Production of *Spirulina platensis* from Growth Media Containing Anaerobically Digested Cattle Waste

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ABSTRACT

The marine microalga, *Spirulina platensis*, was cultured in growth media that included various concentrations of digested cattle waste effluent from a pilot anaerobic digester system. The anaerobically digested cattle waste was diluted with either a synthetic sea salt solution or a diluted oilfield brine solution and culture growth rates were monitored over time. Media treated with digested cattle waste were significantly superior for *Spirulina* growth rate (with yields up to 785 mg dry weight L^{-1} d^{-1}) as compared to the control treatment replicates (with yields up to 235 mg dry weight L^{-1} d^{-1}), which contained no cattle waste. We also demonstrated that *Spirulina* could be cultured in diluted oilfield brine. However, the amount of contaminant algae rose as the concentration of anaerobically digested cattle waste increased in the growth medium.

KEYWORDS: growth rate, Instant Ocean, oilfield brine

The use of animal wastes to culture microalgae is particularly desirable in areas such as the High Plains of West Texas where intensive production of cattle is accompanied by the accumulation of large amounts of wastes. For cattle feedlots and dairies, government regulations have become stricter and now are at a zero discharge level. Failure to comply with these regulations has led to many producers receiving fines from the Environmental Protection Agency (EPA). Mitchell and Richmond (1988) successfully grew *Spirulina platensis* in media prepared from Zarouk medium treated with various concentrations of leachate of raw cattle waste. Pieterse et al. (1982) produced an average of 10 g m^{-2} d^{-1} dried algal material in an

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integrated system treating wastes from a cattle feedlot in South Africa. Pal and Amla (1992) have reported the need for additional nitrogen input when *S. platensis* was produced in a sewage-based media. *Spirulina* culture also required a source of alkaline brine for maximum protection. Some algae farms located near oceans have used filtered sea water to dilute animal wastes. However, inland locations such as the High Plains of West Texas must identify a source of saline water abundant enough and suitable for culturing marine microalgae. Potential sources of saline water are ground water that is high in salinity, and thus not suitable for conventional agriculture, and oilfield brine that can be diluted to the desired salinity with fresh water.

Many systems have been designed for the production of algal biomass, ranging from highly automated operations designed to culture a specific microalga, using a completely defined inorganic chemical medium, to open, outdoor ponds that commonly use some form of organic waste to produce a less specific product (Lee et al., 1992; Radmer and Parker, 1994). This waste grown product has been referred to as "albazoid" (algae, bacteria, zooplankton and detritus) to accurately describe its composition (Richmond, 1986; Soeder, 1986). The culture of microalgae from inorganic chemical media and CO_2 yields a practically monoalgal product of food grade quality that commands a relatively high price. The purchase of nutrients and CO_2 account for as much as 30-40% of the operations cost for food grade microalgal culture (Shelef et al., 1978). Obviously, inclusion of the food grade product in animal feeds would not be cost effective. However, culture of algal biomass from organic nutrients such as cattle (Kilani, 1992; Pieterse et al., 1982), swine (Canizares and Dominguez, 1993; Canizares et al., 1993) or poultry waste may provide a high protein product suitable for use in animal feeds due to lower production costs. This study concentrated on developing growth media for production of an algal culture dominated by *S. platensis*, using resources locally available in West Texas, when possible, for future application in outdoor production ponds.

MATERIALS AND METHODS

Anaerobically Digested Cattle Waste

All cattle waste used in this study was collected from Lubbock Feedlots, Inc., Lubbock, Texas. A pilot digester system was constructed to prepare anaerobically digested cattle waste (ADCW) for research purposes. The system consisted of four 500-liter fiberglass silos to which 30 liters of cattle waste diluted to 3% volatile solids was added daily to yield a 17-day retention time. The silos were sealed and the liquid agitated by recirculating methane produced as a product of microbial digestion to suspend solids in the digester. The effluent was centrifuged at 1500 x gravity to reduce suspended solids and frozen for storage.

Culture Conditions

Experiments were inoculated from a laboratory stock *S. platensis* culture. This culture was dominated by *S. platensis* but also include some *Oscilatoria* spp., a filamentous blue-green alga. We will refer to cultures as *Spirulina* cultures, even

though they were not monoalgal. The laboratory culture was maintained under semi-continuous culture conditions to maintain the cells in an exponential growth phase. This was accomplished by removing a portion of the culture daily and replacing it with an equal amount of fresh medium, while keeping the culture at about 30-35°C under a fluorescent light source.

