GROWTH, NUTRIENT UPTAKE AND PROTEIN PRODUCTION IN LABORATORY CULTURES OF ARTHROSPIRA PLATENSIS (CYANOPHYCEAE)

FINAL REPORT

April 1983 through March 1984

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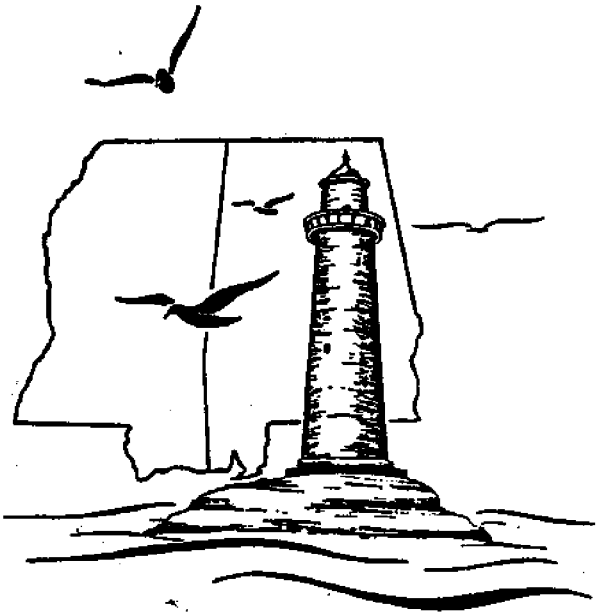
June 1984



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ADMINISTRATIVE SUMMARY

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This final report represents the work carried out under the 1983 project entitled, "Protein Feedstock Production Using an Algae/ Seawater/Wastewater System".

OVERALL OBJECTIVES

The basic aim of this study was to observe and analyze a photosynthetic wastewater bioconversion system utilizing Arthrospira platensis, a bluegreen alga, for the production of single cell protein by achieving the following:

Analysis of various marine blue-green algae found along the Mississippi and Alabama coasts based on growth potential in a wastewater/seawater mixture.

Evaluation of procedures used in maximizing production of protein in the selected alga.

Study of the wastewater treatment relative to NO_3, NH_4 and PO_4 utilized by the algae biomass.

Development of algal culturing techniques is the first step in a program to increase algal biomass yields. As efforts increase in the areas of algal SCP production, culturing technology will play an increasingly important role.

ABSTRACT

Arthrospira platensis, a filamentous blue-green alga, has been shown in our laboratory study to meet certain criteria necessary for its use as a potential protein feedstock in a wastewater/seawater system.

The adaptability of Arthrospira platensis to various percentages of wastewater/seawater mixtures enhances its use in estuarine environments. Optimal growth conditions for A. platensis in the laboratory were demonstrated to be: a temperature of 30° C, salinity range of 5-15 ppt, and a pH range of 8.0-8.5. Cultures were constantly contaminated, but to a lesser degree if NH_4-N levels were kept above 2-3 mg/1; pH above 8.5 and light energy below $0.2 \cdot 10^{16}$ quanta/cm^2/sec. Media was course-filtered but not membrane-filtered.

Maximum biomass productions of 108-138 mg dry wt/1/day were obtained. Protein content of cells ranged between 44% and 49% of dry weight in actively growing cultures.

Under laboratory conditions, reductions of eutrophicating nutrients were approximately 92% for NH_4, 95% for NO_3 and 51% for PO_4.

Contamination was significantly reduced and yields increased slightly when the growth medium was briefly changed from a domestic wastewater/seawater mixture to a tilapia-based culture/seawater medium.

INTRODUCTION

Considerable interest has been shown in single cell protein (SCP) production based on both photosynthetic (algae, bacteria) and nonphotosynthetic microorganisms (algae, bacteria, actinomycetes, yeasts, molds and higher fungi) (Litchfield 1980). Photosynthetic 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 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/hectre/year, 20-30 times the productivity of soybeans (Benemann et al. 1980).

