



CYANOBACTERIAL PROCESS FOR RENOVATING DAIRY WASTEWATER

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Abstract—Dairy operations in Florida face the dual problem of water pollution and air pollution (odors) as a result of the large amounts of manure produced on the farms. Ground and surface waters are contaminated by nitrogen present in seepage and runoff. A low-cost method of treatment of dairy wastewater is to convert the dissolved nutrients to microalgae biomass in engineered ponds designed to maximize photosynthetic production through solar energy. Laboratory experiments conducted on effluent from an anaerobic lagoon of a modern dairy showed that cyanobacteria (= blue-green algae) grow well on dairy wastewater and that nitrogen removal is rapid and complete. Ammonia nitrogen concentrations were reduced from 100 mg l^{-1} to less than 1 mg l^{-1} in seven days. Maximum removal rate was 24 mg l^{-1} per day. Prospects for nitrogen recycling are considered. Copyright © 1996 Elsevier Science Ltd.

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1. INTRODUCTION

Various algal species have been used successfully in the treatment of both municipal sewage and effluent from livestock operations. Such wastewater contains substantial amounts of dissolved nitrogen and phosphorus which can be converted to algal protoplasm in the presence of CO_2 and visible light. Upon removal of the algae by harvesting the cells, the remaining water has thus been renovated through bioremediation. The extent of treatment is proportional to the amount of algal growth and degree of cell removal.

Wastewater treatment with algae has been demonstrated in mass outdoor cultures for municipal sewage and swine manure slurries¹⁻⁴ but not for cattle feedlot or dairy wastes. Filip and Middlebrooks^{5,6} found evidence of an undefined toxic factor inhibitory to algal growth in feedlot run-off and dairy cattle wastes. Shelef *et al.*⁷ and Mitchell and Richmond⁸ succeeded in growing cyanobacteria (also known as blue-green algae) on cattle manure at bench scale and subsequently in small outdoor systems. To test the extent to which cyanobacteria can be grown on the manure of cattle (*Bos taurus*) a combination of laboratory- and pilot-scale cultures

were investigated at the University of Florida. The results of four laboratory experiments are reported here.

2. MATERIAL AND METHODS

The algal medium used here consisted of effluent from the second-stage anaerobic lagoon of a 350-head dairy of the Dairy Research Unit (DRU) at the University of Florida, Gainesville. The null hypothesis was that the selected cyanobacterium, *Arthrospira platensis*, would not grow well on this medium in the laboratory under conditions otherwise conducive to algal growth. As this alga preferentially uses CO_3^{2-} as a carbon source, a secondary objective was to define the range of concentrations of sodium bicarbonate (NaHCO_3) that best served as a carbon source for *Arthrospira*.

Eight 1 l Erlenmeyer flasks were used in the first experiment. The flasks were divided into four duplicate pairs, each pair receiving a different treatment (Table 1). Since the lagoon effluent had an $\text{NH}_3\text{-N}$ concentration of some 120 mg l^{-1} , close to the toxic level for most algae, it was diluted to half this strength with distilled water for the first three treatments. These were flasks 1 and 2 which received 1% bicarbonate, by weight (treatment A), 3 and 4 which received 0.5% bicarb (treatment B) and

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Table 1. Media composition of experiment 1

Flask no.	Treatment	Effluent:distilled H ₂ O	%NaHCO ₃
1	A	50:50	0.0
2	A	50:50	0.0
3	B	50:50	0.5
4	B	50:50	0.5
5	C	50:50	1.0
6	C	50:50	1.0
7	D	100:0	0.5
8	D	100:0	0.5

the control flasks, 5 and 6, which received 0% (treatment C).

Since the question of toxic ammonia levels in dairy wastewater was to be addressed, a fourth pair of flasks (7 and 8) was set up using full strength lagoon effluent (treatment D) and 0.5% NaHCO₃. Earlier work by Lincoln⁹⁻¹¹ has shown that 0.5% lies within the preferred range of NaHCO₃ concentration for *Arthrospira* on swine waste media.

