# PRELIMINARY STUDIES CHARACTERIZING WASTEWATER FROM THE INTENSIVE CULTURE OF CHANNEL CATFISH AND NITRIFICATION IN LABORATORY SCALE SUBMERGED FILTERS

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#### ABSTRACT

A study was conducted to determine the pollutional load generated by catfish cultured under a salinity range of 1000 to 9000 mg/l TDS and to evaluate the effect of high salinity on the process of biological nitrification in laboratory scale submerged filters. Water quality parameters that were monitored included: COD, BOD, PO $_{\rm L}$ -P, Organic-P, NH $_{\rm 3}$ -N, NO $_{\rm 3}$ -N, Organic-N, SS, and VSS. A respirometer study also was performed to determine the influence of high salinity on the exertion of nitrogenous BOD. The results of the wastewater characterization study demonstrated that increasing the salinity from 1000-3000 mg/l TDS stimulated nutrient production, while increasing the salinity from 3000 to 9000 mg/l TDS decreased the nutrient production rates. Catfish nutrient production rates under saline conditions were approximately 57 percent less than those reported in the literature for catfish cultured in fresh water systems. A salinity level of 3000 to 5000 mg/1 TDS was found to optimize nitrification while a TDS level of 5000 to 9000 mg/l was found to inhibit nitrification in a submerged upflow filter. A specific ion(s) in the saline water was not identified as the sources(s) of inhibition to nitrifica-Hydraulic loading rates of 18.9 to 37.9 m/day were found to be acceptable operating rates for the nitrification filter. At a hydraulic loading rate of 18.9 m/day a recycle ratio of 1.0 was found to optimize nitrification while increasing the recycle ratio to 3.0 did not improve filter performance. The results of the respirometer study verified that a salinity range of 3000 to 5000 mg/l TDS was optimum for nitrification under high salinity conditions.

Keywords: salinity, catfish, nutrient production, nitrification, submerged filter, respirometer

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#### INTRODUCTION

#### Background

The Southwest desert of the United States is characterized by an arid climate and limited availability of fresh water. Because of this freshwater limitation, aquaculture, which is very water intensive, has not developed into a prominant industry in New Mexico. However, production of aquatic animals could be accomplished economically using intensive recirculating systems and brackish groundwaters. Total New Mexico groundwater reserves (fresh and brackish) have been estimated at 2.5 x  $10^{13}$  m<sup>3</sup> of which 75 percent is brackish (>1000 mg/l total dissolved solids [TDS]) (Bahr and Herman 1981). Brackish groundwater tends to be closer to the surface of the ground than the fresh water, thereby providing a cheaper and more accessible water supply (Hood and Kister 1962). Brackish groundwaters in New Mexico contain up to 200,000 mg/l TDS but most of the supply is much less than 100,000 mg/l (Hood and Kister 1962). Such is the case in Roswell, New Mexico, where the groundwater has a TDS concentration of 14,000 mg/l. The water is not usable for direct human consumption without extensive dilution or desalination but has the potential to be used for culturing salt tolerant fish species. Some species of fish grow well in brackish water, while other species such as channel catfish could be cultured only in fresh water or in slightly brackish water (<10,000 mg/l TDS) (Allen and Avault 1969, Lewis 1971).

The production of channel catfish in brackish water derives an economic value from a resource that is presently considered a nuisance by conventional agriculturalists. Other benefits can be gained by culturing catfish in slightly brackish water. Brackish water reduces the growth of blue green algae, which sometimes produce off-flavors in the fish flesh and inhibits the

spread of ectoparasites. Algae control also reduces the chance of a devastating epidemic (Avault 1982). In addition, the internal osmotic concentration in the fish will be closer to that of the brackish water, allowing the fish to use less energy in maintaining osmotic balance with a resultant storage of the energy in the tissue (Canagarathnam 1959). Also, the presence of NaCl in the culture water alleviates the stress from nitrite poisoning (Tomasso et. al. 1979).

