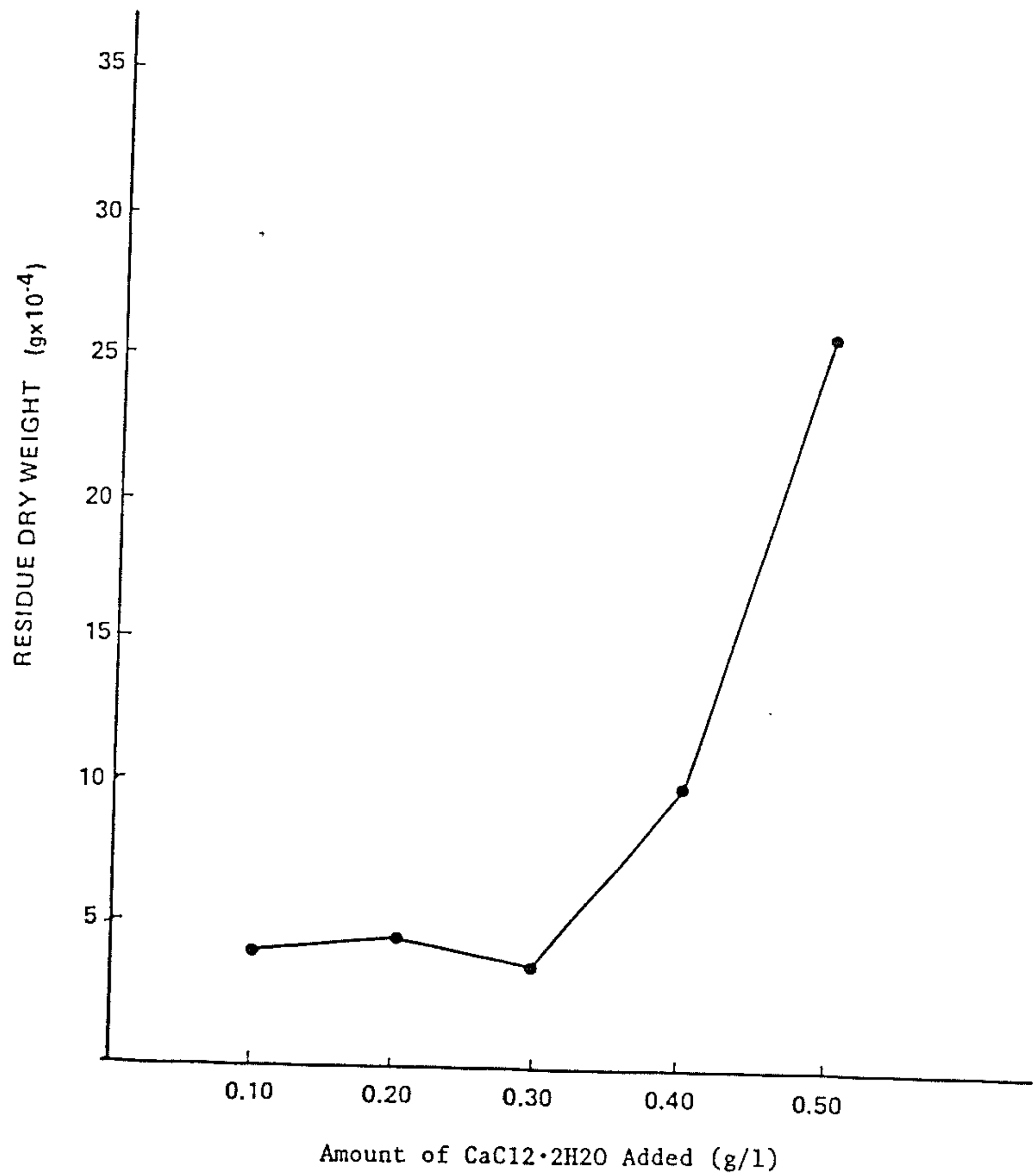

Figure 15. Interaction of CaCl2 and KH2PO4.

ultimately limiting growth. Since RTF water is high in calcium, it is quite possible that appreciable amounts of the phosphate added to the water are being tied up in calcium phosphates.