All eight flasks were filled to 720 ml with their respective media and inoculated with 80 ml of algal culture medium having an *Arthrospira* concentration of 600 mg l⁻¹ dry weight, bringing the inoculum to 10% of the final volume. The flasks were placed at 5 cm distance from a bank of four 34 W fluorescent bulbs and kept under continuous lighting. Temperatures were ambient outdoor levels, ranging from 15° to 25°C. The cultures were mixed gently by continuous aeration. Duration of the experiment was ten days. Media compositions are recorded in Table 1.

Parameters measured on a daily basis were temperature, conductivity, pH, dissolved oxygen, and culture density by direct cell count by microscope and by absorbance at 750 nm. Absorbance readings were adjusted by subtracting those of the uninoculated control to correct for pre-existing, non-algal biomass. Color was judged subjectively and recorded daily, or when a change occurred. Chemical parameters measured at the start and finish of the experiment were chemical oxygen demand (COD), ammonia nitrogen (NH₃-N), nitrate nitrogen (NO₃-N), total Kjeldahl nitrogen (TKN), total phosphorus (TP), and soluble reactive phosphorus (SRP). Ammonia nitrogen concentrations were determined with an Orion #9512 ammonia probe connected to a Fisher model 825 multipurpose meter.

The second experiment was again conducted with eight 1 l flasks filled to 720 ml with half strength effluent from the same lagoon and an

inoculum of 80 ml of algae culture medium having a dry weight density of 600 mg l⁻¹. The independent variable was NaHCO₃ concentration which ranged from 0.6% to 1.2% by weight. The intention was to find the concentration of bicarbonate that produced the densest growth of *Arthrospira* in the shortest time. Flasks 1 and 2 received 1.2% NaHCO₃; 3 and 4, 1.0%; 5 and 6, 0.8%; 7 and 8, 0.6%. All were stirred lightly by aeration and kept under 24 h illumination. The experiment was terminated after seven days.

In order to determine the daily rate of NH₃ removal in the presence and absence of algae, a third experiment was initiated using six 1 l flasks incorporating three duplicate treatments. All were supplemented with 0.75% NaHCO₃. The first treatment, flasks 1 and 2, was full strength lagoon effluent without algae inoculation. The second treatment, flasks 3 and 4, was the same substrate but with an inoculation of 10% algae from an *Arthrospira* culture having a concentration of 3.5 g l⁻¹ (dry weight). The final 825 ml of medium had an initial density of 350 mg l⁻¹ (algal dry weight). This substantial inoculation was designed to shorten the lag time. The third treatment (flasks 5 and 6) was an inorganic control containing 170 mg l⁻¹ (0.01M) NH₃-N as ammonium chloride and neither lagoon effluent nor algae. The null hypothesis in this experiment was that all three treatments would have identical rates of ammonia removal from their respective media.

The fourth and final experiment of this series was designed to eliminate the effect of air sparging on lowering ammonia concentrations, an unquantified variable in previous experiments. A shaker table oscillating at 120 rpm was used to stir the medium, replacing the aeration used previously. To increase sample size, nine flasks were used, three for each treatment. The first treatment was full strength lagoon effluent (flasks 1, 2 and 3) without algal inoculant. The second treatment (flasks 3, 4 and

5) were the same substrate inoculated with *Arthrospira* to a dry weight algal concentration of 250 mg l^{-1} . The third and last treatment (flasks 7, 8 and 9) was an inorganic control identical to that used in experiment three. The null hypothesis was again that all three treatments would have the same rate of NH_3 removal.

3. RESULTS

All treatments in the first two experiments produced significant algal growth. In experiment 1, the control flasks, in which the bicarbonate concentration was 0% produced a bright green population of *Chlorella*, a chlorophyte, rather than the blue-green *Arthrospira* which dominated the rest of the experimental cultures. This was not an unexpected result since *Chlorella* is ubiquitous in lagoons in Florida and is faster growing than the blue-greens at low bicarbonate concentrations. The fact that all treatments showed substantial algal growth disproves the null hypothesis and indicates that cattle manure suspensions are not inherently toxic to algae. The fact that algistats or algicides may exist in cattle wastes is not an unfounded premise, since dense algal blooms are seldom seen in the on-farm wastewater of cattle operations. In cases where such algal blooms do occur, they tend to be dominated by *Microcystis*, a widely occurring genus of blue-green algae.

