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GROWTH AND PROTEIN PRODUCTION IN SELECTED LABORATORY CULTURES OF BLUE-GREEN ALGAE GROWN IN TILAPIA WASTEWATERS

FINAL REPORT

January 1984 through March 1985

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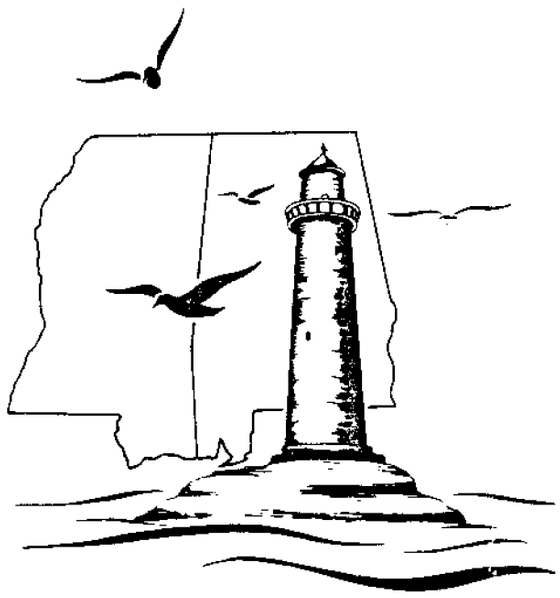

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Prepared by:

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This final report represents the work carried out under the 1984 project entitled, "Protein Feedstock Production Using an Alga/Seawater/Wastewater System.

This report also represents portions of a MS thesis by Priscilla Jordan entitled, "Growth and Protein Production in Laboratory Cultures of Spirulina major grown in Tilapia wastewater.

Published Papers or Papers Delivered:

Rhyne, C. and L. Crump. 1985. Productivity and Protein Production in Arthrospira platensis grown in Tilapia wastewaters.

Abstract, Mississippi Academy of Sciences Annual Meeting, Jackson, MS, February 21, 1985.

Student Thesis:

Jordan, P. Growth and Protein Production in Laboratory Cultures of Spirulina major grown in Tilapia Wastewater, May 1985.

PROJECT OBJECTIVES

The basic aim of this study was to observe and analyze a photosynthetic mariculture system utilizing the blue-green algae Arthrospira platensis and Spirulina major grown in fish wastewater as the algal medium. The objectives were to provide data for critical analysis of an integrated algae/fish mariculture system by the studies of:

1. plant nutrient production by the fish
2. the reduction of these nutrients by the algae
3. algal growth relative to selected environmental parameters
4. control of algal culture contamination
5. protein levels in the algal biomass.

ABSTRACT

Spirulina major and particularly *Arthrospira platensis*, two filamentous blue-green algae, have been shown in our laboratory study to meet certain criteria necessary for their use in an integrated algae/fish/mariculture system.

The wastewaters from 118 liter aquaria holding 12-20 *Tilapia* (*Sarotherodon niloticus*) generated adequate nutrients to allow growth of both algal species. Reductions in the high levels of NO_3, NH_4 and PO_4 in the wastewaters indicated that these plant nutrients were being taken up by the algal biomass.

Growth studies demonstrated that small additions of $NaHCO_3$, NH_4 and NO_3 greatly enhanced the yield of both algal species. It was also found that additions of $NaHCO_3$ (raising pH) and the high concentrations of NH_4 contributed to control of small coccoid green algae, ciliated protozoa and fungal hyphae as contaminants.

Protein levels were found to be 28-59% in *Spirulina* and 43-52% in *Arthrospira* under optimal growing conditions.

INTRODUCTION

Two areas of concern within the realms of environmental quality and human survival are pollution (eutrophication) and protein production. While the two conditions are basically unrelated they are, in fact, tightly coupled when relatively large food production systems are created.

Microalgae have been suggested as a new weapon to fight the worldwide deficiency in proteinaceous matter because of their high reproduction rates, adaptability to various environmental conditions, their high protein levels, and their omnipresence in any aquatic environment where nutrients, carbon source, and irradiance are sufficiently present together with the proper range of temperatures (Oswald 1980, Soeder 1980, Barak 1980).