The work with Spirulina and its potential for feed and food pro-

duction 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).

An important aspect of wastewater/algae-produced protein as a feedstock for both indirect and direct consumption by man is that of toxicological safety. Several studies recently have shown that wastewater-grown algae may be toxicologically safe as protein and vitamin supplements in animal feed (Yanni et al. 1978, Payer & Runker 1978, Mokady et al. 1978, Sandbank & Hepher 1978, 1980, Yanni et al. 1980, Becker 1980, Payer et al. 1980). The direct use by man of wastewater raised algae cells is out of the question from a public health standpoint at this time in the U.S. Feeding the algal biomass to trophic levels below man is a possibility (Mokady et al. 1978, Sandbank & Hepher 1980, Lipstein & Hurwitz 1980, Hwang et al. 1980, Walz & Brune 1980, Berend et al. 1980).

The investigator proposed to study the production of a proven feedstock (cyanophycean protein) from an alga grown in seawater/wastewater mixtures. The study was designed to evaluate the growth poten-

tial, nutrient uptake and protein production in the estuarine blue-green alga Arthrospira platensis grown in varying wastewater/seawater combinations.

MATERIALS AND METHODS

EXPERIMENTAL ORGANISM

Arthrospira platensis was purchased from Carolina Biological Supply Co, Burlington, North Carolina. Relative to the discussion on the systematics of Arthrospira and Spriulina, the alga is assumed to be Spirulina platensis Geitler. The planktonic trichomes of A. platensis are helically twisted, ranging in length between 100 and 400 µm. The diameter of the trichome is approximately 2 to 5 µm (Geitler 1932, Smith 1950, Bold & Wynne 1978). Pigmentation, usually light blue-green, as well as detailed filament morphology was variable, depending upon the medium type and light conditions.

STOCK CULTURE TECHNIQUES

The stock seawater supply used in this study was collected in five gallon polyethylene carboys from a pump system at the Gulf Coast Research Laboratory's Oyster Laboratory located in Biloxi, Mississippi (5-20 ppt.) and from Mississippi Sound, 15 miles offshore (30-35 ppt). The wastewater effluent used was collected in carboys from the Municipal Wastewater Treatment Plant (trickling filter system) in Ocean Springs and the Jackson Wastewater Treatment Plant (activated sludge system) Jackson, Mississippi.

Chemical Analysis of Stock Water Supplies

Stock wastewater and seawater supplies were analyzed periodically to monitor the levels of NH_4-N, NO_3-N, PO_4 and Fe. Monitoring was carried out with the use of a reagent system (Bausch and Lomb Spectro-

kit) and a spectrophotometer (B&L Spectronic-20) (Rhyne and Crump 1983). The pH of water stocks 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). Water was course-filtered, then filtered through 0.45 µm membrane filters (Millipore) and stored in 20 l Nalgene containers at 30 C.

Cultures

Stock cultures of Arthospira platensis were maintained in one and two liter Erlenmyer flasks in a medium composed of 20–25 ppt seawater that had been membrane filtered (0.45µm). Stocks were placed in a Percival reach-in environmental chamber model 160LL. Temperature was regulated to 30° C. Photoperiods of 15:9 were provided with the use of 40 watt cool fluorescent tubes. Light energy was measured using a QSL-100 Quantum Scalar Irradiance meter (Biospherical Inc.). The pH of the stock medium was maintained between 8.0 and 8.5. Glass pipettes attached to rubber tubing and connected to a standard aquarium air pump provided aeration to the flasks. Approximately twice a week, half of the liquid volume of each flask was removed and replenished with equal amounts of fresh medium.

EXPERIMENTAL PROCEDURES

Various parameters were used to determine which would provide optimum conditions for growth, viability and protein yield. The size of flasks (Erlenmyer, 250 and 500 ml) and the amount of medium (200 and 400 ml) added to each flask respectively were the only changes from the stock culture techniques.