In the first experiment, the 1% NaHCO_3 treatment had the fastest growth and by day four had undergone a doubling of cell number and developed a deep green color. The pair of cultures that received full strength lagoon effluent in experiment 1 (flasks 7 and 8, with 0.5% bicarbonate) showed a lag time of 8 days over the other treatments before greening fully. It appears that sublethal ammonia concentrations inhibited algal growth at first, but this was gradually sparged off by aeration and rapid growth followed. By termination on day 10, cell counts indicated that *Arthrospira* had become equally as dense as the other algal populations.

Chemical parameters measured before and after experiment 1 showed a dramatic fall-off in nutrient concentrations. Ammonia nitrogen in the untreated lagoon effluent at the beginning of the experiment amounted to 130.9 mg l^{-1} . The final mean value of $\text{NH}_3\text{-N}$ after treatment A was 2.45 mg l^{-1} for flasks 1 and 2, 3.15 for flasks 3 and 4 (treatment B), 1.75 for flasks 5 and 6

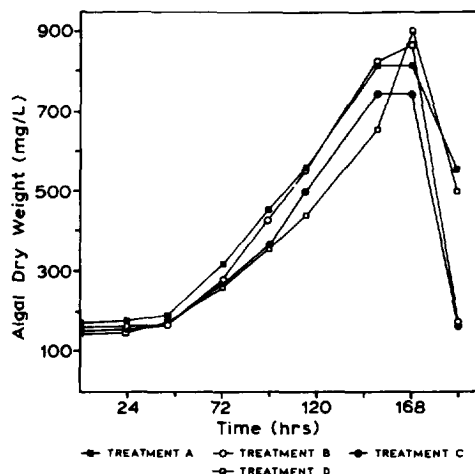


Fig. 1. Growth of *Arthrospira* at concentrations of sodium bicarbonate ranging from 0.6% (A) to 1.2% (D) in steps of 0.2%. No significant difference existed between these treatments.

(C), and 2.24 for flasks 7 and 8 (D). The latter had received full strength rather than half strength medium at the start of the experiment. Nitrate nitrogen, which was present in amounts of less than 2 mg l^{-1} in the raw wastewater, was hardly detectable after treatment.

The second experiment was terminated on day eight, after culture densities had peaked (Fig. 1). While there was considerable variation in final absorbance, treatments having the highest and lowest bicarbonate concentrations showed little difference in algal density. In fact there was no correlation between final optical density and concentration of NaHCO_3 . Since concentrations ranged from 0.6% to 1.2% the premise that an optimum bicarbonate level lies within this interval is not supported.

Chemical data from the second experiment (Table 2) are similar to those of the first experiment. The initial ammonia nitrogen concentration of the half strength lagoon effluent was 45 mg l^{-1} . Treatment one, the two flasks having an NaHCO_3 concentration of 0.6%, contained an average of 2.1 mg l^{-1} ammonia nitrogen at the end of experiment two. The second treatment, flasks 3 and 4, had a final $\text{NH}_3\text{-N}$ concentration of 2.8 mg l^{-1} ; flasks 5 and 6 had 1.75 mg l^{-1} ; flasks 7 and 8 had 3.2 mg l^{-1} at the end of the experiment. These are substantial reductions, but with aeration occurring in each of the flasks, an undetermined amount of NH_3 loss was due to sparging rather than algal uptake. Determination of this amount was delayed until experiment 4.

