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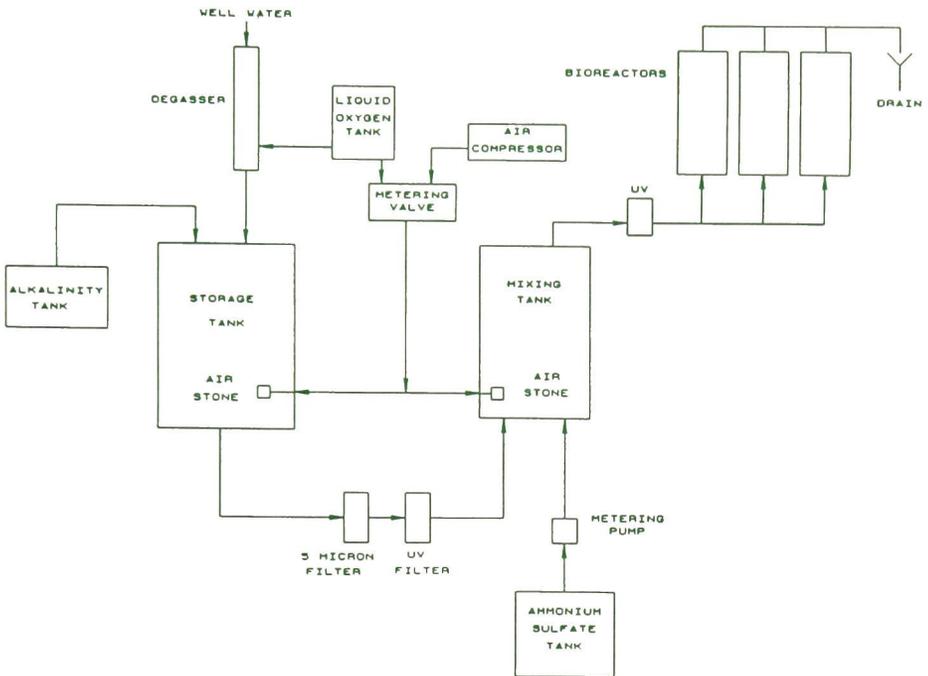


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Oxygen Supplementation for Aquaculture Biofilters

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MAINE AGRICULTURAL AND FOREST EXPERIMENT STATION
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Oxygen Supplementation for Aquaculture Biofilters

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INTRODUCTION

In recirculating aquaculture systems the removal or reduction of ammonia and nitrites is critically important. Ammonia is toxic to fish and shellfish to varying degrees. For example, while the Common Carp (*Cyprinus carpio*) can withstand levels of up to 2 mg/liter of un-ionized ammonia (Hasan and Macintosh 1986), some salmonid species are limited to one tenth of that figure (Rice and Bailey 1980). It is a naturally occurring product of the animals' metabolism, but can also arise from degradation of protein feeds. Nitrites are also toxic and are produced in the conversion of ammonia to nitrate.

The oxidation of ammonia to nitrite by nitrifying bacteria in a bio-filter is a complex series of biochemical reactions. A comprehensive review of the bacteriology of nitrification is presented by Hagopian and Riley (1998). A bio-filter requires three elements in order to function: a population of nitrifying bacteria, a substrate (ammonia), and oxygen. It is generally felt that in recirculating aquaculture systems the process is substrate limited, i.e., there is more than enough dissolved oxygen (DO) for the bacteria to nitrify the relatively low levels of ammonia, but that with higher ammonia concentrations, e.g., in highly intensive systems and in wastewater treatment plants, the oxygen levels are limiting.

It is known that the levels of DO in the water to be treated affect the aerobic process but there is some controversy as to the quantitative effects of these levels, on both the growth and the activity of the nitrifying bacteria. Much of the literature (Anderson and Levine 1986; Forster 1974; Manthe et al. 1988; Wild et al. 1971; Wortman and Wheaton 1991) makes it appear that nitrifiers require very little oxygen to perform their oxidation, and that O_2 is rarely limiting. On the other hand, in activated sludge systems, DO concentrations of approximately 4 mg/liter were found to remove 80% of ammonia in a 6-hour detention time, while 2 mg O_2 /liter removed only 40% (Wheaton et al. 1989).

There is conflicting evidence concerning the negative effects of high concentrations of dissolved oxygen on nitrifying bacteria. This project was developed to determine what happens to an established slime layer in a fixed-film, flow-through bio-filter, exposed to oxygen supersaturation. Specific objectives were to determine qualitatively and quantitatively whether the treatment is actually beneficial after acclimation, and whether rapid fluctuations in DO are detrimental to an acclimated culture.