Photosynthetic single cell protein (SCP) production has been synonymous with algae culturing for several decades. In terms of protein produced per unit area, microalgae are more efficient than any other type of plant (Waslien et al. 1978, Grisanti & Oswald 1978). More specifically, the blue-green algae have been shown, on the average, to contain higher levels of protein than other classes of algae. This is particularly true for Spirulina, Arthrospira and certain species of Oscillatoria when grown under optimal conditions (Waslien et al. 1978, Grisanti & Oswald 1978, Soeder 1980a, Durand-Chastel 1982, Yanagimoto & Saitoh 1982).

A recent gathering of worldwide authorities on "applied algology" met in Akko, Israel to present findings and discuss the state of the art (Shelef & Soeder 1980). Potential uses and products derived from algal biomass that the group discussed were: 1. food and feed; 2. oxygen production; 3. nutrients and minerals, 4. chemicals and pigments; and 5. energy. Some were seen as having immediate application while others were viewed with more remote perspectives. Several aspects were well discussed, these being: 1. increasing algal yields; 2. increasing light conversion efficiency; 3. control of desirable algal species; 4. bacterial and algal biomass relationships, 5. improving harvesting, separation, dewatering and drying; 6. improving digestibility and nutritional uniformity; 7. stabilizing nutritional and toxicological guidelines; 8. potentials in chemicals, pigments, vitamin and food additives from the algal biomass and 9. establishing cost-benefit parameters.

The prospects of protein production by algae and its direct use by humans have received less acclaim recently, mainly due to a general resistance to nutritional innovations. What has recently gained new momentum is algal biomass as a source of protein and vitamins for animals, particularly when the algae are produced as part of a wastewater treatment scheme. However, it should be pointed out that the production of both Chlorella (Chlorophyceae) and Spirulina (Cyanophyceae) has greatly increased as part of the large scale human health food industry, particularly in Japan and Taiwan. This activity has contributed considerable scientific knowledge to SCP production.

In terms of value, algae biomass plays an important role in supplying photosynthetic oxygen to heterotrophic microorganisms

that degrade wastes. This concept first gave rise to algal oxidation and stabilization ponds and later on to the development of high rate algal ponds. Algae not only produce dissolved oxygen useful for purposes of organic matter treatment, but also remove nutrients such as nitrogen and phosphorous, thus reducing the potential of the effluent for triggering eutrophication in receiving bodies of water.

Productivities of 20 g/m^2/day are sustainable in diluted animal wastes. Because green algae are about 8% nitrogen, the corresponding nitrogen recovery is 1.6 g/m^2/day, or about 5.8 metric tons of nitrogen-/hectare/year, 20-30 times the productivity of soybeans (Benemann et al. 1980).

The work with Spirulina and its potential for feed and food production began in the recent past with the discovery by the Belgian Sahara Expedition of 1964-65 that the blue-green alga Spirulina was collected and eaten regularly by natives around Lake Chad. Soon after this discovery, the French Petroleum Institute developed methods for producing Spirulina on a commercial scale. The findings were that Spirulina regularly contained 60% protein, had good digestibility and was harvested easily due to its large size. In 1974, a research program entitled "Combined Systems for Algal Wastewater Treatment and Reclamation-Protein Production" was approved and carried out by the German-Israeli Algae Project (Soeder 1980b). Spirulina production on a commercial scale has been steadily developed by the Mexican company, Sosa Texcoco S. A. Production in 1980 had reached 2 tons/day. Most of the product is shipped to Japan and a few other countries (Soeder 1980b, Durand-Chastel 1980).

Feeding the algal biomass to trophic levels below man is a possibility (Mokady et al. 1978, Sandbank & Hepher 1980, Lipstein & Hurtwiz 1980, Hwang et al. 1980, Walz & Brune 1980, Berend et al. 1980). An area of increasing interest is that of coupling waste nutrients and algal biomass production for the feeding of fish particularly (Kromann 1980, Edwards 1980a 1980b, Montgomery & Gerking 1980). Fish in the genus Tilapia are currently regarded as an attractive species for cultivation (Balarin 1984, Pullin & Lowe-McConnell 1982). The ability of Tilapia to thrive under conditions deleterious to many other fish and to feed on organisms low in the food chain, e.g., algae, are important advantages. Tilapia have short generation times and breed rapidly in captivity. They are frequently reared in ponds without supplemental feeding, subsisting only on naturally occurring phytoplankton and zooplankton supported by periodic fertilization. Many species are able to grow in seawater; therefore, they could be cultivated in under-utilized marine environments or in arid regions lacking freshwater (Mitsui et al. 1981, Murray & Mitsui, 1982).