Algal species tests

Three filamentous blue-green algae were tested in the stock wastewater/seawater medium to investigate their yields relative to the growth

of Arthrospira platensis. Spirulina major was purchased from Carolina Biological Supply Co. and was the same strain used in our earlier study (Rhyne & Crump 1983). Spirulina sp. and Oscillatoria sp. were collected in a brackish water area near the Ocean Springs Wastewater Treatment Plant outfall.

Percentages of wastewater/seawater-Salinity tests

Mixtures of wastewater/seawater were used in growth studies at 5 ppt intervals between 0 and 30 ppt (see table 2 for additional data).

Temperature study

Temperature intervals of 5° C between 20° and 35° C were used to evaluate an optimal temperature (see table 3 for additional data).

Iron concentration study

Iron ($FeCl_3 \cdot 6H_2O$) was added at 0.5, 1.0, 2.0, 4.0, 10 and 20 mg/l levels (see table 4 for additional data).

pH tests

Levels between 7.0 and 10.5 at 0.5 pH intervals were used to test for the optimum pH concentration (see table 5 for additional data).

Nutrient uptake

Samples of the medium were measured spectrophotometrically (B&L Spectrokits) for NH_4, NO_3, PO_4 and iron (see table 6 for additional data).

Growth in different wastewater media

A comparison of growth potential, using the control medium versus a wastewater/NaCl medium, a seawater/fertilizer medium, a fish culture water/seawater medium, and a fish culturewater/NaCl medium was carried out.

A. wastewater/NaCl: 5 g/l NaCl added to Ocean Springs wastewater

B. seawater/fertilizer: "Nutri-leaf" hydroponic fertilizer, Miller Chemical and Fertilizer Co., Hanover, PA (20-20-20 plus trace elements)

was added at the level of 30 mg/l N, P and K.

C. fish culturewater/seawater: water from a 40 liter aquarium holding approximately 150 g. of juvenile (2-4 cm long) tilapia, Sarotherodon niloticus, in 5 ppt seawater.

D. fish culturewater/NaCl: 5 g NaCl added to Jackson tapwater from a 40 liter aquarium holding tilapia (see table 7 for additional data).

Contamination level study

Contamination usually involving motile and nonmotile green algae was studied using the same wastewater media types in the above test (see table 8 for additional data).

Growth in nitrate or ammonia nitrogen media

Wastewaters from Ocean Springs and Jackson generally supplied either high or low nitrate-N or ammonia-N. A 10 g/l NH_4Cl and a 10 mg/l KNO_3 concentration was added separately to a fish culturewater/-seawater medium as a test in evaluating the nitrogen source best utilized by our cultures of Arthrospira platensis (see table 9 for additional data).

Protein levels

Four determinations were made on actively growing cells in the wastewater/seawater media using the method of Kochert (1980b).

Replication

All growth studies, nutrient uptake and other tests were carried out using triplicate flasks unless otherwise indicated.

ANALYTICAL METHODS

A. Extraction of low-molecular weight components

Three samples of cells were collected and centrifuged to yield a packed volume of 0.5 ml. Supernatants were discarded and tubes containing the cells were placed on ice. Ten ml of ice cold 0.2 N $HClO_4$ were added to the pellets and vortexed to resuspend the cells. After

15 min at 4° C, samples were centrifuged in chilled centrifuge tube holders and the supernatant was carefully removed. This procedure was repeated with an additional 10 ml of perchloric acid (Kochert 1980a).

B. Extraction of lipids

Ten ml of chloroform/methanol solution (1:1 v/v) was added to the pellets from the $HClO_4$ extraction and allowed to stand for 5 min at room temperature. Samples then were centrifuged and supernatants discarded. This procedure was repeated with 5 ml of chloroform-methanol (Kochert 1980a).