MATERIALS AND METHODS

Apparatus

The experiments utilized three submerged fixed-film bio-reactors. The proposed procedure was to establish nitrifying bacteria populations in these reactors, supply them with carefully controlled amounts of substrate and DO, and measure the effects of varying DO levels on nitrification of this substrate.

Water for the experiments was supplied from a well and had a DO of 5 ppm @ 19.5°C. A packed column degasser was designed for oxygen absorption and concurrent nitrogen stripping at atmospheric pressure. The water was piped to the top of a 2.1-m length of 25.4-cm-inside-diameter Schedule 40 PVC pipe and applied through a distribution plate into a column of multi-pronged, high-surface-area material. Water trickled by gravity through this plastic media into a stilling well where pure oxygen was injected through porous diffusion stones to purge excess nitrogen and CO₂ and to produce the required DO levels for the experiments. From the stilling well the water was pumped into three 3,800-L storage tanks.

A 1,300-L buffer tank contained sodium bicarbonate, in a solution of trace nutrients necessary for nitrifying bacteria growth. This solution was metered into the storage tanks and mixed with the oxygenated well water. From the storage tank the oxygenated, buffered, nutrient-rich solution was pumped through a 5-micron particle filter and a UV sterilizer into a mixing tank, into which a peristaltic pump metered a concentrated ammonium sulfate solution (16,200 mg/liter at 21.2°C, pH 6.65). The diluted synthetic wastewater was pumped from the mixing tank to the bio-reactors (see Figure 1).

The reactor vessels were constructed from Schedule 40 PVC pipe, with dimensions as shown in Table 1. The packing consisted of 38-mm-diameter hollow high-density polyethylene spheres. The area of the entire inner reactor wall was included in the total media surface area because this may be a significant contributor to the total amount of nitrification attachment sites. The flow rates are approximated from the mean values over the entire life of the experiment.

Each reactor was capped on top with a threaded round cap and on bottom with a threaded flat plug. A 0.64-cm-thick round plastic distribution plate separated the packing from the bottom of the reactor. Water was applied uniformly over the cross section of the column through 100 1.5-mm holes drilled in the distribution plate. A tall, thin design was chosen to ensure an even water contact over the growing media. Consistent nitrifier activity throughout the filter was

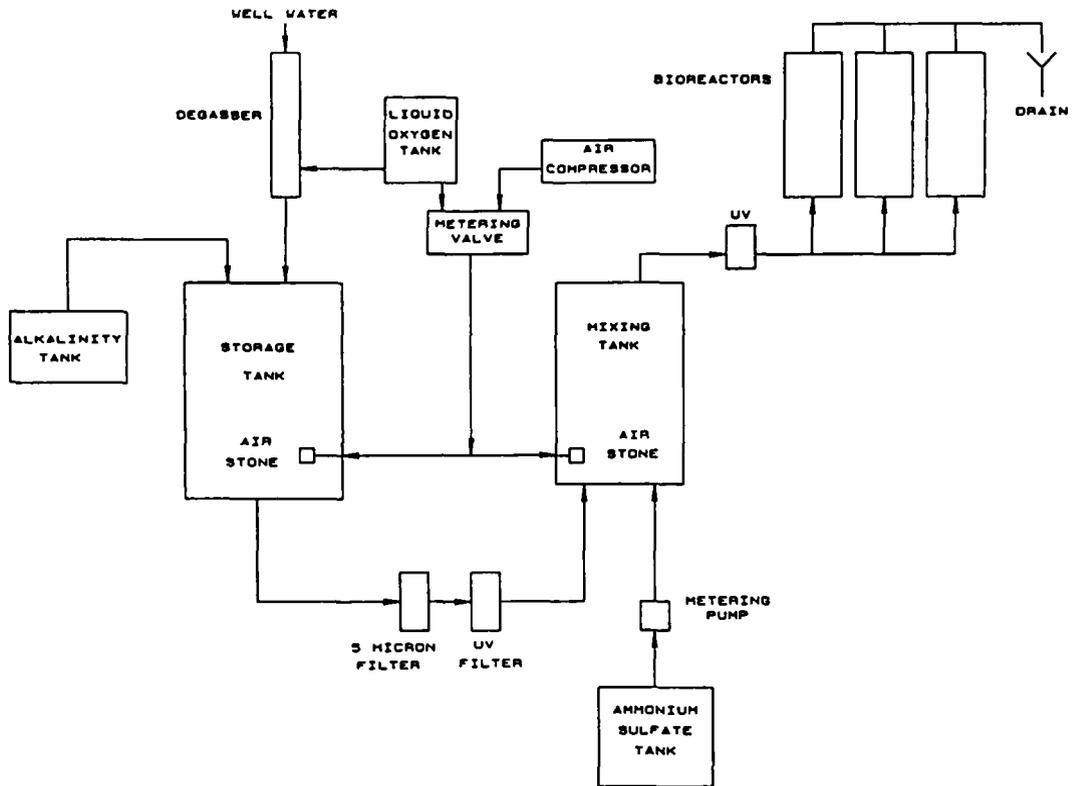


Figure 1. Schematic diagram of experimental apparatus.