A similar experiment was performed to determine the effect of the added bicarbonate on turbidity. It can be seen (figure 16) that increased concentrations of bicarbonate added to RTF water (made up to full Zarrouck's media strength, but without $CaCl_2$ or bicarbonate) cause increased filter residue dry weight. Concentrations of sodium bicarbonate of 5 and 10 g/liter, at least, should be completely soluble and thus leave no filter residue. Either the bicarbonate's solubility is reduced in the saline water or some chemical interaction resulting in precipitation, is taking place. The end result is the same-reduced light reaching the algae and ultimately reducing growth.

Potential solutions to these problems include: pretreatment of the RTF water to reduce calcium levels, a common procedure in RO technology; using different sources of phosphate that may decrease interactions with calcium; using constant, low concentrations of bicarbonate; and, perhaps most effective of all, reusing the media. The media would then be essentially pretreated because the free calcium in the water already has interacted with added phosphates. Any other chemicals in the original well water that would interact with the added fertilizer ingredients already will have reacted. Reuse of the media makes good economic sense as well since less pumping for new water is required and less trace elements may need to be added.

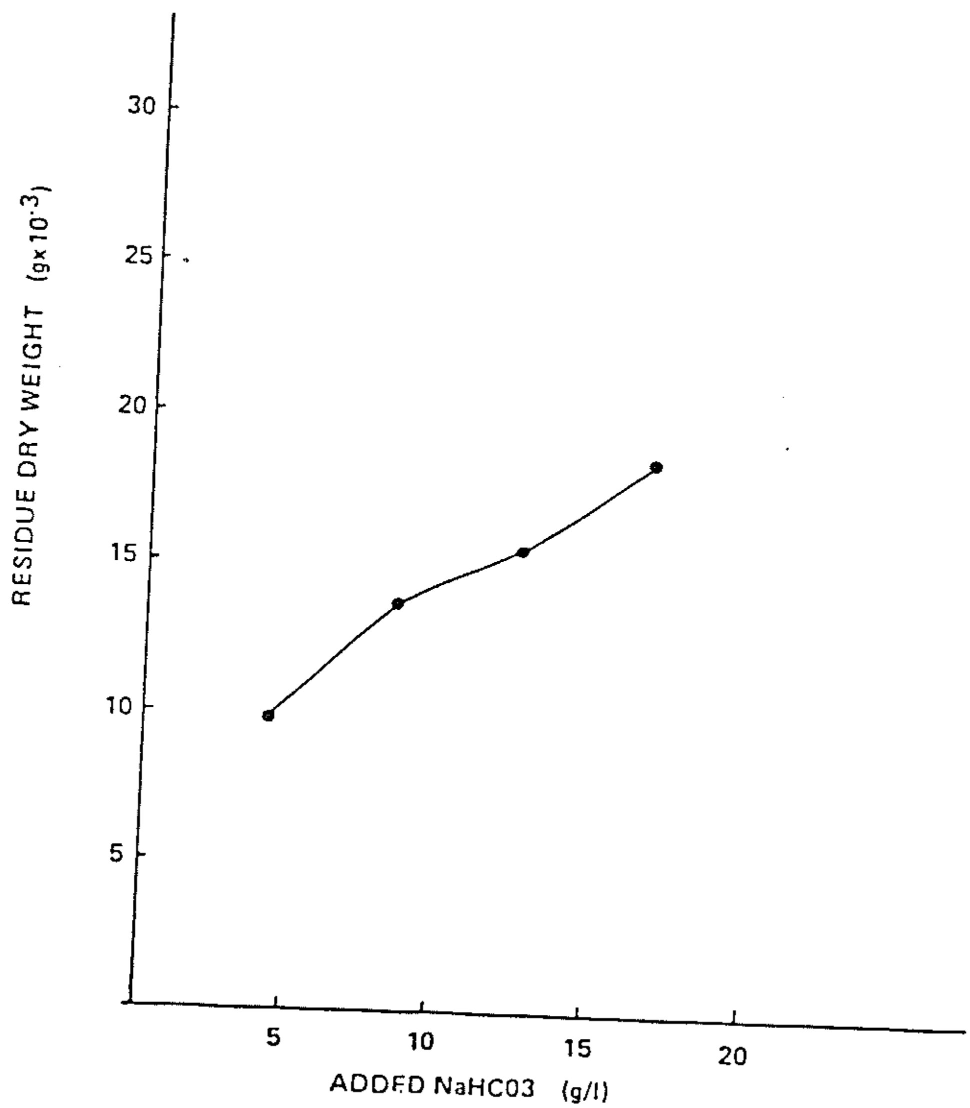

Figure 16. Effect of increased concentration of added bicarbonate on turbidity in RTF water.

4.0 Pilot-Scale Cultivation