Replicate cultures were grown in 500-mL Erlenmeyer flasks plugged with cotton to protect cultures from airborne contaminants. Each replicate was maintained at a volume of 200 mL. The flasks were placed on a rotary shaker at 120 revolutions min^{-1} and illuminated at a light intensity of about 0.756 lumen cm^{-1} by fluorescent light in a white light chamber. Light intensity was measured with a Simpson (Elgin, Illinois) model 408 illumination level meter. A diel cycle (16 hours light to 8 hours dark) was maintained by an automatic timer and light chamber temperature ranged from 25-32°C.

Experimental Design

Experiments consisted of four replicates in each treatment category in a completely randomized design (CRD). Each replicate started as 175 mL of the appropriate growth medium and 25 ml from the laboratory stock culture as an inoculum. The 25-mL inoculum in a total volume of 200 mL yielded a starting culture density of 12.5% of the dry weight of the stock culture for each replicate. The cultures were allowed a 48-hour initial growth interval with no sampling occurring.

Fifty milliliters were removed from each replicate for evaluation after 48, 72, 96, 120 and 144 hours. Fresh medium was added to replace the medium that was removed for evaluation and the replicates were placed back on the shaker table. Parameters measured were pH, temperature, and dry weight per volume (mg L^{-1}) of biomass. The pH and temperature were measured with a Cole Parmer (Chicago, Illinois) Model 5986-60 pH and temperature meter. Dry weight was determined by filtering the cells from 25 mL of culture medium through a 0.45-μm membrane filter in a Whatman glass fritted vacuum filtration apparatus. The filters were dried at 105°C overnight and then weighed. Dry weight in milligrams per liter was used to determine growth rate of algae for each replicate as a measure of growth for each 24-hour period.

The daily dry weight and growth rate values were analyzed by analysis of variance (ANOVA) and a Fisher's protected least significant difference (LSD) means separation test to determine significant differences ($P < 0.05$). Also, a standard hemocytometer was used to determine the amount of single celled contaminant algae present in each treatment after 144 hours.

Synthetic Sea Salts

This experiment was conducted to determine the optimum concentration of ADCW in a synthetic sea salt (Instant Ocean; Sarrebourg, France) solution. The experiment consisted of four replicates in each of five treatment categories. All replicates contained 1 g urea L^{-1} , 0.10 g K_2HPO_4 L^{-1} and 6 g $NaHCO_3$ L^{-1} to insure that nitrogen (N), phosphorus (P) and carbon (C) would not be limiting factors for culture of *Spirulina*. Additionally, each replicate contained 8 g synthetic sea salt L^{-1} to yield a salinity, as total dissolved solids, of 15 g L^{-1}. The five experimental treatments were 0 (control), 12.5, 25, 50 and 100% ADCW (% of total volume).

The remainder of the medium was composed of water purified by a 1-μm sediment filter, a 1-μm carbon filter and a 0.05-μm membrane filter. Starting pH was 8.37 for the control replicates, 8.35 for the 12.5% and the 25% ADCW replicates, and 8.33 for the 50% and the 100% ADCW replicates. Stock culture density was 465 mg L^{-1} and 25 mL was used as the starting inoculum. Therefore, the starting culture density was 58 mg L^{-1} for each 200-mL replicate.

Oilfield Brine

This experiment was conducted to determine the feasibility of a *Spirulina* growth medium consisting of diluted oilfield brine and ADCW. The diluted oilfield brine solution consisted of 80% purified fresh water, 10% ADCW, 10% oilfield brine (175 g total dissolved solids L^{-1}) and 100 mg L^{-1} IGP-HCB6 (Industrial Grain Products; Lubbock, Texas), a commercially available product containing petroleum degrading bacteria. The pH was 7.84 and salinity was 20 g L^{-1}. The cattle waste present in the solution served as a nutrient source for the bacteria that were included to degrade residual hydrocarbons present in the brine. The solution was placed on a magnetic stirrer and was aerated at a temperature of 28.5-30.6°C for 48 hours. The solution was then treated with 4 g Na_2CO_3 L^{-1} , which immediately caused the solution to turn milky and flocculate heavily. The suspended, flocculated solids were precipitated with 80 mg $Al_2(SO_4)_3$ L^{-1}, which resulted in a clear, straw-tinted solution with a pH of 7.39 and salinity of 18 g L^{-1}. This solution was treated with an additional 4 g Na_2CO_3 L^{-1} and 80 mg $Al_2(SO_4)_3$ L^{-1} to repeat the flocculation, precipitation process, resulting in a pH of 9.82 and a salinity of 19 g L^{-1}. The solution was filtered through a 0.45-μm membrane filter and stored in a glass aspirator bottle. A growth experiment was performed with this solution serving as the diluent for ADCW. Treatment levels were 0% ADCW (control) and 25% ADCW with the remainder of the growth medium containing diluted oilfield brine solution. There were four replicates for both treatment levels. The treatments were supplemented with urea at 1 g L^{-1}, 0.10 g K_2HPO_4 L^{-1}, and 6g $NaHCO_3$ L^{-1}. Starting pH was 8.87 for the control replicates and 8.88 for the 25% ADCW replicates. Stock culture density was 500 mg L^{-1} and 25 mL was used as the starting inoculum. Therefore, the starting culture density was 62.5 mg L^{-1} for each 200-mL replicate.