The investigators proposed to study the production of proven feedstock (cyanophycean protein) from two blue-green algae, Arthrospira platensis and Spirulina major, grown in Tilapia wastewater-based media. The study was designed to evaluate the protein production and growth potential of the algae and the nutrient production (NH_4, NO_3, PO_4) by the cichlid fish, Sarotherodon niloticus, as well as the utilization of those nutrients by the algae.

MATERIALS AND METHODS

EXPERIMENTAL ORGANISMS

Algae: Both Arthrospira and Spirulina were orginally purchased from the Carolina Biological Supply Company, Burlington, North Carolina, but numerous physiological strains have developed over time in our laboratory. Both species grow as a helicoid filament with Arthrospira platensis having a much more open coiling arrangement than the tight coils in Spirulina major. Both genera are placed in either the Nostocales or Oscillatoriales of the Cyanophyceae depending upon which author is consulted (Geitler 1932, Smith 1950, Bold and Wynne 1978). Pigmentation usually appears light blue-green in both species depending upon the medium type and light conditions.

Fish: The cichlid fish Sarotherodon niloticus (Tilapia) were kindly donated to us by Dr. Cornell Ladner of the Mississippi Bureau of Marine Resources. The tilapia were approximately 150g and 2-3 cm in length at the beginning of the study and grew to 13-20 cm long 12 months later.

STOCK CULTURE TECHNIQUES

Stock Water Supply

The stock 5 ppt. seawater supply used in this study was collected from 113 liter aquarium tanks in laboratory housing the fish, Sarotherodon niloticus (Tilapia). The filtering systems included an under-gravel system which was later changed to a charcoal and

cellulose fiber system outside the tank. The aquarium water was collected in five gallon polyethylene carboys from Mississippi Sound 15 miles offshore at 30 ppt. and diluted to 5 ppt. with dechlorinated Jackson, MS tap water.

Chemical Analysis of Stock Water Supplies

Stock aquarium water supplies were analyzed before and after each experiment. Concentrations of PO_4 and Fe were determined using a reagent system (Bausch and Lomb Spectrokit) and a spectrophotometer (Bausch and Lomb Spectronic-20). The NH_3N and NO_3N were monitored using the LaMotte Chemical test kit, a reagent system (Bausch and Lomb Spectrokit) and spectrophotometer.

Analysis of PO_4 in stock and culture water supplies involved adding ammonium molybdate and ascorbic acid to the sample. The reaction was allowed to proceed for 9 to 11 minutes before measurement at 880 nm.

The analyses of Fe concentrations were carried out using the 1, 10-phenanthroline reductant method, which was allowed to proceed for 4 minutes before measurement at 510 nm.

The nesslerization method was used to determine amount of ammonia levels, which included a dechlorinating reagent and the nessler reagent. Samples were read at 410 nm.

The cadmium reduction method was used for measurement of nitrate. This method involves the application of reductant sulfanilic acid and amine coupling reagent. The results were read at 540 nm.

The stock water supply was filtered through (Whatman) glass filters, and the pH was monitored with a (Fisher Accumet Model 610) pH meter. Salinities were measured with an automatic temperature-compensated hand-held refractometer, model 10419 (American Optical).

Cultures

Arthrospira and Spirulina filaments were transferred to 5 ppt. diluted seawater where all experiments were carried out. Stock cultures were grown in growth chambers where the temperature was maintained at 30°C ± 1 C. A photoperiod of 16:8, L:D was maintained. Twenty watt plant light fluorescent bulbs (Sylvania) were utilized.

Experimental cultures were grown in 250 ml Erlenmyer flasks in 5 ppt. seawater obtained from the Tilapia aquarium. The water was filtered through (Whatman) glass filters (1.0 and 1.2 u pore).