There were four daily continuous turnover rates evaluated: 25 percent, 50 percent, 75 percent, and 100 percent or put another way: 4, 2, 1.3, and 1 day residence times, respectively. All runs used half RTF water. It can be seen in figure 17, that there is a decrease in standing algal density (on a dry weight basis) of UTEX #2342 over time at all turnover rates evaluated. This indicates that the algae are being "washed out" of the raceway. That is, average algae growth rates were slower than the turnover rates evaluated. Thus, under the test conditions experienced by the algae during these runs, species #2342 cannot be grown in sustained continuous culture at turnover rates greater than or equal to 25 percent daily turnover.

Even though the algae are being washed out at all turnover rates tested, at a 25 percent daily turnover rate, the washout rate is slower than for any other turnover rate. This may indicate that sustainable continuous culture of Spirulina at pilot scale under these test conditions, may be possible at lower daily turnover rates, such as 10 percent to 15 percent.

However, good daily production can be achieved even at these lower turnover rates. For, instance, it can be seen in figure 18 that excellent average daily production rates ($g/m^2/day$) were demonstrated at all turnover rates. However, in general, the faster the turnover rate, the faster that washout occurred. Since 25 percent daily turnover rate appears to be just above the sustainable turnover rate for these test conditions, these numbers will be used as most indicative of the production potential of Spirulina cultivation at pilot scale. Even after four days on continuous culture, species #2342 is