RESULTS

Synthetic Sea Salts

After an initial 48-hour growth period, all treatments prepared with synthetic sea salts and ADCW had developed into rapidly growing cultures. Culture dry weight increased as concentration of ADCW increased. There was some decline in daily growth rates in all treatments. However, all treatments, except for the 100% ADCW replicates were growing at a high rate by the completion of the experiment. After 144 hours of culture, all treatments that included ADCW had significantly higher final daily growth rates and dry weights than the control. During the 144-hour period, temperature and pH did not vary with treatment. Treatments, three, four and five all had final dry weights in excess of 1,000 mg L^{-1}. However, growth rate is ultimately the deciding factor for treatment performance. The 25% ADCW

and 50% ADCW treatments had significantly higher final growth rates than all the other treatments (Figure 1). Contaminant load (unicellular algae mL^{-1}) after 144 hours of culture were, 6.6×10^5 for the control, 8.7×10^5 for the 12.5% ADCW treatment, 1.6×10^6 for the 25% ADCW treatment, 2.2×10^6 for the 50% ADCW treatment and 3.9×10^6 for the 100% ADCW treatment.

Diluted Oilfield Brine

Spirulina in control and ADCW treated cultures grew quickly and by the 48-hour point had treatment mean dry weights in excess of 300 mg L^{-1} (Figure 2). Additionally, both peaked for mean daily growth rate after about 72 hours. However, both control and ADCW treated replicates showed a steady increase in growth rate after an initial drop following the growth rate peak. The ADCW treated replicates had a significantly higher final dry weight upon completion of the experiment. Contaminant load (unicellular algae per milliliter) after 144 hours, as determined by a standard hemocytometer count on a pooled treatment sample, was 0 for the control and 4.8×10^4 for the treated culture. All growth media used were supplemented for P with 0.10 g K_2HPO_4 L^{-1}. Above this level, formation of a white flocculant occurred in media formulated with synthetic sea salt. No cultures showed signs of P-limited growth, such as change in trichome size or shape.

DISCUSSION

Nutrient Balance

Ideally, the sole limiting factor for an algal culture should be solar irradiance. However, this is seldom the case in any algal culture, since as the biomass increases and is removed from the pond, the macro- and micro-elements required for optimum growth are present in diminished quantities. Therefore, large outdoor cultures should be monitored constantly. Richmond (1986) used the level of nitrogen as a guideline for adding, in equivalent amounts, the entire formula of the growth medium. This is a relatively easy method to maintain a nutrient balance in a food grade algal culture with a defined inorganic medium. However, when animal waste is used to increase the nutrient level, the concomitant increase in organic matter (particulate and dissolved) serves to increase contamination by undesirable biota such as heterotrophic unicellular algae (i.e., *Chlorella spp.*) and their predators (i.e., cladocerans, ciliates, rotifers, etc.). This results in a complicated food chain that leads to either a decrease in the output of the desired species or total loss of the *Spirulina* culture. For a medium that contains all required macro- and micro-nutrients, there is no significant difference between mean daily growth rate for the 25% ADCW treatment and the 50% ADCW treatment even though the 25% treatment had a much lower contaminant load. The treatment with the lower contaminant load should contain more *Spirulina* biomass per unit volume and thus be a more valuable product. To maintain a cattle waste based culture dominated by *Spirulina*, it is important to add organic and inorganic nutrients to supply N, P, and C while keeping the concentration of organic waste as low as possible.