Experimental cultures were grown in a Percival reach-in environmental chamber, and a wooden chamber without temperature control. Temperatures were maintained in the Percival chamber at 30°C, and all photoperiods in experimental cultures were 16:8, L:D except where stated. Similar fluorescent bulbs were used for the experimental cultures as for the stock cultures. Light energy (quanta/cm^2/sec) was measured using a Quantum Scalar Irradiance Meter QSL-100 (Biospherical, Inc.). Glass pipettes, attached to rubber tubing, connected to a standard aquarium air pump (Whisper 800) provided aeration to the flasks. Sodium bicarbonate in the amounts of 1.0-5.0 g/l was added to each flask, except where noted. The duration of each experiment was approximately 7 days.

EXPERIMENTAL PROCEDURES

Media

All experiments were carried out in 5 ppt. aquarium wastewater, having been diluted from 30 ppt. seawater by dechlorinated Jackson tap water. Sodium bicarbonate was added to all culture media, except where stated. The nutrient concentrations (NO, NH_4, PO_4, and Fe) varied each time water was removed from the aquarium for the growth

experiments.

Plant Nutrient Production

Levels of NH_4, NO_3 and PO_4 were followed by analysis over an 8 day period in two aquaria. Water was removed in one study in order to analyze the effect of aeration on NH_4 and NO_3 production without fish present (see Table 1 for additional data).

Photoperiods

Photoperiods of 12:12, 18:6, 20:4 and 24:0 L:D were tested in attempts to obtain optimum growth conditions.

Light Energy

Light energy intervals of 0.08, 0.3, 0.6, 0.8, 1.0, 1.2 and 1.8 quanta/cm^2/sec were tested to evaluate optimum yield of Arthrospira and Spirulina.

Temperature Studies

Temperature intervals of 2 C between 26 C and 36 C were used to evaluate optimum temperature.

Chemical Additions

Potassium nitrate, ammonium chloride, sodium bicarbonate and trace metals $FeCl_3$, $MgSO_4 \cdot 7H_2O$, $MnCl_2 \cdot 4H_2O$, $CuSO_4 \cdot 7H_2O$, $CoCl_2 \cdot 6H_2O$ and Na_2EDTA) were added at varying concentrations to cultures to analyze their effect on the yield of both Arthrospira and Spirulina (see Tables 5, 6, and 7 for specific concentrations of each).

Contamination Level Study

Contamination, usually involving both motile and nonmotile green algae, small ciliated protozoa and fungal hyphae, was crudely quantified, using medium combinations of low/high pH and low/high NH_4 levels.

Protein Levels

Seven determinations of protein levels were made on actively growing cells in 5 ppt. aquarium seawater using a modified method of Kochert (1980 b).

Replication

All experimental tests were repeated and carried out using triplicate flasks.

ANALYTICAL METHODS

A. Extraction of low-molecular weight components

Several samples of cells were collected and centrifuged (2,000g) to yield a packed volume of 0.5 milliliters. Each supernatant was discarded, and the tubes containing the cell pellets were added and resuspended by vortexing. After incubating for 15 minutes on ice, the samples were centrifuged at 2,000g in a pre-cooled tube. The supernatant was carefully removed and the procedure was repeated with an additional 10 ml of 0.2N $HCLO_4$ (Kochert, 1980a).

B. Extraction of lipids

To the pellets from the $HCLO_4$ extraction, 10 ml of chloroform-methanol solution (2:1 v/v) were added. The pellets were resuspended and allowed to stand for 5 minutes at room temperature. The extraction was repeated with an additional 5 ml of chloroform-methanol, discarding the supernatants (Kochert, 1980a).

C. Determination of Proteins

The acid-extracted lipid-free pellets were allowed to air dry before adding 1 ml of 1N NaOH, (The original procedure of Kochert, 1980b called for adding 1 ml of 1N NaOH, and not the additional

centrifugation step). We found by making these changes, our samples did not form a precipitate. The sample was heated for 10 minutes in a boiling water bath to dissolve the pellet. Additional centrifugation at 3,000g for 10 minutes was done after dissolving the pellet. The dye binding assay was used to assay aliquots of the sample for protein concentrations.

Protein Determination

Coomassie Brilliant Blue G-250 (Sigma) exists in two different color forms. When the dye binds to protein, the red form converts to blue. The protein dye complex has a high extinction coefficient, which gives great sensitivity to the assay.