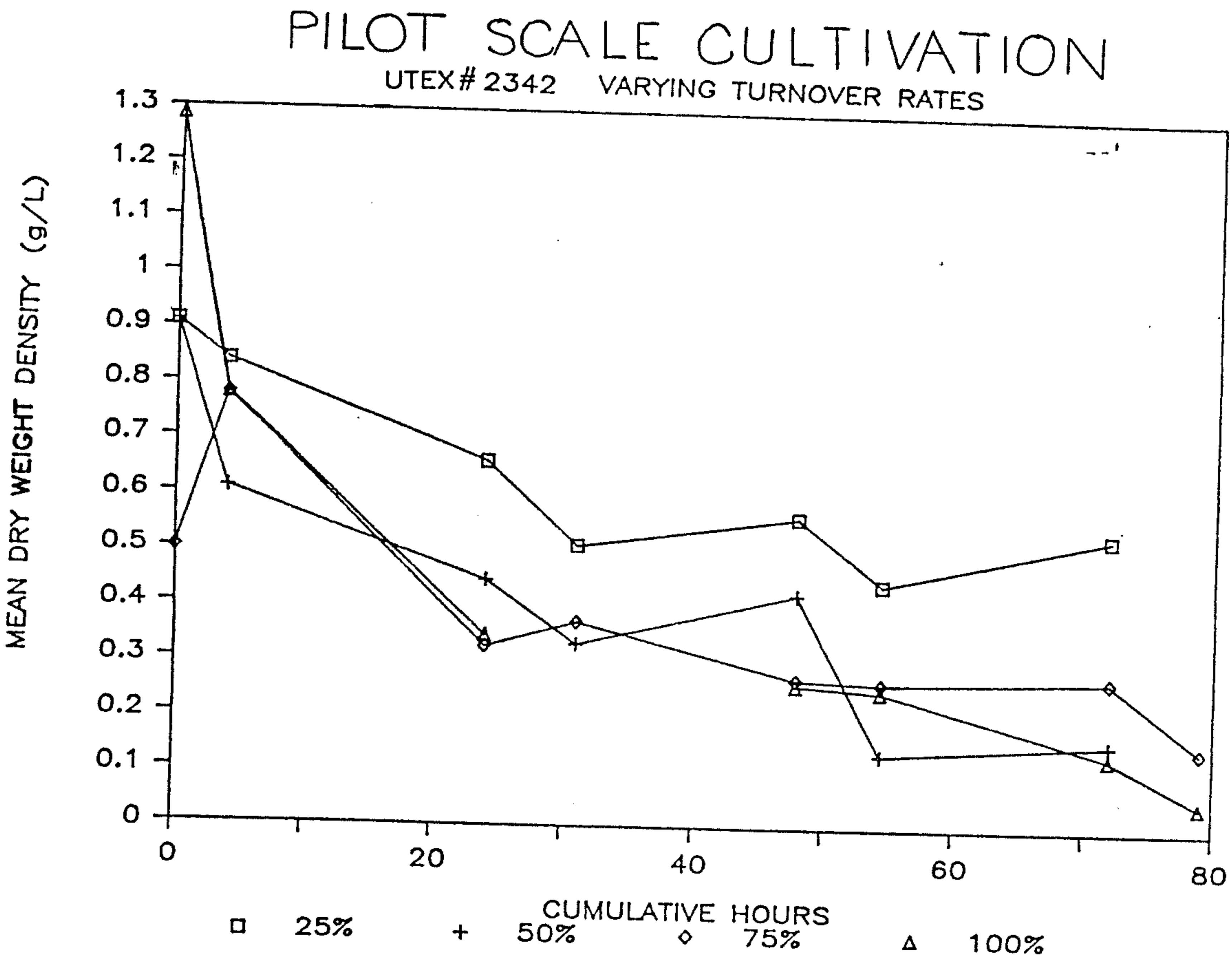

Figure 17. The pilot scale cultivation of UTEX #2342 at four turnover rates.