Table 1. Bio-reactor physical parameters.

Reactor #	Inside Dia/ Height (cm/cm)	Number of Bio-balls	Total Surface Area (m ²)	Volume (L)	Median Flow (L/min)	Retention Time (min)
1	7.7/80.6	59	0.59	2.20	0.505	4.36
2	7.7/125.7	100	0.90	3.14	0.845	3.72
3	10.2/114.3	157	1.23	4.84	0.984	4.92

desired for reliable filter performance evaluation. The water flowed up the packed bed; flow was controlled with needle valves on the reactor outlet line before passing to the drain. The temperature in the reactor room was held at a relatively constant 21.2°C with one electric space heater.

Experimental Methods

All tanks and lines were disinfected with common liquid bleach (Chlorox), then flushed with well water for 24 hours. A pure strain of *N. europaea* in liquid form was obtained from the American Type Culture Collection in Rockland, MD (ATCC #25978). Flasks of well water and chemicals similar to the standard medium suggested by ATCC were inoculated with the culture and incubated at 26°C for 34 days. These batch cultures were then introduced into the three bio-reactors which were sealed and rested for three days. At this time sterilized water was pumped through the system.

Nitrite production was relied upon as sole indicator of nitrification activity, so it was crucial that the oxidation of nitrite to nitrate be completely stifled. It has been shown (Anthonisen et al. 1976) that all nitrification is inhibited by free ammonia levels of 10–150 mg NH₃/liter while inhibition of only nitrification begins at between 0.1–1.0 mg NH₃/liter depending on temperature and other factors. Therefore, it was necessary to maintain free ammonia levels below 10 mg NH₃/liter.

The activity of the cells in each reactor was estimated from measurements of the transformation rate of ammonia to nitrite. Measurement of pH, DO, nitrite-nitrogen, and nitrate-nitrogen was conducted twice daily from samples taken at the reactor inlet and outlet. Temperature, alkalinity, and ammoniacal-nitrogen were measured at the inlet. Flow rate through the reactors was determined by the time to fill a 1-L flask. Temperature and pH were measured with

a Sentron meter (Sentron, Inc., Federal Way, WA); nitrite, DO, and ammonium analysis was conducted with a Hach DR 2000 Spectrophotometer (Hach Co., Loveland, CO). Alkalinity was measured by titration with sulfuric acid. Nitrate levels were tracked using a pH/ISE meter with a Hach nitrate-ion selective electrode. The DO concentrations used are shown in Table 2.

Testing was repeated during two experimental runs, referred to as "Phases" (See Table 3). For the first 82 days after inoculation, air was used as the aeration gas. All three bio-reactors received the same treatment.

Table 2. Average partial pressures and dissolved oxygen levels for each treatment.

Treatment #	pO ² (atm)	DO (ppm)
1	0.21	9.0
2	0.42	18.0
3	0.62	27.0
4	0.82	36.0

Table 3. Duration and treatments for the two phases.

Phase	Beginning date	Ending date	Beginning day #	Ending day #	Treatment
I	July 20	Sept 21	83	146	Initial to High DO
II	Sept 21	Oct 16	146	171	Sequentially Increasing DO

RESULTS

Results are presented as mg NO₂ - N/m².min against time. These are corrected for surface area and flow rates in the three reactors. Statistical analysis indicated no significant differences between reactors; therefore data from all three reactors are lumped together.