The biology of channel catfish has been studied extensively because of their popularity as a commercially culturable food fish. Optimum growth in fresh water occurs at temperatures between 28 and 30°C (Andrews et al. 1972, Sutton and Lewis 1982). Channel catfish adapt readily to intensive tank and raceway culture (Andrews et al. 1972, Stickney et al. 1972, Tackett 1974) and, unlike salmonids, can withstand crowding in poorer quality waters and still maintain good growth and food conversion efficiencies (Andrews et al. 1971). High-density tank culture, as opposed to pond culture, permits the elimination of undesirable species of fish, reduces food wastage, aids detection and treatment of disease, allows complete and economical harvesting and eliminates the possibility of contaminants from outside sources (Andrews 1972).

Intensive indoor recirculating systems require less space and lower water flow rates, which lower the pumping costs and reduce the problems associated with the pollution potential of fish hatchery effluents. These systems also provide for increasing the carrying capacity of a water supply and regulating temperature, which decreases the rearing time significantly. Except for aquatic mammals, most aquatic species are poikilotherms (their body temperatures are essentially that of their environment). Like any reaction that is temperature dependent, the metabolism and the activity of the fish increase with a rise in temperature thereby increasing their oxygen demand and

their excretion of metabolites. Excretion of metabolites is also increased by increasing the carrying capacity of the fish culture system, i.e., increasing the number of fish and the feed rate. Excretion of metabolic end products in fish occurs mainly through the gills. The principal products eliminated through the gills are ammonia and urea (75-95 percent of the total nitrogen excreted). The remaining products, which include creatine, creatinine and uric acid, are excreted through the kidney (Gigger and Speece 1970). Urea decomposes into ammonia  $(NH_3)$  and  $CO_2$ 

Excretion of ammonia (NH  $_3$ -N) by catfish ranges from 0.08 to 0.8 gm/day/kg fish with an average of 0.36 gm/day/kg (Gordon 1974, Murphy and Lipper 1970, Page and Andrews 1974, Ruane et. al. 1975). Ammonia exists in two forms; free unionized ammonia,  $NH_3$ , and ammoniumion,  $NH_4^+$ . The equilibrium between free ammonia and ammonium ion is highly pH dependent. The higher the pH, the higher the concentration of free ammonia. The unionized free ammonia (NH<sub>3</sub>) is toxic to fish because it readily diffuses across the gill membranes. The reported mean lethal dose  $(\mathrm{LD}_{50})$  for unionized ammonia ranges from 1.1 to 2.4 mg/l for channel catfish (Wheaton 1982, Piper et. al. 1982). This range in the LD<sub>50</sub> is probably due to differences in water characteristics particularly pH. In various species of fish, ammonia has been shown to cause degenerative tissue damage to gills and kidneys, reduce oxygen carrying capacity of hemoglobin, increase oxygen consumption, respiratory rate, heart rate, and increase urine output. It has been reported that exposure of channel catfish to a concentration of 0.05-1.0 mg/l unionized ammonia reduced the growth significantly while 1.2 mg/l unionized ammonia resulted in complete mortality during a 31 day growth trial (Piper et. al. 1982, Tomanso et. al. 1981).

In a closed aquaculture system, the ammonia accumulates in the system, therefore, the water should either be changed or the ammonia should be removed.

The ammonia can be removed physically, chemically or biologically. The ammonia can be removed physically by stripping the water with air after raising the pH of the water to more than 10.0. This process is not generally feasible in an aquaculture recycle system using brackish water because raising the pH induces heavy precipitation of the dissolved minerals sush as CaCO<sub>3</sub> and Mg(OH)<sub>2</sub>. The ammonia can be removed chemically using zeolite known as clinoptilolite, which is a specific ion exchanger for ammonium ion.

A potential problem for clinoptilolite in a brackish water system is that the dissolved salts may out-compete the ammonium ion resulting in incomplete removal of ammonia.