Phosphorus removal was far less dramatic than the removal of nitrogen. The initial total-P

Table 2. Experiment 2: final values of six parameters after eight days

Sample No.	NaHCO ₃ (%)	pH	NH ₃ -N (mg l ⁻¹)	NO ₃ -N (mg l ⁻¹)	Total-P (mg l ⁻¹)	SR-P (mg l ⁻¹)
1	0.6	10.02	<2.5	0.98	13.24	12.07
2	0.6	10.03	<2.5	1.40	16.01	12.63
3	0.8	10.29	<3.0	1.40	11.46	11.26
4	0.8	9.95	<3.0	0.28	17.28	13.42
5	1.0	10.01	<1.5	0.70	12.37	11.96
6	1.0	10.21	<3.0	1.40	11.04	11.89
7	1.2	9.93	<3.5	2.10	11.91	11.89
8	1.2	10.36	<3.0	1.40	14.40	12.94
9 (B)	—	7.18	44.5	2.80	22.93	29.93

SR-P = Soluble reactive phosphorus

B = Baseline: untreated lagoon effluent (control).

in half-strength lagoon effluent was 22.9 mg l⁻¹ in experiment 2. The final mean concentration in supernatant from the eight algal cultures was 13.5 mg l⁻¹, a reduction of only 41%. Reduction in total-P was equally as low or lower than in experiment 1.

One exception in the first experiment was the control containing 0% bicarbonate in which *Chlorella*, a green alga, was the dominant form rather than the blue-green *Arthrospira*. Total phosphorus removal amounted to 72%. Luxuriant uptake, which is common in the chlorophytes, can account for this greater removal capacity.

The results of the third experiment are given in Table 3.

While there is clearly more rapid removal of ammonia in treatment 2, the *Arthrospira* culture, final concentrations are the same, 0.0 mg l⁻¹, in all. Therefore the null hypothesis cannot be rejected and it cannot be said that algae was necessary for the removal of ammonia. Air sparging in the inorganic control caused the same effect, but required twice the time. It should be noted that aeration in outdoor ponds is expensive. Algae ponds should be stirred by paddlewheels which use about 10% as much electric power and achieve the same result in less time.

The fourth, and final, experiment was designed to eliminate the effect of aeration and to show just how efficient algae can be in the

removal of ammonia nitrogen by uptake alone. The results are shown in Fig. 2. Because of a rather high ammonia concentration at time 0, there was a considerable lag time in the algal treatment. However, after one week (168 h), a critical point was reached in the concentration of algae and a rapid increase in density took place. The final absorbance of 0.65 corresponds to an algal concentration of about 1 g l⁻¹, dry weight. This occurred in three days. At the same time the ammonia concentration plummeted to less than 0.5 mg l⁻¹. The other two treatments, lacking algae, failed to bring the concentration of ammonia below 75 mg l⁻¹ (see Fig. 3).

ANOVA evaluation of the fourth experiment by Duncan T test showed that the two treatments without algae do not differ significantly from each other, but both showed significantly less nitrogen removal than the algal treatment. It should be noted that the dramatic fall-off in ammonia concentration in the *Arthrospira* culture was due almost entirely to nitrogen uptake by the algae. In this case the null hypothesis predicting similar results for all treatments can be rejected and the superiority of the algal treatment in nitrogen removal readily accepted.

3.1. Odor control

Odors, not only from dairy farms, but from confined livestock operations in general are rapidly becoming a source of irritation to the

Table 3. Experiment 3. Ammonia concentrations in mg l⁻¹ with elapsed time

Flask No.	Treatment No.	Ammonia concentration (mg l ⁻¹)				
		0.0 h	18.5 h	45.0 h	68.0 h	72
1	1	110	59	6	2	<1
2	1	110	48	16	4	<1
3	2	96	40	17	<1	<1
4	2	95	27	<1	<1	<1
5	3	150	73	26	16	<1
6	3	150	75	11	2	<1

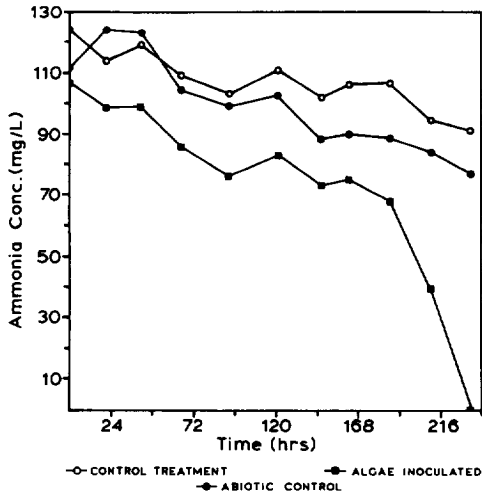


Fig. 2. Concentration of NH_3 over time in culture media consisting of lagoon effluent uninoculated (control), inoculated with *Arthrospira* (algae inoculated), and inorganic nitrogen NH_4Cl (inorganic control).

public in the U.S. Most of these odors, stemming from manure and wastewater systems, are difficult to control and eradicate. Aeration is commonly used to oxidize and thereby deodorize these substances. However, mechanical aeration by currently available technology is expensive, consumptive of fuel energy, and relatively ineffective. The same is not true of algal systems, especially in the mild sunny climates of the south.