The new culture was first subjected to an abrupt increase in oxygen tension on Day 92. When the aeration gas was suddenly changed from air to pO₂ (approximately 82%), the bacteria responded by producing more nitrite. However, as Figure 2 clearly illustrates,

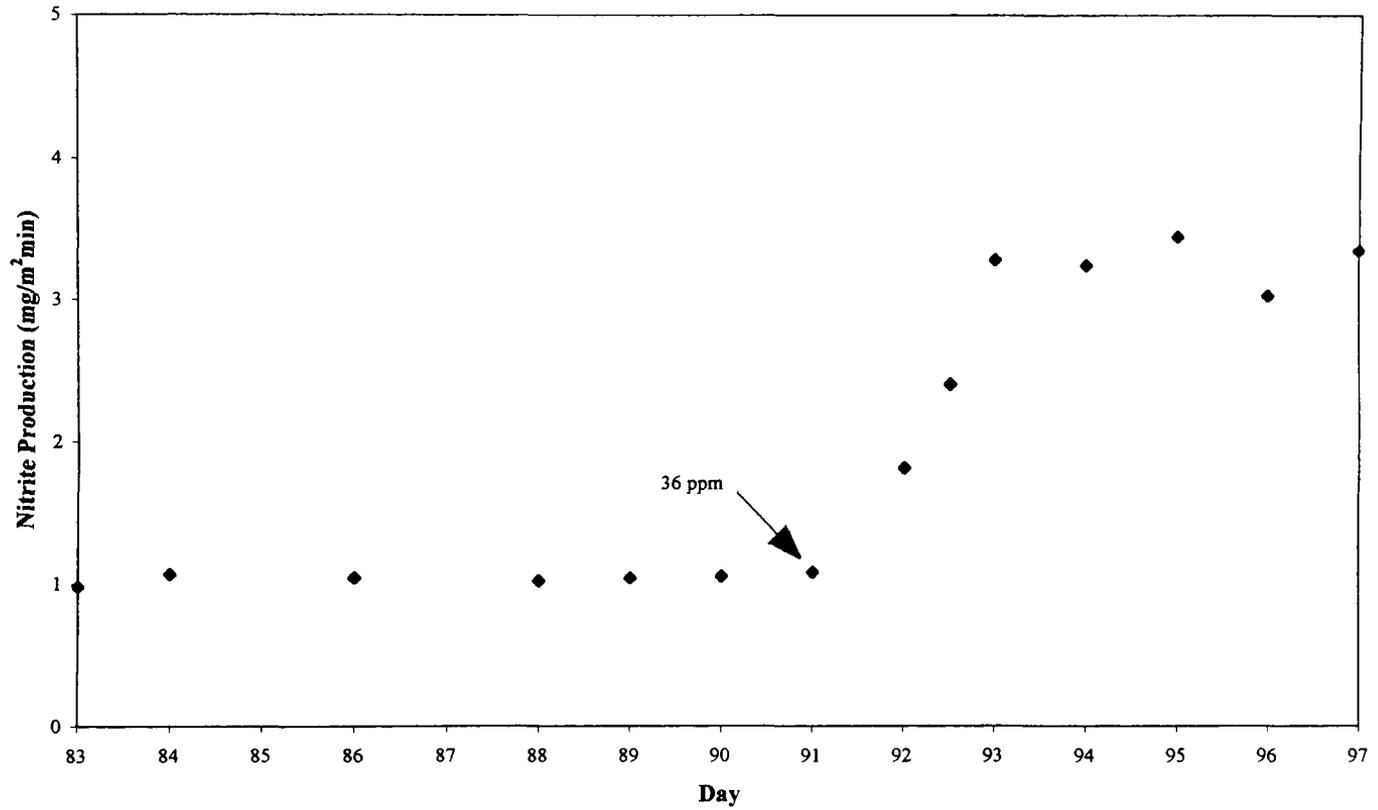


Figure 2. Time series response to initial hyperoxic exposure. Arrow indicates when hyperoxia was applied.

two days were required in order for the new rate of activity to reach steady-state conditions. When the DO was reduced from this high pO_2 level to ambient levels on Day 146, the full response was immediate. Figure 3 reveals no restabilization delay. Figure 4 shows a step-wise response to increases in DO during Phase II, with again no restabilization delay. Figure 5 shows a continuing increase in nitrification at the highest level of DO.

Nitrifiers are slow growing and over the short-term one may consider the kinetics of activity separately from the kinetics of growth. However, this experiment was conducted over a period of months and the activity in the bio-reactors continually increased with time. The system showed up to a 10x increase in nitrite production over the length of the experiment (see Figures 2 and 5).