For a brackish water system, the ammonia may best be removed biologically by the nitrification process. Nitrification is mainly performed by bacteria of the genera Nitrosomonas and Nitrobracter. Nitrosomonas species oxidize ammonium ion  $(NH_4^+)$  to nitrite  $(NO_2^-)$  and Nitrobacter species oxidize the nitrite to nitrate  $(NO_3^-)$  according to the following equations:

$$NH_4^+ + 1.5 O_2 \xrightarrow{Nitrosomonas} 2 H^+ + H_2 O_1 + NO_2^-$$
  
 $NO_2^- + 0.5 O_2 \xrightarrow{Nitrobacter} NO_3^-$ 

It is generally agreed that the rate of nitrification is controlled by the conversion of  $NH_4^+$  to  $NO_2^-$  and that nitrite oxidation to nitrate occurs much more rapidly, consequently, the concentration of nitrite will remain very low (< 1 mg/l).

There are five main factors that can potentially affect the rate of the nitrification process in a brackish water aquaculture system; ammonia and nitrite concentrations, pH, temperature, dissolved oxygen, and salinity. Inhibitory effects of ammonia and nitrite were investigated by Anthonison

et. al. (1976). They concluded that inhibition of nitrification was caused by free unionized ammonia ( $\mathrm{NH}_3$ ) at a concentration of 10 mg/l and undissociated nitrous acid ( $\mathrm{HNO}_2$ ) at a concentration of 0.2 mg/l. The concentration of both the ammonia and the nitrous acid are controlled by the pH of the water, consequently, pH has a great influence on the nitrification process and the degree of inhibition observed at any given ammonia or nitrite concentration.

Nitrification is strongly affected by the pH of the system. The reported pH optimum varies widely, but generally, nitrifiers favor a mildly alkaline Srna and Baggaley (1975) collected data about the relation between the pH and the rate of removal of ammonia. They found that pH has less effect on the Nitrobacter species than Nitrosomonas, and that Nitrobacter prefer lower pH levels (6.6-6.7) than the Nitrosomonas (7.8). Huang and Hopson (1974) found that nitrification at a rate of 85 percent of the optimum occurred at a pH ranging between 8.4 and 9.0. However, Haug and McCarty (1972) noticed that nitrifiers could adjust to pH values as low as 5.5 to 6.0. An abrupt change of pH from 7.2 to 6.4 had no adverse effect on nitrification, while an abrupt change from 7.2 to 5.8 inhibited nitrification but the rate of nitrification was restored when the pH was returned to 7.2. In a closed fish culture system, the  ${\rm CO}_{2}$  produced by the fish, and the hydrogen ions produced by the nitrifiers will cause the system pH to drop steadily with time as illustrated in the equations shown below (USEPA 1975). A decrease in pH occurs through the generation of carbonic acid  $(\mathrm{H_2CO_3})$  and nitric acid  $(\mathrm{HNO_3})$  or the consumption of alkilinity (HCO<sub>3</sub>).

$$C0_2 + H_20 \rightarrow H_2C0_3$$

$$NH_4^+ + 1.83 \, O_2^- + 1.98 \, HCO_3^- \rightarrow 0.021 \, C_5^- H_7^- NO_2^- + 0.98 \, NO_3^- + 1.041 \, H_2^- O_3^- + 1.88 \, H_2^- CO_3^-$$
(bacteria)

It is generally accepted that through nitrification 7.14 mg alkalinity (as  $CaCO_3$ ) is destroyed per mg of ammonia completely oxidized.

Temperature has a strong effect upon nitrification and the specific growth rate of nitrifiers. Wong-Chong and Loehr (1975) found that the deactivation of <u>Nitrobacter</u> occurred at temperatures lower than that needed for the deactivation of <u>Nitrosomonas</u> and that the temperature dependency of both genera was a function of pH. Stankewich and Gyger (1978) have summarized the results of several researchers studying nitrification. From their analysis it was determined that the growth rate of ammonia oxidizers changes by roughly 10 percent for a change in temperature of 1°C and nitrification becomes severely reduced at temperatures below 15°C (Tomayo et. al. 1981).