Materials

Coomassie Brilliant Blue G-250

Bovine Serum Albumin (2x crystallized) (Sigma)

Solutions

1. Protein reagent-100mg of Coomassie Brilliant Blue G-25 was dissolved in 50 ml of 95% ethanol, to this solution 100 ml of 85% (w/v) H_3PO_4 were added. The resulting solution was diluted to a final volume of one liter with distilled water.
2. Protein standard solution-100mg of bovine serum albumin was dissolved in water to a final volume of 100 ml.

Method

A standard curve was generated by pipetting a range (10-100 ug) of protein concentrations from the protein standard solution into a series of marked 12 x 100 mm test tubes. The volume of each tube was adjusted to 0.1 ml with water

into a series of marked 12 x 100 mm test tubes. The volume of each tube was adjusted to 0.1 ml with water. A reagent blank of 0.1 ml of protein reagent were rapidly added to all tubes and mixed immediately by vortex mixer. Absorbance at 595 nm was measured after 2 minutes at room temperature. A standard curve was generated by plotting the weight of protein standard against the corresponding absorbance using a weight correction factor.

The samples with unknown protein content were pipetted into separate marked test tubes in 0.1 ml amounts. Five ml of protein reagent were added, mixed and incubated for 2 minutes at room temperature. The absorbance was measured at 595 nm. Each unknown concentration was determined graphically.

RESULTS

A. Production of Essential Plant Nutrient by Tilapia

Depending on the number of fish, aeration, amounts of fishfood used and volumes of aquarium water exchanged periodically; the macro and microelements in the Tilapia aquarium water appeared to be at adequate levels, as growth of both Arthrospira and Spirulina proceeded well. The production of NO_3, NH_4, and PO_4 was followed in certain aquaria at different times during the study. Table 1 shows the gradual increase in all three nutrient levels for two aquaria through the eight day study. When aquarium water was isolated and aerated in a separate flask, the NO_3 levels showed a gradual increase and NH_4 a rapid decrease.

B. Reduction of Plant Nutrients by Algae

Several analyses of the NH_4, NO_3 & PO_4 levels in selected algal cultures were carried out in order to determine if the high concentrations were being utilized by the algae. The averages for NO_3 uptake in Arthrospira was 94%, NH_4 98% and PO_4 48%. Uptake rates of Spirulina were 99% NO_3, 89% NH_4 and 52% PO_4.

C. Growth Studies of Arthrospira and Spirulina

1. Photoperiod: The yield of Arthrospira increased as photoperiod was lenghtened to 24 hours; while biomass production in Spirulina was maximum at a 16:8 photoperiod (Table 2).

2. Light Energy: As light energy levels increased from 0.08 quanta/cm^2/sec, yields were found to be maximum for Arthrospira at 0.80

and 1.20 quanta/cm^2/sec for Spirulina at 34 C.

4. Sodium Bicarbonate Additions: A concentration of 2.5 g/l $NaHCO_3$ produced maximum yields for Arthrospira, while a concentration of 1.25 g/l produced the greatest growth in Spirulina with little growth at levels of 5 and 10 g/l (Table 5).

5. Trace Element Additions: Additions of appropriate levels (0.1-1.0 mg/l) of Fe, Mg, Mn, Cu, Zn, Co and EDTA to the medium (aquarium water) showed no significant increases in yield compared to the control flasks. Data not shown.

6. Nitrate Additions: Table 6 shows that Arthrospira growth decreased as nitrate levels increased, while maximum growth in Spirulina was seen at 150 mg/l NO_3.

7. Ammonia Additions: As NH_4 levels were increased over controls, yields increased for Arthrospira, while growth decreased as NH_4 levels were increased for Spirulina (Table 7).

D. Media Effects on Contamination and Growth

High pH and high NH_4 concentrations demonstrated the best growth and lowest contamination, while low pH and low NH_4 levels showed the highest amounts of contaminations and lowest growth rates (Table 8).

E. Protein Levels in Algal Biomass

When grown under optimal conditions, Arthrospira produced 43-52% of its dry weight as protein with an average of 48%. Spirulina values ranged between 28 and 59% protein for an average of 44% (Table 9).