It is expected that as the culture grows, the film thickness reaches a maximal value due to fluid shear stress. However, it was apparent from the sampling routine that biomass was accumulating with time. More bacterial flocks were observed to be washed out with the effluent as the experiment progressed. Also, nitrifying growth became thicker in the transparent vinyl tubes that carried the influent and effluent. The loss from slough-off never exceeded or balanced growth inside the filters. Probably as a result of the near-optimal conditions and the long detention times, the culture thrived and the nitrification rate steadily increased. When the experiment was terminated after 209 days, the bio-reactors and the tubes leading to and from them were virtually "choked" with a thick culture of *Nitrosomonas europaea*. This observed accumulation, and the resulting loss of pressure head through the bio-filters, taken together with the increase of activity with time, suggested a correlation between activity and biomass that would make it necessary to correct the results for growth. By compensating nitrite production for biomass inflation, one may obtain a truer reflection of the effect of the intended independent variable (i.e., dissolved oxygen). Using time as a proxy for growth, the degree to which the data responded to bacterial population density could be estimated. To this end, multiple linear regression analysis was conducted on DO and Day # for each phase. The results are plotted in Figure 6.

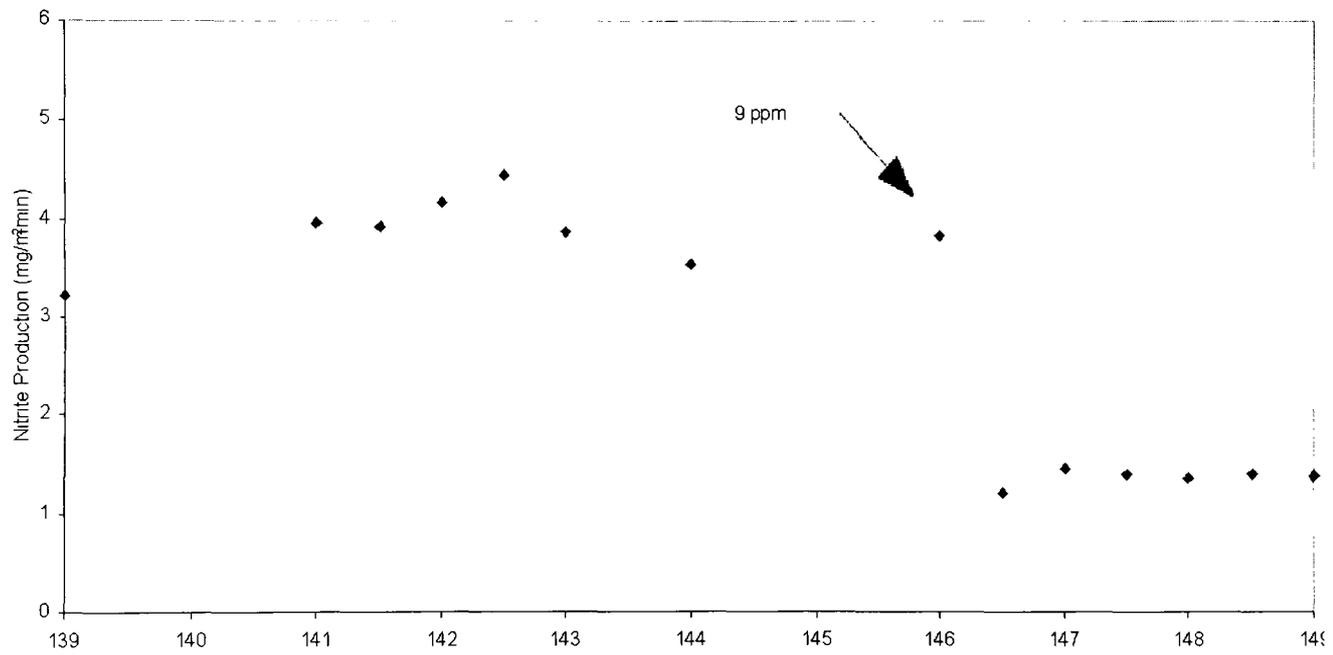


Figure 3. Time series response to a return to normoxia. Arrow indicates when air was applied.