In large outdoor algal cultures, an average of 1.6 kg of molecular oxygen is released into

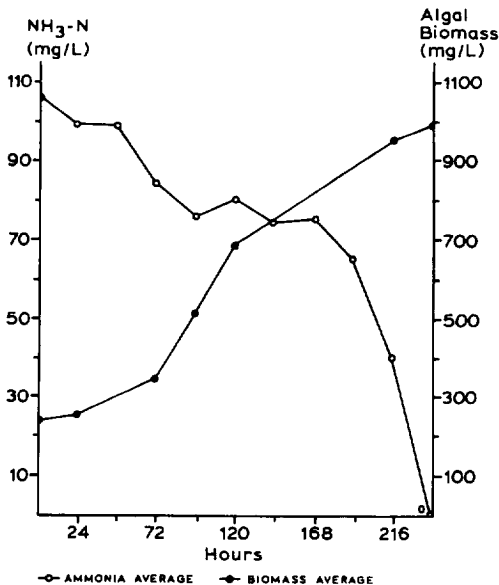


Fig. 3. Biomass and ammonia nitrogen concentrations over time in the algae treatment of experiment four. Biomass and ammonia nitrogen concentrations over time in the algae treatment of experiment 4.

solution for each kilogram of new algal biomass produced. The weight of O_2 produced is up to 1 ton $\text{ha}^{-1} \text{day}^{-1}$ and the culture medium becomes supersaturated with oxygen early in the day with concentrations of dissolved oxygen reaching 20–40 mg l^{-1} .

Under such conditions, bad smelling compounds are rapidly oxidized and deodorized. Since the process is powered by solar energy there is no cost involved. DO concentrations, being several times normal saturation, are far higher than can be obtained by aeration at any cost, and odors are eliminated faster and more economically than by mechanical means. This unique and natural way to eliminate bad odors is clearly unrivaled in warm and sunny weather. Strangely, in spite of the rise in recent public complaints about manure odors, algae systems have yet to be exploited.

4. CONCLUSIONS

The evidence presented here shows conclusively that anaerobically digested cattle manure, appropriately diluted, can support algal growth. Such growth is rapid and uninhibited in the presence of $\text{NH}_3\text{-N}$ at concentrations of less than 75 mg l^{-1} , but growth inhibition occurred at a concentrations above 100 mg l^{-1} . Likewise, it was found that NaHCO_3 supplementation need not exceed 0.5% of the culture medium to be adequate for *Arthrospira* growth. However, a complete absence of bicarbonate supplementation leads to the takeover of the culture by chlorophytes (e.g. *Chlorella* spp.) in spite of heavy inoculation with cyanobacteria. Quantification of laboratory results demonstrates that algal uptake of ammonia nitrogen can be rapid and complete, lowering concentrations by more than 24 mg l^{-1} per day, once the algae population reaches a critical density of about 250 mg l^{-1} (Fig. 3).

More importantly, from the standpoint of biomass production, laboratory productivities averaged some 70 g m^{-3} per day (Fig. 3), or 0.7 tons/ha (0.28 tons/acre) per day, indicating a potential productivity of *Arthrospira* an order of magnitude greater than the typical biomass crop which seldom reaches 0.1 tons/ha per day (0.04 tons/acre). Our best outdoor yields amount to about one-third of laboratory yields (24 g m^{-2} per day) under optimum conditions. Also, it is significant that at least half of the dry weight of algal biomass is edible protein. This

enhances the prospects for production of a protein feed as a byproduct of the cyanobacterial treatment of dairy wastewater effluent.

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