Table 1. Production of Plant Nutrients in Tilapia Aquaria

Aquarium/Nutrient		Days					
		1	2	3	4	6	8
I	NO_3	4.3	5.1	5.9	7.3	11.2	14.9
	NO_4	9.9	12.3	15.8	18.0	21.9	29.5
	PO_4	0.9	2.0	2.9	3.9	5.1	5.3
II	NO_3	6.8	9.1	10.9	13.4	16.9	19.9
	NH_4	14.9	20.1	27.3	34.5	42.3	50.1
	PO_4	1.1	3.1	5.9	7.3	8.1	9.1
	Water removed from aquarium I & aerated in separate flasks						
	NO_3	14.3	15.8	17.9	18.1	20.1	28.5
	NH_4	23.5	20.0	17.0	14.3	9.1	4.0

Well established 117 liter aquarium systems
Aquarium I: 14 fish (10-15 cm long)
Aquarium II: 15 fish (12-18 cm long)
No makeup water added to aquaria during study

Table 2. Yield of *Arthrospira platensis* & *Spirulina major* at Different Photoperiods

Photoperiods (Light:Dark)	Biomass (mg dry wt./l)	
	Arthrospira	*Spirulina*
(12:12)	409.0	N.D.
(16:8)	501.8	894.5
(20:4)	683.1	585.0
(24:0)	829.3	138.3

7 day growth period
Temperature: 28°C (dark period) to 32°C (light period)
N.D.: no data

Table 3. Yield of Arthrospira platensis & Spirulina major at Various Light Energies

Light Energy 1×10^{16} quanta/cm^2/sec	Biomass (mg dry wt./l) Arthrospira	Spirulina
0.08	320.4	369.0
0.12	321.8	Trace
0.30	302.0	95% Green Algae
0.60	502.1	95% Green Algae
0.80	742.3	299.0
1.00	589.2	366.0
1.20	435.7	627.0
1.80	389.0	433.0

7 day growth period
Temperature 28°C dark to 32°C light period
Photoperiod: 16:8 - Spirulina
24:0 - Arthrospira

Table 4. Yield of *Arthrospira platensis* and *Spirulina major* at Different Temperatures

Temperature °C	Biomass (mg dry wt./l)	
	Arthrospira	*Spirulina*
36	317.3	ND
34	401.8	572.0
32	801.9	347.0
30	598.2	474.0
28	278.3	106.0
26	189.3	311.0

7 day growth period
Photoperiod: 16:8 - *Spirulina*
24:0 - *Arthrospira*

Table 5. Arthrospira platensis and Spirulina major Yield After Sodium Bicarbonate Additions

$NaHCO_3$ grams/liter	Biomass (mg dry wt./l) Arthrospira	Spirulina
Control (No $NaHCO_3$)	328.8	Trace
1.25	521.1	437.0
2.50	634.0	136.0
5.00	598.2	26.0
10.0	473.8	42.0

7 day growth period
Temperature 32°C
Photoperiod: 16:8 - Spirulina
24:0 - Arthrospira

Table 6. The Effects of Nitrate Levels on Arthrospira platensis and Spirulina major

NO_3 mg/L	Biomass (mg dry wt./l) Arthrospira	Spirulina
Control	901.3	319.6
30	608.9	271.5
150	315.0	992.0
300	282.1	334.0
600	208.8	476.3

7 day growth period
Temperature 30°C
Photoperiod: 16:8 - Spirulina
24:0 - Arthrospira

NO_3 levels in controls: <3 mg/l
NH_4 levels in controls: 18.5 mg/l

Table 7. The Effects of Ammonia Levels on Arthrospira platensis and Spirulina major

NH_4 (mg/l)	Biomass (mg dry wt./l) Arthrospira	Spirulina
Control (no additions)	589	742
10	842	534
50	982	381
100	948	203
250	722	no growth

7 day growth period
Temperature 32°C
Photoperiod: 16:8 - Spirulina
24:0 - Arthrospira