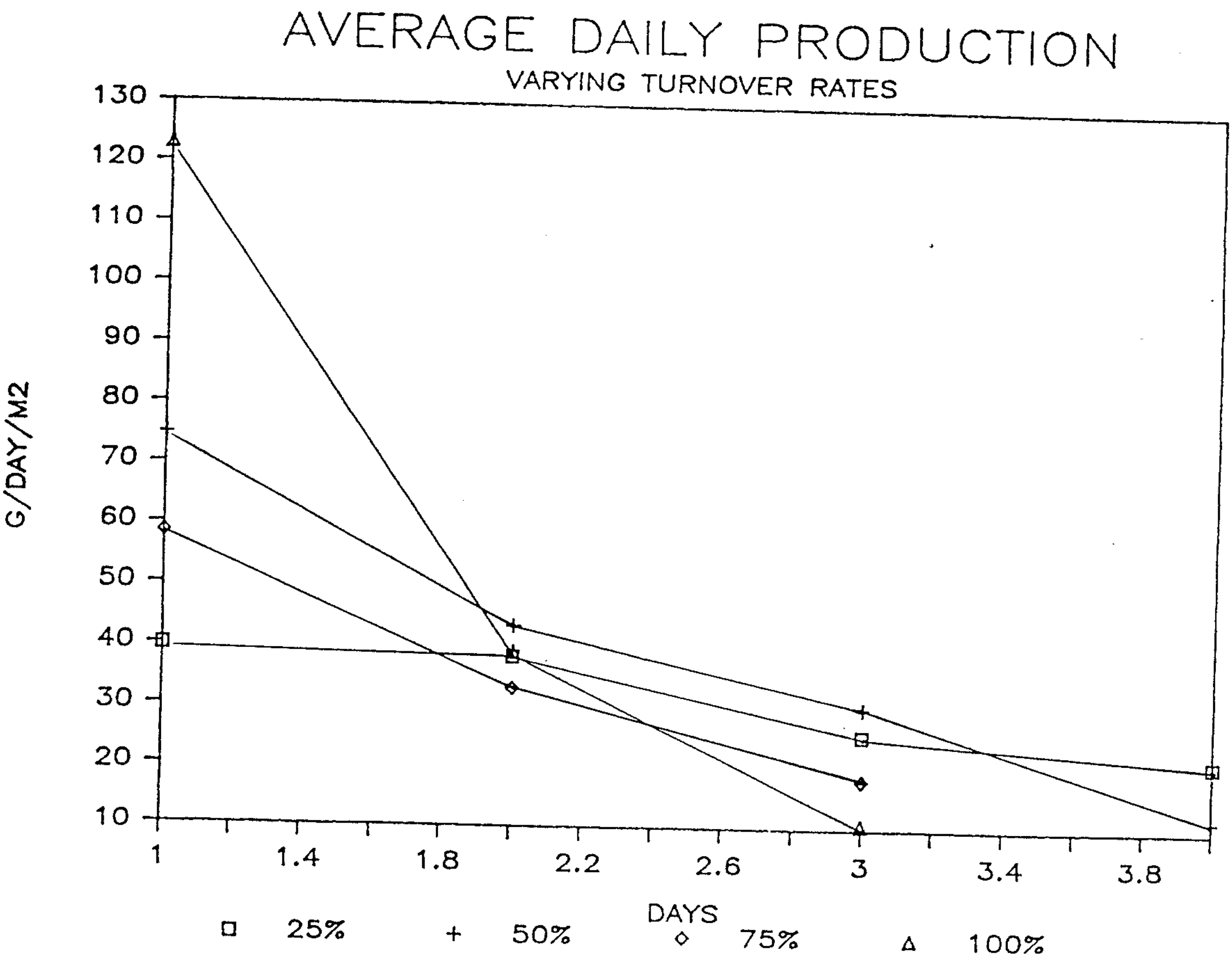

Figure 18. Average daily production rates (uncorrected) of UTEX #2342 at four turnover rates.

producing at the rate of a little over 20g/m^2/day on a dry weight basis. In the beginning of the experimental run, it was producing at the rate of 40 g/m^2/day. Over a four-day period, at a daily turnover rate of 25 percent, species #2342 produced an average of 30 g dry weight/m^2/day. Because all experimental trials of different turnover rates were begun when there was a high standing density of algae, it became obvious that at least some of the average daily production had a component consisting of harvesting of initial biomass. In order to determine how much actual net daily production there was, the following relationship was used: A + B = C + D where:

A = Initial amount of algae (calculated as initial algae density in mg/l multiplied by algal raceway volume in liters);

B = New algal production (calculated by difference);

C = Total amount of algae harvested (calculated as sum of daily production over the experimental period in mg); and

D = Remaining amount of algae (calculated as final algal density in mg/l multiplied by algal raceway in liters).

Thus, total new production is the difference between the sum of the total amount of algae harvested and the remaining amount of algae minus the initial amount of algae. A corrected average daily production rate is calculated by dividing total new production by the area of the raceway (50 m^2) and by the number of days of the run.