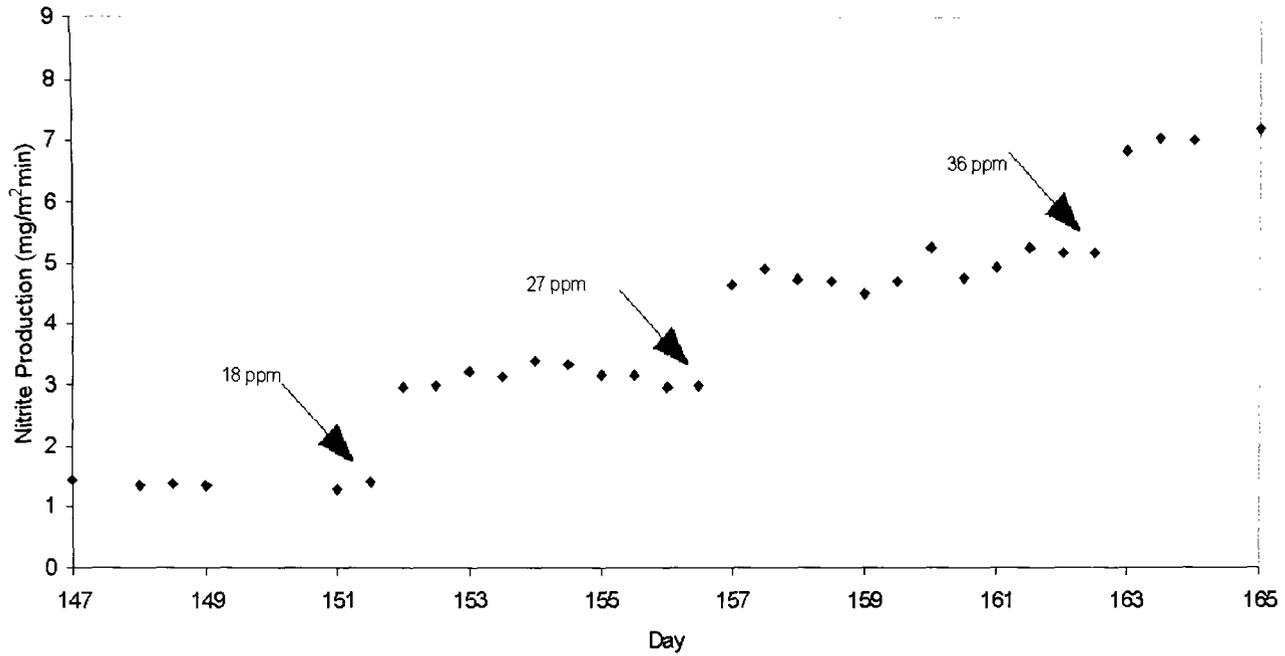


Figure 4. Time series response to stepwise increase in DO. Arrows indicate when the three hyperoxic treatments were applied.

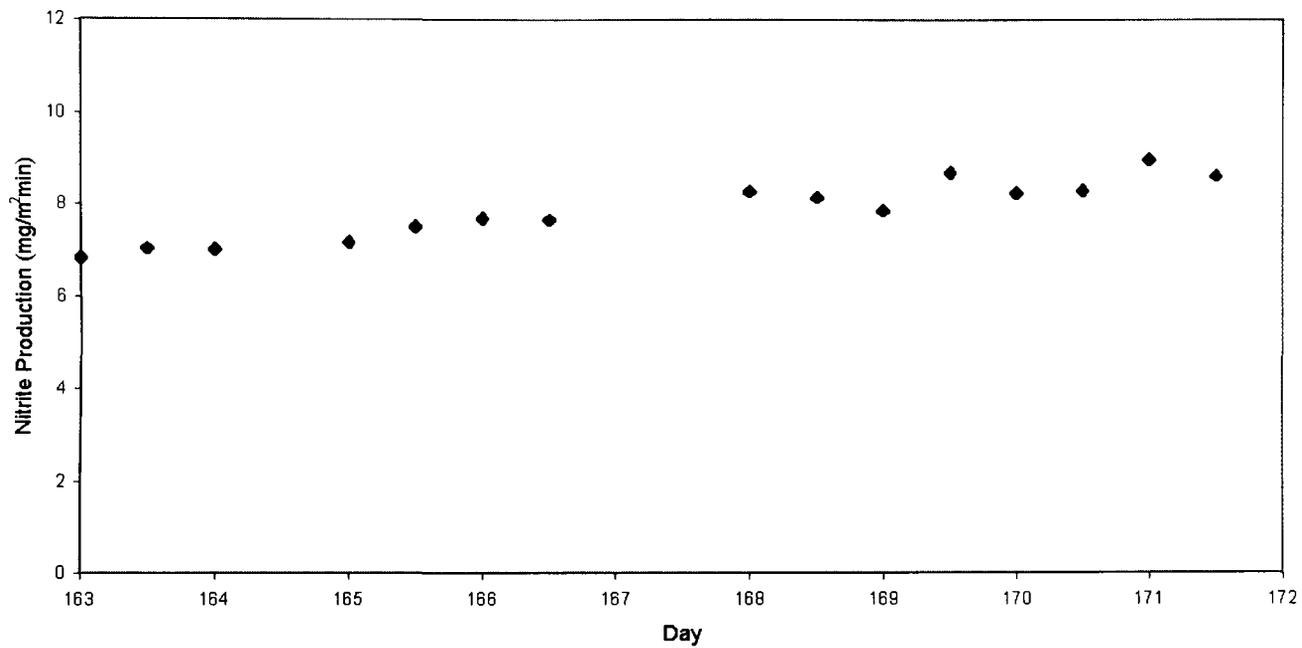


Figure 5. Time series increase in activity under constant hyperoxic exposure indicating the development of a thicker biofilm.