NO_3 levels in controls: 5.4 mg/l
NH_4 levels in controls: 2.1 mg/l

Table 8. Media effects on contamination and yield of Arthrospira cultures

Media (NH_4 mg/l)	Contamination	Yield (mg dry wt./l)
low pH 7.0; low NH_4 3.9	high	320
high pH 9.5; low NH_4 4.3	moderate	491
high pH 9.6; high NH_4 30.2	low-absent	694
low pH 7.3; high NH_4 34.8	high	402
very high pH 10.9 high NH_4 39.8	absent	little growth

5 day growth period
Temperature 32°C
Photoperiod: 24:0
pH adjusted up by addition of $NaHCO_3$

Table 9. Protein Analysis of Arthrospira platensis and Spirulina major

Medium Samples	% Protein* Arthrospira	Spirulina
#1	43	47
#2	49	46
#3	50	31
#4	52	43
#5	43	28
#6	50	59
#7	52	55
	$\bar{x}$ 48	$\bar{x}$ 44

7 day growth period
32°C Temperature
Photoperiod: 18:6 - Spirulina
24:0 - Arthrospira
*Total protein as % of dry weight

DISCUSSION

The laboratory studies focused on one half of an integrated algae/fish wastewater mariculture system. The entire process would involve utilizing wastewater containing CO_2 and plant nutrients from Tilapia aquaria as a medium for the production of high quality protein and mineral-rich algal biomass. This biomass would then be fed to the fish. The spent algal medium would also contain high levels of oxygen (during periods of photosynthesis) to be added back to the aquarium. The intent of the investigators was (1) to analyze the growth and protein production levels in the algal biomass, (2) to determine the concentrations of NO_3, NH_4 and PO_4 produced by the Tilapia in the wastewater, and (3) to study the utilization of these nutrients by the algae.

The purpose behind this integration system is basically the conserving of plant nutrients that are repeatedly linked with environmental pollution (eutrophication) (Kromann 1980). The utilization of these nutrients is the basis for a commercial mariculture system with low salinity-tolerant Tilapia, which grow rapidly on protein-rich algal biomass. This system was studied in parts by Murray and Mitsui (1982), Mitsui et al. (1981).

In attempts to provide data for critical evaluation of a small scale laboratory system, we studied certain requirements thought to be important in the success of such an integrated model. The loading of the aquarium with a given number of fish, certain rates

of feeding, and certain volumes of water renewal provided an adequate level of all inorganic compounds known to be required in the growth of the two blue-green algae used in the study. While growth was attained, added levels of either NH_4 or NO_3 were added for optimal yields. Sodium bicarbonate was also an important addition to the aquarium water as a culture medium.

Ammonia levels increased as the fish grew and more feed was used. If aeration was reduced, less NO_3 accumulated in the aquaria; however, when the aquarium water was isolated and aerated well, NH_4 quickly shifted toward NO_3.

Uptake of these nutrients was high, 50-99%, which was identical to the findings for each of the two species studied earlier (Rhyne and Crump 1983, Rhyne 1984). Ammonia was converted to NO_3 under high aeration periods; however, a certain amount of loss to the atmosphere was most likely occurring.

Photoperiod studies demonstrated an interesting contrast between the two species Table 2. Arthrospira increased its yield up to the 24 hour light regime, while Spirulina yield decreased significantly at longer photoperiods and was optimal at 16:8.

The amount of light energy seen to be optimal for Arthrospira was 0.80 and 1.20 quanta/cm^2/ sec for Spirulina, Table 3. Again, the two species had different requirements with Arthrospira showing an optimal growth at a lower energy level.

The temperature study showed somewhat similar preferences between the two species, with Arthrospira growing best at 32 C and Spirulina at 34 C.

Sodium bicarbonate addition studies indicated that 2.5 g/l was optimal for Arthrospira, and 1.25 g/l yielded the best growth

for Spirulina. The compound supplied carbon and increased the pH, two factors that appear to be very important in both increasing growth and helping control contamination. The addition of $NaHCO_3$ appeared to be extremely important to the growth of Spirulina, Table 5.

A large difference in NO_3 utilization for growth was seen in the contrast between the two species. Arthrospira growth was reduced when NO_3 is added at 30 mg/l and above, while at 150 mg/l NO_3 growth was greatly enhanced in Spirulina. When NH_4 was added, the opposite effect is observed. Arthrospira growth was enhanced at 10-50 mg/l, while Spirulina yield is reduced at levels of 10 mg/l NH_4 and above. The same effect of NH_4 versus NO_4 on Arthrospira was also observed in an earlier study (Rhyne 1984).