When production figures are then corrected for the harvesting of "old" biomass, it can be seen (figure 19) that high net production rates were demonstrated by #2342 at 25 percent and 75 percent

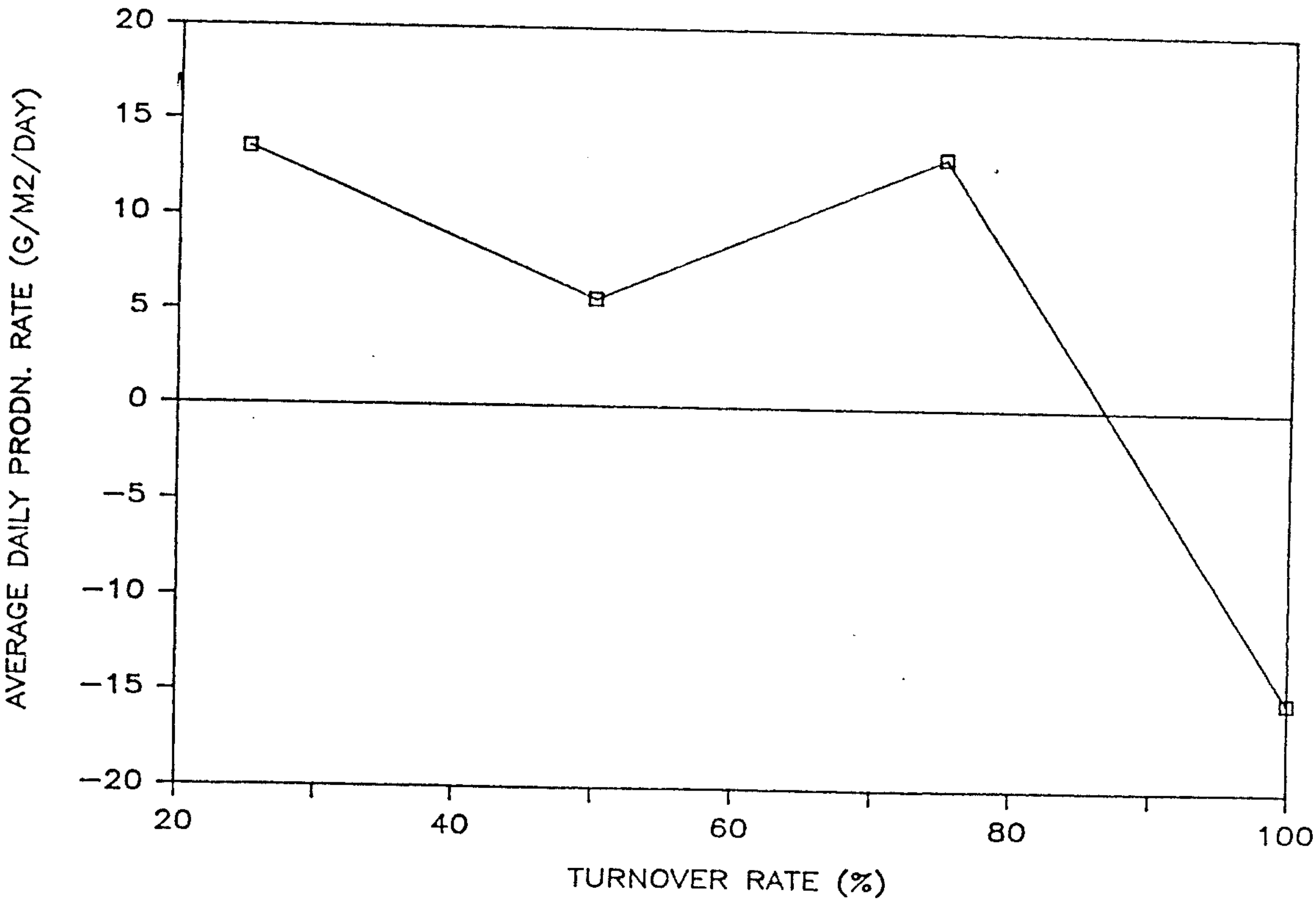

Figure 19. Corrected average daily production rate of UTEX #2342 at four turnover rates.

turnover rates: 13.57 g (dry weight)/m^2/day and 13.32 g/m^2/day respectively. At a 50 percent turnover rate, the average daily production rate was 5.68 g/m^2/day. Only at 100 percent daily turnover rate was there no net production. No statistical analyses were performed on this data to determine whether there was a significant difference between these growth rates.