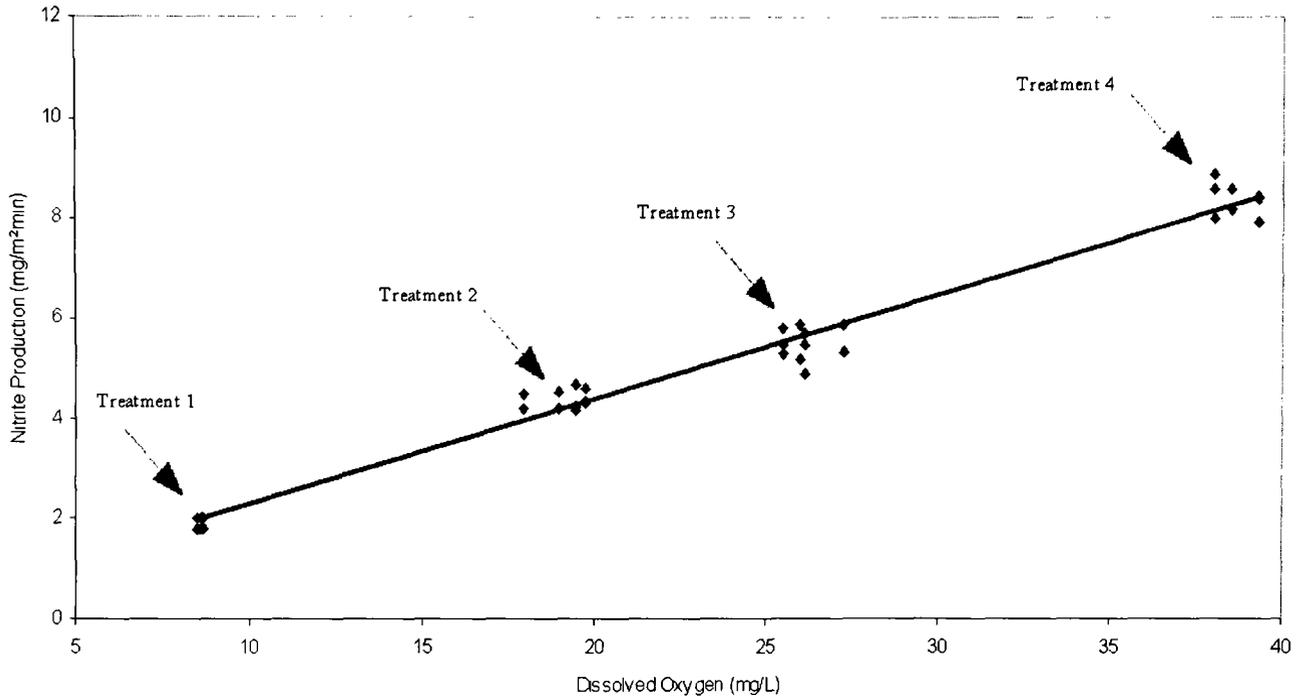


Figure 6. Activity response to multiple-day DO treatments.

DISCUSSION

The results showing that submerged, fixed-film nitrifiers can tolerate high dissolved oxygen levels confirms previous work. Our experience shows no inhibition, but a distinct initial acclimation period. When the system was first challenged with oxygen supersaturation (Figure 2), there was a gradual increase to a new steady-state nitrite production level. This adjustment was observed for two days but no adaptation time was required upon subsequent increases or decreases in dissolved oxygen (Figures 3 and 4). Furthermore, the experience of this study confirms the Jones and Paskins (1982) finding that nitrifying bacteria retain a tolerance to artificially high DO even after multigenerational growth at normal DO values. Nitrifying bacteria are slow growing and normally require a relatively long time to respond to changes in factors which effect metabolism.

High DO levels were not only tolerated by the bacteria, the treatment also had a stimulatory effect on activity. Nitrite production increased rapidly when high oxygen levels were applied. The effect was linear and continued to the highest pO_2 levels achieved, almost 90%. The magnitude of the slope increased with time as the nitritation rate under atmospheric saturation increased. In other words, the response could be predicted by roughly extending a straight line from the origin through the treatment #1 results. This fact indicated that the positive effect of the treatment was defined by the density of the bacterial population in the bio-reactor.