The maximum daily average growth rate of 785 mg L^{-1} is the equivalent of 785 g dry weight m^{-3}. In a commercial production pond of 0.3-m depth, this

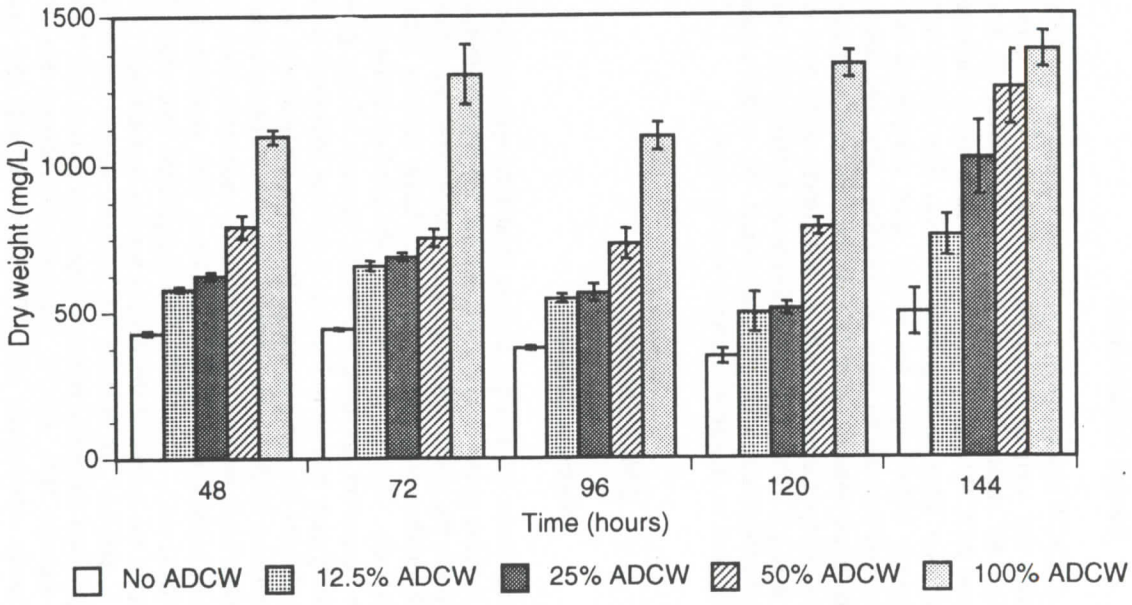

Figure 1. Mean dry weight and standard error (mg L^{-1}, n=4) of *Spirulina* after 48, 72, 96, 120, and 144 hours of culture in growth media formulated with synthetic sea salts and 0, 12.5, 25, 50, and 100% anaerobically digested cattle waste (ADCW). For ADCW of 0%, pH was 9.11-9.15, and temperature was 31.5-32.3°C; for 12.5% ADCW, pH was 9.02-9.22, and temperature was 32.4-32.9°C; for 25% ADCW, pH was 8.92-9.12, and temperature was 33.0-33.4°C; for 50% ADCW, pH was 8.86-8.99, and temperature was 32.2-33.3°C; for 100% ADCW, pH was 8.76-8.87, and temperature was 32.2-34.4°C.