C. Determination of protein

To the acid-extracted lipid-free pellets one ml of 1 N NaOH was added. The pellets were then placed in a boiling water bath for 10 minutes to dissolve the pellets. Aliquots of the samples were assayed for protein by the dye-binding method of Kochert (1980b).

RESULTS

A. Growth Studies of Selected Blue-Green Algae

As can be seen in Table 1, Arthrospira platensis and Spirulina major demonstrated the best growth in a 5 ppt wastewater/seawater mixture. Other percentages allowed similar or reduced growth for Spirulina sp. and Oscillatoria sp. As Spirulina major had been previously studied by us (Rhyne & Crump 1983), Arthrospira platensis was chosen for the 1983-84 study.

B. Growth Studies of Arthrospira platensis

1. Salinity: Table 2 shows the salinity levels at which growth was greatest. The 5-10 ppt. range shows a higher yield than other concentrations tested.

2. Temperature: A temperature of 30° C was shown to yield a higher biomass than 20°, 25° or 35° C (Table 3).

3. Iron Additions: A range of 1-2 mg/l Fe, as $FeCl_3$, appeared to be optimal concentration for maximum growth as opposed to 0.5, 4.0, 10 and 20 mg/l Fe (Table 4).

4. pH levels: A range of 8.0 to 9.0 appears to be optimal, with testing between 7.0 and 10.0 (Table 5).

5. Growth in various Wastewater Media: Growth of Arthrospira appeared somewhat higher in seawater/fertilzer; fish culturewater/seawater and fish culturewater/NaCl mixtures as opposed to the two domestic wastewater compositions (Table 7).

6. Growth in NO_3 & NH_4 Nitrogen Based Cultures : Growth appears

to be greater when NH_4-N levels were high and NO_3-N levels were low, as shown in two domestic wastewater media types and the fish culture media. This also appears to be true based on the results when additions of NH_4Cl or KNO_3 were made (Table 9).

C. Reduction of Plant Nutrients by Algal Biomass: The algae reduced the levels of both NO_3 and NH_4 by 90% and PO_4 by approximately 50% (Table 6).

D. Protein levels in Algal Biomass: Four determinations based on actively growing Arthrospira in wastewater/seawater media produced values of 44.3, 45.0, 47.1 and 49.4 % of the dry weight.

E. Foreign Algal Contamination: High level contamination was observed in both wastewater cultures, while considerably less was observed in both fish culture media. Contamination was constantly high in wastewater media when water was not membrane filtered (Table 8). Low levels of contamination in the wastewater/seawater could be achieved only if the pH was constantly held above 8.5 and NH_4-N levels kept above 2-3 mg/l. At the same time, light energy levels had to be maintained below $0.2 \cdot 10^{16}$ quanta/cm^2/sec.

Table 1. Growth of Selected Blue-Green Algae in a Wastewater/Seawater Mix*

Species	Yield** (mg dry wt/l)
Arthrospira platensis	689
Spirulina major	632
Spirulina sp.	398
Oscillatoria sp.	417

* Ocean Springs wastewater/25 o/oo seawater from MS Sound= 5 o/oo
** Yield after 5 day growth period, based on $\bar{x}$ of 3 flasks

Table 2. Yield of Arthrospira platensis in Wastewater/Seawater Mixtures

Salinity (ppt)	Yield* (mg dry wt/l)
0 (wastewater only)**	511
5	698
10	670
15	599
20	402
25	288
30 (seawater only)***	103

* Yield after 5 day growth period, based on $\bar{x}$ of four replicate flasks
** Wastewater from Ocean Springs Treatment Plant
*** Seawater from MS Sound

Table 3. Yield of Arthrospira platensis grown at various temperatures

Temperature (C)	Yield* (mg dry wt/l)
20	201
25	485
30	671
35	594

* Yield after 5 day growth period, based on $\bar{x}$ of duplicate flasks

Table 4. Yield of Arthrospira platensis in Iron Enriched Culture Media

Iron (mg/l, $FeCl_3$ $6H_2O$)	Yield* (mg dry wt/l)
0.5	501
1.0	631
2.0	601
4.0	545
10.0	417
20.0	405