The combined effects of high NH_4 and pH were seen to be conducive to maintenance of very low contamination of the cultures and increase growth rates. As long as the aquarium water was treated with enough $NaHCO_3$ to raise pH (originally ca. 6.0-7.0) to over 9.0 but below 10.5, the culture grew well. When conditions allowed a lower pH and NH_4 was reduced through normal uptake, contamination increased and growth decreased. Very high levels of both pH and NH_4 had a detrimental effect on growth but contamination was absent. High levels of NH_4 at high pH are known factors in ammonia toxicity to both aquatic plants and animals.

Protein analysis showed a range of 28-59% for Spirulina and 43-52% for Arthrospira. The range was considerably narrower for Arthrospira with slightly more protein being produced than in Spirulina. Several investigators have found various strains of both species to exceed 50% protein (Becker and Venkataramen 1984, Nakamura 1984, Soeder 1980a & 1980b).

In an earlier study (Rhyne 1984) data were shown indicating that certain advantages did lie with an algae/wastewater/mariculture system over that of a domestic-commercial wastewater utilizing system. The disadvantages that would be resolved with a mariculture/wastewater system are: (1) the inherent variability of domestic wastewater chemistry due to wastewater processing out of the control of the investigator, (2) the constant contamination of cultures by small coccoid green algae, if wastewaters are not membrane filtered, (3) the problem of bringing either liquid (seawater or wastewater) to the site of the other for mixing, and (4) the problem of human pathogens associated with farming human wastewaters.

The model as discussed appears to work well, particularly for Arthrospira, in that NH_4 is the nitrogen component produced effectively and quickly from urea breakdown in the aquaria. We have found that our strain of Tilapia could easily live and grow at high NH_4-N levels. The high NH_4 concentrations were detrimental to the growth of Spirulina without the NH_4 being shifted to NO_3 by heavy aeration.

Sodium bicarbonate at only 2.5 g/l increased the yield of Arthrospira over controls. The indirect effect of this addition also helped minimize contamination by raising the pH.

As no study has been carried out looking critically at the use of Tilapia wastewaters as the medium for the production of their own food, we feel that the investigation contributes usable data in the analysis of an integrated fish/algae mariculture system.

SUMMARY

Analysis of data generated by a laboratory-oriented algae/fish/mariculture system demonstrated that Spirulina major and particularly Arthrospira platensis could be grown successfully in the wastewaters of the aquarium-raised cichlid Sarotherodon niloticus (Tilapia).

Nutrient Generation of Fish: Substantial levels of NO_3, NH_4 and PO_4 were produced by the Tilapia in the 118 l aquaria as well as other required elements needed in the growth of both blue-green algae species.

Nutrient Uptake by Algae: Ammonia reductions for Spirulina and Arthrospira were 89-98%, 94-99% for NO_3 and 48-52% for PO_4.

Growth Studies of Algae: After analyzing for optimum conditions for photoperiod, light energy, temperature, $NaHCO_3$, NO_3, NH_4, PO_4 and selected trace elements additions, the greatest yield of Spirulina was 137 mg dry wt./l and 140 mg dry wt./l for Arthrospira in seven days.

Contamination Control: High pH and NH_4 concentrations not only increased growth over controls but also helped control the growth of small coccoid green algae, small ciliated protozoa and fungal hyphae based on the addition of $NaHCO_3$.

Protein Levels in Algal Biomass: Under optimal growing conditions, Spirulina produced 28-59% of its dry weight as protein and Arthrospira 43-52%.

RECOMMENDATIONS

The laboratory data strongly suggest that fish wastewater can serve as the medium for the growth of protein-rich algal biomass and is technically feasible. The feeding of blue-green algae as the entire feed ration to Tilapia and observing good growth and longevity has been shown to be technically possible in the literature.

What is needed is the combining of these integrated components into a small field study. An advantage is that Arthrospira and Tilapia are highly adaptable to low to moderate salinity and high NH_4 levels at relatively high temperatures. These conditions help lend themselves to an integrated system of raising fish protein in semi-tropical to tropical estuarine areas of the world.

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