Nitrification is significantly affected by the concentration of dissolved oxygen. The oxygen requirement for nitrifying bacteria is approximately 4.6 kg per kg of ammonia (NH $_4^+$ -N) oxidized to nitrate. Haug and McCarty (1972) have suggested that the rate of nitrification is independent of dissolved oxygen concentration as long as the stoichiometric requirement is met; but it is well established that nitrification is inhibited by low levels of dissolved oxygen concentration. Jenkins (1963) reported that at concentrations lower than 0.5 to 0.6 mg/l, nitrification does not occur. However, reports are variable and in several cases details of other influental environmental factors are incomplete. It has also been determined that requirements in saltwater biofilters was higher than that required for fresh water biofilters. For a saltwater biofilter, the oxygen requirement was 12.5 kg  $_{02}$  /kg NH $_{3}$ -N while for a fresh water biofilter the  $_{02}$  requirement was 7.8 kg  $_{02}$  / kg NH $_{3}$ -N (Poole).

Helder and Vries (1983) studied the effect of salinity on nitrification using ammonia solutions at 25°C and a salinity of 15,000 mg/l. They found that

<u>Nitrosomonas</u> were able to adapt to salinity though they reacted to salinity changes initially with an increased lag phase. Rosenthal and Otte (1979) found that changing the salinity of the water from 0 to 8000 mg/l in four days affected nitrification significantly during the first three days. The results of the research showed the initial accumulation of ammonia followed by a maximum nitrite concentration several hours later. While ammonia oxidation regained its former efficiency during the period of the salinity increase, nitrite oxidation was still unbalanced six days after the final salinity was reached. The study concluded that <u>Nitrobacter</u> was more sensitive to salinity changes than Nitrosomonas.

Jones and Hood (1980) studied the effect of the environmental conditions on nitrifying bacteria isolated from an estuarine bay and a fresh water marsh. They found that the estuarine isolate exhibited optimum activity at 40°C whereas the fresh water isolate was most active at 35°C. The estuarine isolate had an optimum pH of 8.0 while the fresh water isolate had an optimum pH of 8.5. Both isolates were able to grow over a wide range of salinity. The estuarine isolate showed the greatest activity between 0.5 to 1 percent salinity whereas the freshwater isolate showed optimum activity at 0.3 to 0.5 percent salinity. At 0 percent salinity, activity of the estuarine isolate declined by 95 percent. Although the fresh water isolate did not grow well at 0 percent salinity it retained 60 percent of its activity at this salinity. Neither strains were active at a salinity of 3 percent. Nitrite proved to be inhibitory to both the isolates at greater than 5 mg/l NO<sub>2</sub>-N. Nitrate had no significant effect on the activity of either isolate at up to 2000 mg/l NO<sub>3</sub>-N.

Several studies have been conducted to determine the effect of seawater salinity on the nitrification process, however, there is limited research studying the effect of high salinity groundwaters with chemical analysis

similar to that of the water found in Roswell, N.M. Therefore the main purpose of this research was to study the effect of salinity on the nitrification process as a means of treatment of wastewater generated in an intensive catfish culture operation.

### Research Objectives

The specific objectives outlined for the research project are listed below:

- 1. Determine the pollutional load generated by a catfish culture operation by characterizing the quality of the wastewater generated by an experimental, intensive catfish culture system located at the Roswell Test Facility (RTF) in Roswell, NM. This system was operated at salinities ranging between 1000 and 9000 mg/1 TDS.
- 2. Evaluate the effect of high salinity on the process of biological nitrification by operating laboratory scale submerged filters under conditions simulating the catfish culture conditions at the RTF (salinity levels ranging from 1000 to 9000 mg/1 TDS).

### Scope of Study

The first phase of the study was conducted at the RTF located in Roswell, NM. Under a separate study (Turner 1985), fingerling channel catfish (Ictalurus punctatus) were grown in circular raceways originally filled and continuously flushed with brackish groundwater ranging in salinity between 1000 and 9000 mg/1 TDS. The catfish growth studies were conducted over a six month period. Water samples to characterize the pollutional load generated by the catfish were collected and analyzed during the last three months of the study. Water quality parameters that were monitored included: chemical oxygen demand (COD), five day biochemical oxygen demand (BOD<sub>5</sub>), phosphate phosphorus (PO<sub>4</sub>-P), organic phosphorus (Organic-P), ammonia nitrogen (NH<sub>3</sub>-N),

nitrate nitrogen ( $NO_3$ -N), organic nitrogen (Organic-N), suspended solids (SS), and volatile suspended solids (VSS).