* Yield after 5 day growth period, based on $\bar{x}$ of duplicate flasks

Table 5. Yield of Arthrospira platensis grown in different pH levels

pH level*	Yield** (mg dry wt/l)
7.0	312
7.5	398
8.0	503
8.5	540
9.0	412
9.5	303
10.5	40

* adjusted using $NaHCO_3$, wastewater/seawater mix was normally 7.0-7.5

** Yield after 5 day growth period, based on $\bar{x}$ of triplicate flasks

Table 6. Reduction of Plant Nutrients by Arthrospira platensis

	Percent Reduction		
	NH_4	NO_3	PO_4
Test 1*	94	95	50
Test 2**	91	94	53

* Chemical analysis at beginning of test: NH_4 5.8 mg/l; NO_3 57.3 mg/l; PO_4 3.9 mg/l

** NH_4 8.7 mg/l; NO_3 47.1 mg/l; PO_4 4.2 mg/l

Table 7. Yield of Arthrospira platensis in different wastewater media

Media*	Yield** (mg dry wt/l)
Wastewater/Seawater (control)	650
Wastewater/NaCl	685
Seawater/fertilizer***	720
Fish culturewater/seawater****	718
Fish culturewater/NaCl	709

* adjusted to 5 o/oo
** Yield after 5 day growth period, based on $\bar{x}$ of triplicate flasks
*** 30 mg/l each of N, P and K; "Ferti-leaf" commercial hydroponic
**** Water from aquaria containing Tilapia (Sarotherodon niloticus) NH_4 3.5 mg/l; NO_3 54.3 mg/l; PO_4 4.1 mg/l

Table 8. Yield of Arthrospira platensis and contamination level in different wastewater growth media

Media*	Contamination**	Yield*** (mg dry wt/l)
Wastewater/Seawater	high	628
Wastewater/Seawater/NaCl	high	640
Seawater/fertilizer	moderate	734
Fish culturewater/seawater	low	730
Fish culturewater/Seawater	low	720

* adjusted to 5 o/oo (water not membrane filtered)
** Yield after 5 day growth period, based on $\bar{x}$ of triplicate flasks
*** Contamination based on microscopic inspections, low= 5 cells/field moderate= 5-15 cells; high= 15 cells

Table 9. Yield of Arthrospira platensis in Nitrate and Ammonia Nitrogen Based Cultures

Media	Yield* (mg dry wt/l)
Jackson Wastewater/Seawater	
35 mg/l NH_4-N; 0.5 mg/l NO_3-N	701
10 mg/l NH_4-N; 0.15 mg/l NO_3-N	680
1.1 mg/l NH_4-N; 8.5 mg/l NO_3-N	408
0.3 mg/l NH_4-N; 15.5 mg/l NO_3-N	211
Ocean Springs Wastewater/Seawater	
1.1 mg/l NH_4-N; 41 mg/l NO_3-N	351
2.0 mg/l NH_4-N; 65 mg/l NO_3-N	310
10.3 mg/l NH_4-N; 11 mg/l NO_3-N	588
Fish Culture/Seawater	
16 mg/l NH_4-N; 0.4 NO_3-N	670
1 mg/l NH_4-N; 20 mg/l NO_3-N	417
10 mg/l NH_4Cl added to above medium for total of 11 mg/l NH_4-N; 20 mg/l NO_3-N	615
10 mg/l KNO_3 added to second medium above for total of 1 mg/l NH_4; 30 mg/l NO_3	391

* Yield after 5 day growth period, based on $\bar{x}$ of duplicate flasks

DISCUSSION

Oswald (1980) demonstrated algal growth as being a highly economical method of advanced wastewater treatment and the most effective known way to fix solar energy in the form of biomass. The potential of algae to provide abundant food, feed and energy has raised questions concerning harvest methods, predator infestations, contamination, species selection and economy of scale.