Of course, these values are only indicative of the potential annual production rate. However, given more optimum water temperatures, it may well be possible to sustain a high average daily production rate as demonstrated by #2342 at 25 percent turnover. This could be accomplished in several ways:

1. a lower average continuous daily turnover rate, such as 15 percent, may be sustainable; or
2. a semicontinuous harvest of algae to maintain an optimum biomass density.

A semicontinuous harvesting approach may result in somewhat less than maximum production, but could increase production reliability by reducing culture "crashes" due to washout stresses possible in continuous culture.

We have demonstrated that Spirulina species #2342 grows well at pilot scale in 7,000 ppm TDS water. At pilot scale, under less than optimum water temperatures for this species, species #2342 produced a daily average of 13.6 g/m^2/day over a four-day period. On an annualized, corrected basis (see discussion above), #2342 could produce close to 50 metric tons/hectare/year or 22 tons/acre/year if test conditions could be replicated year round. This is possible if geothermal waters and/or temperature controlled greenhouses are used. If grown outdoors without these aids, annual production would be closer to 20 m tons/ha/yr.

It is not clear why there was a greater average daily production rate at a 75 percent daily turnover rate than at a 50 percent daily turnover rate. Water temperatures were, on average, one to two degrees Celsius higher (average of 25°C) during the 75 percent turnover trial, but this shouldn't account for the great difference in average daily production. The pH was more optimum (average of 8.95) during the 50 percent turnover trial than during the 75 percent turnover trial (average of 7.88). Ambient light levels were roughly the same during both the 50 percent and 75 percent turnover rates, as was conductivity. The answer may lie in decreased light levels experienced by the algae. Because algae densities initially and throughout the trials were higher during the 50 percent turnover trial than during the 75 percent turnover trial, the average algal cell received less light during the 50 percent turnover trial. This extra light limitation could well account for the lower productivity. On the other hand, the 50 percent turnover trial productivity is lower than the 25 percent turnover trial productivity, yet initial algal densities were identical. At the higher turnover rate (50 percent), washout was occurring at a faster rate than at the 25 percent turnover. Thus, it appears that, given equal initial algal densities, productivity was inversely proportional to turnover rate. On the other hand, when initial algal densities varied significantly, the extra light limitation caused by higher algal densities can override the effect of turnover rate on production. In an ideal world, all trials would have been conducted under identical environmental conditions including identical initial algal densities. Unfortunately, the very nature of outdoor, pilot scale investigation often preclude such precise control.

In summary, we have demonstrated that Spirulina species #2342 is capable of producing at pilot scale, under less than optimum conditions at the rate of greater than 13 $g/m^2/day$. This productivity was demonstrated while #2342 was being grown in 7,000 ppm TDS water. This productivity equals or exceeds published values. Thus, we have demonstrated the technical feasibility of commercial cultivation of Spirulina in New Mexico utilizing the extensive saline groundwater resource of New Mexico.

SUMMARY AND CONCLUSIONS

Summary

The data presented here indicates which combination of species/water type/water temperature should be used to achieve maximum growth rates using New Mexico's saline groundwaters.

Additional laboratory studies indicate that, in general, full Zarrouck's level of nutrients is not necessary for good growth. Even 10 percent of full Zarrouck's level of nutrients is sufficient in light-limited cultures of #1928 and #2342 such as those of the experimental flasks. Any inexpensive source of nitrogen, may be used in preparing Zarrouck's media. Further, any cost-effective carbon source may be used for Spirulina production; carbon concentration at lower levels than used in Zarrouck's media are effective in supporting good growth.