The second phase of the study was conducted in the laboratory of the civil engineering department at New Mexico State University (NMSU). The effect of high salinity on biological nitrification was evaluated by operating five laboratory scale submerged nitrification filters using brackish groundwater from Roswell, NM, diluted with tap water from Las Cruces, NM, and supplemented with reagent grade ammonium sulfate. The five salinity levels that were evaluated included TDS concentrations ranging from 1000 to 9000 mg/l. The performance of each filter was evaluated by monitoring the concentrations of the following parameters: NH<sub>3</sub>-N, nitrite nitrogen (NO<sub>2</sub>-N), NO<sub>3</sub>-N, pH, and dissolved oxygen (DO) concentration. In addition to the filter studies, respirometer studies were performed to determine the influence of high salinity on the exertion of nitrogenous biochemical oxygen demand (BOD).

#### MATERIALS AND METHODS

## Wastewater Characterization

The study to characterize the wastes generated by catfish cultured under varying salinities was conducted at the RTF located in Roswell, N.M. Fingerling channel catfish (Ictalurus punctatus) were cultured in circular fiberglass raceways 1.39 m in diameter and which held a working volume of 760 liters of water. Water flowed into each tank at a rate of 5.7 1/min resulting in an exchange rate of 2.3 hours. Salinity levels selected to conduct the catfish growth studies included TDS concentrations of 1000, 3000, 5000, 7000, and 9000 mg/l. Each salinity level was studied in triplicate yielding a total of 15 culture tanks. The predetermined salinity levels were created by blending Roswell drinking water with water from a deep brack-The characteristics of the two separate water ish well located at the RTF. sources are shown in table 1. The water fed into each tank was heated to a temperature of 26-28°C and was sprayed at an angle to create a clockwise circular flow pattern and surface aeration. Supplemental aeration to each raceway was supplied by three, 12 cm airstones connected to an air compressor. The initial fish stocking level was 100 catfish having a mean length of 157 mm and a mean weight of 35.6 gm. The specific details of the catfish growth studies have been published by Turner (1985).

The catfish growth studies were conducted over a period of six months. Water samples to characterize the pollutional load generated by the catfish were collected and anlyzed during the last three months of the study. Water samples were collected as 24-hour composites made by randomly sampling each tank from the five salinity groups. Because each salinity group consisted of triplicate tanks, an individual tank was sampled at a frequency of every three

Table 1

Concentrations of various ions contained in Roswell city water and the brackish well at the RTF

Constituent	Concentration, mg/l <sup>a</sup>			
	City Water	Brackish Water		
Sodium, Na	67	4449		
Potassium, K	1	23		
Calcium, Ca	184	525		
Magnesium, Mg	51	156		
Chloride, Cl	97	6948		
Sulfate, SO <sub>4</sub>	451	1488		
Bicarbonate, HCO3	238	190		
Dissolved Solids	1055	14,240		
Total Hardness, CaCO <sub>3</sub>	670	1950		
рН	7.65	7.63		

<sup>&</sup>lt;sup>a</sup>Source: S. Isaacs, Chemist, Roswell Test Facility, tested December 20, 1983.

hours or eight times in a 24-hour period. During collection, all samples were preserved by refrigeration at 4°C. Water quality analysis was performed either at the RTF, the Roswell wastewater treatment plant, or the civil engineering laboratory at NMSU. COD, PO<sub>4</sub>-P, Organic-P, SS, and VSS measurements were made at the RTF within 24 hours of sample collection. Once a composite sample was made, it was preserved by acidification to pH 2.0 with sulfuric acid and refrigeration at 4°C. BOD<sub>5</sub> determinations were made within 24 hours of sample collection by the Rowell wastewater treatment plant personnel. Samples for BOD analysis were preserved by refrigeration at 4°C while those for nitrogen analysis were preserved by acidification to pH 2.0 with sulfuric acid and refrigeration at 4°C (or on ice while in route between laboratories). NH<sub>3</sub>-N, Organic-N, and NO<sub>3</sub>-N concentrations were determined within 72 hours of sample collection at the civil engineering laboratory at NMSU.