There are certain criteria that have to be met in order to realize effective wastewater/mariculture systems. The major requirements are: (1) moderate to high productivity; (2) adaptability to high wastewater levels in seawater; (3) reasonable resistance to contaminating algae; (4) high levels of plant nutrient uptake; (5) be easily harvested and (6) relatively high production of a usable feedstock such as protein. Each of these areas will be discussed in light of the data collected.

(1) Moderate to high productivity

Our results show a maximum of 108-138 mg dry weight/1/ day based on 5 day growth studies using a wastewater/seawater mixture. Yields of 144-147 mg/1/day were seen in a seawater/fertilzer medium, while the fish culturewater/seawater medium supported yields of 144-146 mg/1-/day. The growth rates are similar to those found by us (Rhyne & Crump 1983) for Spriulina major in wastewater/seawater systems. The somewhat higher yields in the fertilizer and fish culturewater-based media show that Arthrospira platensis is capable of greater growth. Growth at these levels corresponds theoretically to approximately 18-24 g/m/day based on a square meter 16 cm (6 inches) deep. Outdoor cultures of

Spirulina have been shown to yield 15-18 g/m^2/day in Thailand, 10-20 g/m^2/day in Mexico (Aaronson 1982); 9-12 g/m^2/day (Seshadri & Thomas 1979), and 8-12 g/m^2/day (Becker & Venkataraman 1984). These published values indicate that Spirulina and Arthrospira are not fast growing species.

(2) Adaptability to high wastewater levels in seawater

A high level of wastewater (ca. 66-85%) corresponding to 5-10 ppt showed a maximum yield for Arthrospira (Table 2). This is most likely due to the alga's preference for low to moderate salinity conditions.

(3) Resistance to contaminating algae

Resistance was low as long as the pH was below 8.5 and NH_4-N levels were less than 2-3 mg/l. Light energy less than $0.2 \cdot 10^{16}$ quanta/cm^2/sec helped reduce the incidence of foreign algae. Small motile and nonmotile green cells became very common when the media were course-filtered only. When the medium was briefly changed to a seawater/fertilizer or fish culturewater/seawater mixture, contamination was greatly reduced to less than 5 and often less than one contaminating cell/field (Table 8). Contamination was not significantly reduced by the use of NaOH, NH_4OH, and UV light treatments without greatly reducing the yield of Arthrospira.

(4) High levels of plant nutrient uptake

High levels of NO_3 and NH_4 usage were observed (Table 6), as 91%+ was removed by the end of two 5 day studies. It is believed that the NH_4-N was utilized by the algae. However, we think that the NO_3-N reduction was aided by loss to elemental N by way of the aeration process. Phosphate showed a 50-53% reduction, presumably due to active cell uptake in the cultures. The 91-94% reduction of NH_4-N by cell

activity demonstrates the usefullness of this alga in ameliorating nitrogen based eutrophication.

(5) Easily harvested

Earlier studies in our laboratory have shown that recovery was approximately 100% efficient using a 75 µm diameter mesh screening and a 90% efficiency rate using a 120 µm mesh. The relatively large size of Spirulina and Arthrospira has been commented on by others (Becker & Venkataraman 1984) as being an advantage over smaller algal cells such as Chlorella and Scenedesmus. Microscreening is a significantly cheaper method of harvesting as opposed to filtering and centrifugation.

(6) Relatively high production of usable feedstocks (protein)

Protein determinations show values of 44.3, 45.0, 47.1 and 49.4 % of dry weight. These levels are somewhat higher than those found in commercial tropical fish food formulations and considerably higher than commercial catfish chow meals. As amino acid profiles were not performed, we do not know the quality of this particular SCP source. Depending upon the culture history, water quality and strain of Spirulina, methionine may well be slightly deficient in this alga as brought out by Becker & Venkataraman (1984).