Towards the end of the experiment, after approximately 200 days, with a dissolved oxygen level of 35 ppm, nitrification rate was more than $25 \text{ mg N/min} \cdot \text{m}^2$ and still showed no indication of falling. This contradicts previous findings (Siegrist and Gujer 1987); however, this contradiction may be traced to the erroneous assumption that every bacteria in a bio-reactor experiences the conditions that are measured from the synthetic wastewater. These bacteria live within a complex slime matrix. During a treatment of pO_2 approximately 82% an entire range of dissolved oxygen concentrations from zero to approximately 36 ppm was experienced. Those bacteria that were located on the top layer of a biofilm were exposed to something near the intended supersaturation level, while those buried at the bottom of the biofilm were subject to anaerobiosis. It is for this reason that a plateau in DO vs. nitrite production was not achieved. Siegrist and Gujer (1987) noted a steady increase in the ammonium uptake rate of their trickling filter with oxygen levels, but after a concentration of 25 mg/liter was reached, there was a leveling off of the response. Theoretically, a pressurized reactor could have been used in this study to drive the

dissolved oxygen level well above the 40 ppm limit and reach a point where the linear response would stop.

The theory being presented here is that the observed improvement in nitrite production is simply a manifestation of the activation of dormant cells deeper in the biofilm, rather than an evenly distributed increase in all of the bio-reactor cells. If concentration gradients are present inside biofilms, the legitimacy and practical usefulness of traditionally held figures are in question. True kinetic constants for a fixed-film system may only be experimentally quantified.

If the preceding argument is correct, there are actually four ways to increase the nitrification rate in a fixed-film scenario, rather than three. One may increase the maximum rate constant, provide substrate above rate-limiting levels, or increase the amount of biomass in the filter. But according to the theory advanced above, one may also increase the number of bacteria that are experiencing substrate levels greater than the rate-limiting level. Throughout each phase, there was an increase in nitrite production with time, even during the highest levels of supersaturation. Regression analysis on the data from the last 9 days of Phase II (see Figure 6) reveals a 28% net increase in biomass over 8.5 days in at least 82% pO₂ conditions. The evidence for biomass accumulation under continuous hyperoxidation, as well as under alternatively high/ambient DO tensions, is strong.

SUMMARY AND CONCLUSIONS

The conditions of this experiment differed from those of a recirculating system in several ways. First, the nitritation energy source was well above allowable concentrations for finfish. Second, as a result of the ammonia level being approximately 50x what one would find (or what would be desirable) in an actual aquaculture operation, the activity of nitrite-oxidizers was suppressed. There were no suspended solids present in the medium, which might have played a mitigatory role against oxygen toxicity and other adverse factors. Finally, most of the growth was observed to be in flocks, rather than in a biofilm, so it is not likely that the protective effects of adhesion were at play. With these caveats in mind, we conclude the following:

- There is a distinct adaptation period upon an abrupt introduction to high oxygen tensions.
- The ability of a biofilm to perform up to its full potential is maintained during multigenerational growth at a different DO level.

- The activity of a biofilm will not follow standard biochemical kinetics. Diffusion-limited, pseudo-kinetic parameters will prevail through the activation of buried cells.
- Where there is excess substrate present, as in highly intensive culture systems, pure oxygen amendment will significantly increase the maximum nitrification rate of a biofilm.
- A nitritation rate of 36 mg NO₂ - N/m² .min is possible in a packed column.
- Biofilm growth does occur under hyperoxic conditions.

Complete nitrification is essential to maintain adequate water quality in recirculating aquaculture production systems. It is not possible to optimize the rate of bacterial activity, since their physiological requirements differ from those of fish (e.g., pH, TAN, temperature). For these reasons, fish farm filters are routinely oversized. It is imperative that the reduced nitrogenous compounds are kept well below toxic concentrations.

Oxygen supplementation will not harm the nitrifying ability of an aquaculture filter. The net result will be an improvement in their competition against other aerobes. This relatively simple, inexpensive, modification to the conventional recirculating aquaculture filter could lead to an improvement in nitrogen toxicity elimination. In very practical terms, one might be able to reduce the bio-reactor surface area necessary for any given waste stream. DO supersaturation will optimize the performance of nitritation bacteria in a fixed-film. The higher the ammonium level delivered to the biofilm, the more crucial this will be. The current interest in reducing bio-filter size and/or increasing nitrification rates would support the need for more extensive large-scale testing, but in the final analysis, the design of attached growth reactors must be based on empirical results from pilot-scale systems because of substrate mass transfer limitations into the biological film.

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