All water quality analyses were performed in accordance with the procedures outlined in Standard Methods for the Examination of Water and Wastewater (18) with the exception of COD. The specific sections from Standard Methods identifying the analytical procedures are listed below: BOD<sub>5</sub>/Section 507; PO<sub>4</sub>-P/Section 424E; Organic-P/Section 424C (III); NH<sub>3</sub>-N/Section 417A,B; SS/Section 209D; VSS/Section 209G. All nitrogen species determinations were made using a Buchi digestion/distillation unit model no. 320.

COD was measured by a modification of a procedure developed by the Hach Chemical Co. The method is accepted by the U.S. Environmental Protection Agency (EPA) for National Pollution Discharge Elimination System (NPDES) reporting but has not been added to the latest edition of Standard Methods. Modification of the Hach procedure was necessary because the high cholride content of the saline water introduced an error in measuring COD. Chloride ion reduces dichromate. Reduction of dichromate is the basis for measuring

the exertion of COD. Because the catfish growth study monitored the effect of a progressively increasing salinity, even without the presence of organic matter, the water samples would exert an increasing COD due to the increasing chloride content of the treatment levels. Under the final experimental conditions, the highest chloride content expected was 4600 mg/l. In a COD test, chloride interference is normally inhibited by adding mercuric sulfate (HgSO,) which precipitates as mercuric chloride (HgCl<sub>2</sub>). Mercuric sulfate is added at a ratio of 10:1 ( ${\rm HgSO}_4:{\rm Cl}^7$ ). A problem in the analytical procedure was encountered because the high salinity samples required 2.3 times the normal amount of mercuric sulfate. The mercuric sulfate and the mercuric chloride precipitate are essentially insoluble in the COD reagent-water mixture. The ultimate problem was encountered when the color transmittance measurement was made using a spectrophotometer (Bausch and Lomb Spectronic 70). slow settling precipitate caused a positive interference. The extent of the interference was tested by analyzing COD standards made from Roswell saline water ( $Cl^{-2} = 4600 \text{ mg/1}$ ) and distilled water using approximately 92 mg mercuric sulfate (per two ml sample) and comparing the results to distilled water standards containing only 40 mg mercuric sulfate (standard amount for a regular COD test). Through this testing procedure it was determined that the digested samples required setting overnight to allow the precipitate to completely settle. The results showed that chloride interference even at levels as high as 4600 mg/l, could be successfully eliminated by complexing with mercuric sulfate. Based on these results, all COD analyses made during the project were made by adding approximately 92 mg of mercuric sulfate per two ml sample. The mercuric sulfate was added using a measuring spoon calibrated (based on sodium chloride) to dispense 0.05 gm. Two spoonfuls were added for each two ml sample.