Various other parameters were studied such as temperature, iron additions, pH and nitrogen sources. One ml/l $FeCl_3$, a pH of 8.5 and a temperature of 30° C were the optimum levels for these parameters. These three findings are consistent with our data on Spirulina major (Rhyne & Crump 1983). Ammonia-N and not nitrate-N was (Table 9) the preferred nitrogen source in this alga. The opposite was true for our findings with Spirulina major.

Several disadvantages of the wastewater/seawater media led us

to the brief testing of the seawater/ fertilizer and fish culturewater-/seawater media: 1. the inherent variability of domestic wastewater chemistry due to the wastewater plant processing, out of the control of the P.I. Ammonia levels fluctuated considerably creating lower or cessation of algal growth; 2. the constant contamination of the cultures by very small (3-12μm) diameter spherical green cells, when water was not membrane-filtered. Membrane filtering is essentially out of the question under conditions of larger water volumes. Both of these negative qualities will create difficulties when monocultures of algae are being grown under high rate and nutrient conditions; 3. the problem of bringing either liquid to the site of the other for mixing. This logistic problem creates a considerable loss of economic feasibility. It would appear that an algae/fish/mariculture system holds some potential as a wastewater system that meets the necessary criteria for high protein algal biomass production.

PROJECT SUMMARY

Analysis of data generated by the laboratory-oriented culture system demonstrated that Arthrospira platensis could meet most criteria needed for a successful wastewater/seawater system.

Adaptability to wastewater/seawater concentrations: Arthrospira platensis grew well at levels up to 66-85% wastewater in seawater. Iron as $FeCl_3$ was needed in the wastewater media for continued high yields. Ammonia-N, and not nitrate-N, was found to be the preferred nitrogen source.

Biomass Production: Up to 140 mg/l/day dry weight was observed during 5 day tests. This is theoretically equivalent to 23 g/m^2/day in a system 16 cm deep.

Protein Production: Yields up to 49% of the dry weight were obtained in actively growing cells.

Harvesting: Greater than 90% efficiency has been observed in microscreening Arthrospira platensis through a 120 μ standard mesh screen.

Resistance to Contamination: Little resistance to contamination was observed. Lesser amounts of contamination were seen as long as the pH was above 8.5, NH_4-N was above 2-3 mg/l and light energy was below $0.2 \cdot 10^{16}$ quanta/cm^2/sec.

Nutrient Uptake: After five day experiments, 91-95% of both NH_4 and NO_3-N and 50-53% of the PO_4 were calculated to have left the culture system due to both algal activity and denitrification.

Biomass production increased slightly when the growing medium was changed to a tilapia-based culture medium allowing up to 147 mg/l/day. More importantly, contamination was reduced significantly.

RECOMMENDATIONS

Arthrospira platensis shows some potential in the combined efforts of ameliorating plant nutrient levels in wastewater/seawater mixtures and producing a high level of single cell protein. However, a large scale field operation consisting of mixing seawater and wastewater would not be a feasible approach in raising Arthrospira for production of protein. To date, the variability of essential nutrients such as ammonia in municipal wastewaters is large creating unstable growing conditions.

The investigator thinks that the production of Arthrospira platensis will have to be increased substantially from the present 140 mg/l/day to at least 0.5 to 1.0 g/l/day before serious thought can be given to scaling up this system. Contamination will also have to be controlled at levels more conducive to monoculture production. Increased biomass production may have to come from high growth rate strains either found naturally or bioengineered. Smaller aquaculture systems, perhaps 50-100,000 l/day based on wastewater from a mariculture system such as the one briefly studied here, might well lead toward a more feasible and productive system.

Development of algal culturing techniques is the first step in a program to increase algal biomass yields. As efforts increase in the areas of algal single cell protein production, culturing technology will play an increasingly important role.

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