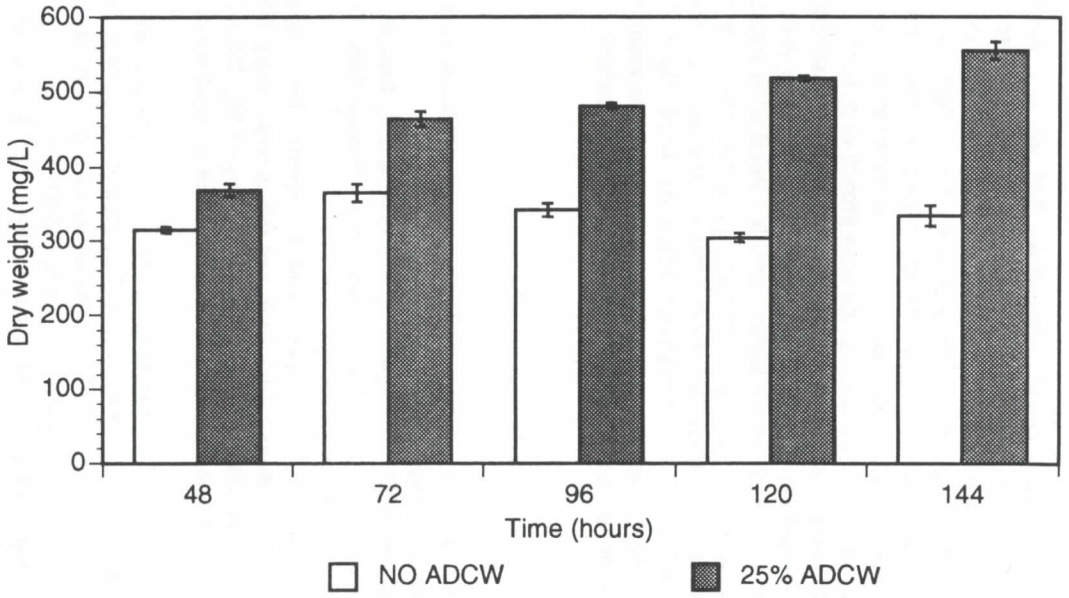

Figure 2. Mean dry weight and standard error (mg L^{-1}, n=4) of *Spirulina* after 48, 72, 96, 120, and 144 hours of culture in growth media formulated with diluted oilfield brine and 0, and 25% anaerobically digested cattle waste (ADCW). For ADCW of 0%, pH was 9.34-9.41, and temperature was 31.3-35.9°C; for 25% ADCW, pH was 9.35-9.47, and temperature was 31.8-36.4°C.

concentration of *Spirulina* would be the equivalent of 235.5 g m^{-2}--well beyond the typical 10-20 g m^{-2} and the extraordinary 54 g m^{-2} achieved by some commercial producers (Richmond & Becker 1986). It is unknown if similar rates can be achieved in large scale production, but at least we know that the biological potential exists if contaminant algae can be controlled.

Recommendations

As the density of a *Spirulina* culture rises above 500 mg dry weight L^{-1}, photosynthetic efficiency is diminished. Therefore, instead of removing a fixed amount of the culture and replacing with fresh medium daily, we recommend that the culture be diluted daily to maintain a culture concentration of 500 mg L^{-1}. Dry weight should be measured before dilution, with the output or growth rate being equivalent to the dry weight in milligrams per liter minus the original 500 mg L^{-1}. This modification in procedure would allow continuous growth of *Spirulina* without the adverse effects of crowding and shading caused by extremely dense cultures.

Outdoor culture of *Spirulina* in diluted oilfield brine will be necessary to determine if *Spirulina* can indeed be cultured in the highly variable natural environment of an outdoor pond and to produce quantities great enough to prepare experimental diets. Laboratory cultures have some protection from gross contamination. However, outdoor cultures are subjected to a continuous attack by weed algae, such as *Chlorella spp*. Therefore, we recommend that outdoor cultures be established to develop management techniques to maintain a dominant *Spirulina* culture.

REFERENCES

Canizares, R.O., and A.R. Dominguez. 1993. Growth of *Spirulina maxima* on swine wastes. BioresourceTechnology 45:73-75.

Canizares, R.O., L. Rivas, A.R. Dominguez, L. Travieso, and F. Benitez. 1993. Aerated swine-wastewater treatment with K-carrageenan-immobilized *Spirulina maxima*. Bioresource Technology 47:89-91.

Fedler, C.B., N.C. Parker, H.L. Schramm, and J. Borelli. 1991. Integrated production of algal protein, omega-3 fatty acids, and fish in west Texas. US Dept. of Commerce, Economic Development Administration. Project No. 08-06-02714.

Kilani, J.S. 1992. Studies on the treatment of dairy wastes in an algal pond. Water SA 18:57-62.

Lea, S.I., B. Koopman, and E.P. Lincoln. 1992. Effect of physiochemical variables on algal autoflotation. Water Science Technology 26:1769-1778.

Mitchell S.A., and A. Richmond. 1988. Optimization of a growth medium for *Spirulina* based on cattle waste. Biological Wastes 25:41-50.

Pal, D., and D.V. Amla. 1992. Commercial fertilizer N:P:K and alternative nitrogen sources for cultivation of Spirulina platensis in sewage. Cryptogamie 13:247-255.

Pieterse, A.J.H., J.L. Roux, and D.F. Toerien. 1982. The cultivation of algae using waste water from feedlots. Water SA 8:202-207.

Radmer, R.J., and B.C. Parker. 1994. Commercial applications of algae: opportunities and constraints. Journal of Applied Phycology 6:93-98.

Richmond, A. 1986. Outdoor mass cultures of microalgae. p. 285-329. In A.

Richmond (ed.), Handbook of Microalgal Mass Culture. CRC Press, Boca Raton, FL.

Richmond, A., and E.W. Becker. 1986. Technological aspects of mass cultivation --a general outline. p. 245-263. In A. Richmond (ed.) Handbook of Microalgal Mass Culture. CRC Press, Boca Raton, FL.

Shelef, F., G. Oron, and R. Moraine. 1978. Economic aspects of microalgae production from sewage. Arch. Hydrobiol. Beih, Ergeben, Limnol. 11:281-294.

Soeder, C.J. 1986. A historical outline of applied algology. p. 25-41. In A. Richmond (ed.) Handbook of Microalgal Mass Culture. CRC Press, Boca Raton, FL.