#### Nitrification Study

The nitrification study was conducted in the civil engineering laboratory at NMSU using five laboratory scale submerged upflow filters. Each filter was constructed of 4.4 cm diameter (inside) plexiglass, 17.1 cm in length, and packed with 6 mm diameter ceramic beads to a depth of 15.9 cm. The clean filter void volume was measured as 130 ml. Wastewater was fed into each filter using Cole Parmer metering pumps model nos. C-1760LP and C-1530LP. The filters and the synthetic wastewater were contained in a walk-in environmental chamber which was maintained at a temperature of 30°C. A temperature of 30°C was selected to coincide with that used to culture the catfish at the RTF. The wastewater used throughout the experimental program consisted of a synthetic feed made by combining Roswell saline water (14,000 mg/l TDS, see table 1) with Las Cruces, NM, tap water (400 mg/l TDS) in varying ratios to obtain TDS concentrations of 1000, 3000, 5000, 7000, and 9000 mg/l. Reagent grade ammonium sulfate ((NH $_4$ ) $_2$  SO $_4$ ) and potassium dihydrogen phosphate ( $\mathrm{KH_2PO_4}$ ) were added to yield  $\mathrm{NH_3-N}$  and  $\mathrm{PO_4-P}$  concentrations of 15 and 3.75 mg/l, respectively. From a toxicity standpoint an  $NH_{3}-N$ concentration of 15 mg/l was considered to be the maximum level tolerable by a catfish culture system. At a temperature of 30°C and a pH of 7.0 (conditions similar to those at the RTF) unionized ammonia  $(NH_3)$  is 0.8 percent of the total ammonia. Under these combined conditions (NH $_3$ -N = 15 mg/l, pH = 7.0, temp =  $30^{\circ}$ C), the concentration of unionized ammonia would be 0.15 mg/l. This concentration is reported to be the level at which the growth rate of catfish begins to decrease significantly (Piper et. al. 1982). Based on this rational all nitrification filter studies were conducted using an  $\mathtt{NH}_3\text{--}\mathtt{N}$  concentration of 15 mg/l.

Each filter was seeded with a nitrifying culture isolated for municipal wastewater secondary effluent and soil from an alfalfa field. The seed was developed by mixing two liters of secondary effluent from the Las Cruces, N.M. wastewater treatment plant and 100 gm of soil with ten liters of an enrichment medium containing 0.01M KH<sub>2</sub>PO<sub>4</sub>, CaCO<sub>3</sub>, and MgSO<sub>4</sub>·7H<sub>2</sub>O and 0.05M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and NaCl. The enrichment medium was aerated for six weeks using compressed air before seeding the filters. The presence of nitrifiers was verified by qualitatively measuring NO<sub>3</sub>-N. The filters were seeded by recirculating the enrichment medium through each filter at a rate of 5.0 ml/min. Oxygen was provided by directly aerating the enrichment medium. The NH<sub>3</sub>-N and PO<sub>4</sub>-P concentrations of the recycle were periodically adjusted to 1400 mg/l and 310 mg/l, respectively. The pH of the recycle was maintained between 7.0 to 7.2 by daily adjustments using 1N Na<sub>2</sub>CO<sub>3</sub>. The recycle was continued for 30 days until significant growth was established in the filters.

Once the filters were seeded, the enrichment medium was replaced with synthetic wastewater having TDS concentrations of 1000, 3000, 5000, 7000, and 9000 mg/l and NH $_3$ -N and PO $_4$ -P concentrations of 100 and 25 mg/l, respectively to maintain a N:P ratio of 4:1. The N:P ratio was selected according to the generally accepted stoichiometric elemental composition of common bacteria (Gaudy and Gaudy 1984). The water was continuously recycled through the filters to further develop and acclimate the seed to a specific salinity level. The pH and NH $_3$ -N concentration of the waters were periodically monitored and adjusted. The pH was maintained between 7.0 to 7.2. The NH $_3$ -N concentration was allowed to decrease to below 15 mg/l then was adjusted to 100 mg/l by adding (NH $_4$ ) $_2$ SO $_4$ . Distilled water was periodically added to makeup losses due to evaporation. Approximately every two to three weeks the entire solution was replaced with a fresh medium. The acclimation recycle process was maintained for approximately 60 days.

The final nitrification study consisted of four experiments. During the first three experiments the synthetic wastewater was continuously recycled through the filters. In the fourth experiment the filters were operated in a flow through/recycle combination to simulate a continuously operating wastewater treatment system. During all experiments the volume of the feed water prepared was 15 liters. The initial NH<sub>3</sub>-N concentration of the feed waters was always 15 mg/l. Oxygen was provided by aerating the feed solution with compressed air. The pH of all systems was monitored frequently and was periodically adjusted to 7.0 to 7.2 using 1N Na<sub>2</sub>CO<sub>3</sub>. NO<sub>3</sub>-N concentrations were measured by the Hach Chemical Company cadmium reduction method using a Bausch and Lomb Spectronic 70 spectrophotometer. Total and volatiles solids were measured using the procedures outlined in Standard Methods for the Examination of Water and Wastewater (198); sections 209D and 209G, respectively.