Pilot scale investigations over a short operational period under less than optimal conditions indicate that Spirulina species, growing in saline groundwater, have a potential daily production rate of $13 g/m^2/day$.

Conclusions

1. All four species of Spirulina tested can grow in all four water types tested when full Zarrouck's media ingredients are added to each of the water types.
2. The optimum temperature range for the growth of those species of Spirulina tested, of the temperatures tested, is 27°C to 42°C.
3. There is little, if any, net growth at 20°C for any species in any water type. Death occurs in all water types for all species after several days at 10°C.
4. Spirulina species UTEX #2342 is the best overall species to use at temperature of 42°C, regardless of water type.
5. A coinoculant of species #2340 and #2342 is recommended as the best overall approach to Spirulina cultivation in New Mexico's saline groundwaters. More specific recommendations can be made in certain, site-specific situations.
6. Both species #1928 and #2342 were not limited in growth by concentrations of Zarrouck's media as low as 10 percent of full levels.
7. Any cost-effective source of nitrogen can be used to grow Spirulina species #$1928 and #2342.
8. Bicarbonate may be preferrable to carbonate as a carbon source for both species #1928 and #2342, if its use is cost-effective.

9. The interaction of the fertilizer ingredients with the saline water types tested results in turbidity of the media. The problem is most severe in full RTF well water. This turbidity can result in decreased growth via reduced light levels and reduced nutrient availability.
10. This turbidity makes accurate, replicable algal dry weight determinations difficult. Optical methods of algal biomass determination are more replicable. Fluorometry, which is sensitive to in-vivo chlorophyll a, but not to salt precipitates, appears to be the most reliable method of algal biomass determination.
11. The pilot-scale system at the RTF is complete and operational. All subsystems have performed as expected, except for the harvest subsystem which needs additional development.
12. The computerized measurement and control system currently has the capability of providing three data points for each of three parameters (conductivity, pH, and turbidity) for each of four sampling points every hour. In addition, continuous measurements of PAR (photosynthetically active radiation) at the top and bottom of the Spirulina culture water column are made. Continuous measurements of temperature at five points in each of the greenhouses are also made.
13. The greenhouses generally are 10°C warmer than ambient during winter. This is important in maintaining water temperatures closer to optimum during the colder months of the year.

14. Unialgal cultures of Spirulina have been grown on pilot scale at the RTF. These cultures have lasted as long as 11 weeks and reached densities exceeding 1.4 g/liter (dry weight). Average daily production rates of greater than 13 $g/m^2/day$ were documented.
15. The technical feasibility of producing Spirulina in New Mexico using a variety of saline groundwaters has been demonstrated.

RECOMMENDATIONS

1. Continued development of the harvest system is necessary. A low-cost harvest and solar drying system for the Spirulina will enhance the economic feasibility of commercial cultivation of Spirulina in New Mexico.
2. A more thorough understanding of the chemistry of RTF water interacting with fertilizer ingredients is necessary to reduce or eliminate turbidity problems.
3. The use of both dry weight and ash-free dry weight should be evaluated as a means for improving algal biomass weight determinations.
4. Continued development of the computerized measurement and control system will enhance the ability to acquire and analyze experimental data. This will ultimately lead to quicker progress toward project goals.
5. Since Spirulina growth is optimal at higher temperatures (27°C to 42°C) and its temperature tolerance range is relatively narrow, methods for maintaining optimum temperatures in

large-scale cultures are necessary. The development of passive solar greenhouses is strongly recommended. Gaining practical experience with using geothermal water for its heat content as well as a growth media is strongly recommended.

6. Research on culture systems that are low in both capital costs and operating costs is recommended.
7. Market research should be performed to determine the size and location of potential markets for Spirulina grown in New Mexico.
8. A small commercial size Spirulina culture operation should be established and operated for at least one full year to determine actual operating and capital costs, as well as actual annual production.

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