Experiment No. 1. The objective of this experiment was to determine the influence of salinity on the nitrification process under a fixed loading condition. During this experiment the initial synthetic feed was continuously recycled for a period of eight days at a hydraulic loading rate of 4.7 m/day. This loading rate produced an initial nitrogen loading rate of 0.42 kg NH<sub>3</sub>-N/m<sup>3</sup>-day. Samples were collected once per day and were analyzed for their NO<sub>3</sub>-N concentration. Distilled water was added every day to make up losses due to evaporation.

Experiment No. 2. The objective of this experiment was to determine the combined effect of salinity and hydraulic loading rate on the nitrification process. During this experiment the initial synthetic wastewater was continuously recycled through the filters for a 24 hour period. At the

end of 24 hours samples were collected and analyzed for their  $No_3$ -N concentrations. A fresh wastewater was made and was then recycled for an additional 24 hours. Five hydraulic loading rates were evaluated. A summary of the loading conditions is presented in table 2. The loading rate was progressively increased from 4.7 to 37.9 m/day. During this experiment, three days were allowed for acclimation to the increased loading rate. On the and fifth days, samples were collected and analyzed. The objective of this experiment was to evaluate the in-Experiment No. 3 fluence of salinity on the nitrification process under a fixed hydraulic loading condition. During this experiment the initial synthetic wastewater was continously recycled for a period of 48 hours at a hydraulic loading rate of 18.9 m/day. This loading rate produced an initial nitrogen loading rate of 1.66 kg  $NH_3$ -N/m $^3$ -day. During this experiment only salinity levels of 3000, 5000, and 7000 mg/l were tested. Samples were collected every six hours and were analyzed for their NO2-N concentrations. Distilled water was added periodically to make up for losses due to evaporation.

Experiment No. 4. The objective of this experiment was to simulate a continuously operating nitrification system by operating the filters in a flow through/recycle mode. In the previous experiments the filters were operated in a purely recycle mode and therefore produced no final effluent. During this experiment a final effluent was discharged by the filters. Only two salinity levels were evaluated, 3000 and 5000 mg/l TDS. Both filters were operated at a hydraulic loading rate of 18.9 m/day. The recycle was created by pumping effluent from the filter to the influent feed line to be combined with the fresh feed. Recycle ratios (recycle flow/fresh feed flow,  $Q_r/Q$ ) that were evaluated included 0.33, 1.0, and 3.0. Each recycle ratio was evaluated for a period of three days with a one day acclimation period

TABLE 2

Summary of conditions created by varying the hydraulic loading rates during experiment no. 2

Filter Hydraulic Loading Rate, m/day	Initial Nitrogen Loading Rate, kg NH <sub>3</sub> -N/m <sup>3</sup> -day	Void Volume Detention time, min	Hydraulic Passes per Day
4.7	0.42	26	55
9.5	0.83	13	111
18.9	1.66	6.5	221
28.4	2.49	4.3	333
37.9	3.32	3.3	443

between different ratios. Samples were collected from the final effluent line once per day and were analyzed for their NO<sub>3</sub>-N concentration. At the end of this experiment the packing material from all five filters was removed to determine the total and volatile solids concentration of the contents. The percent volatile solids level was determined to obtain an estimate of biological mass contained within each filter.

#### Respirometer Study

A respirometer study was conducted using a Pneumatic Computerized BOD (PC/BOD) Respirometer developed by Drohbyczer (1985). Continuous exertion of nitrogenous BOD was measured over a period of five days. Salinities of 1000, 3000, 5000, 7000, and 9000 mg/l TDS were tested. Each PC/BOD Respirometer bottle was filled with one liter of synthetic wastewater containing 15 mg/L NH $_3$ -N and 3.75 mg/l PO $_4$ -P. Seed for each reactor was provided by adding 50 ml of the feed waters being recycled through the submerged nitrification filters.

#### RESULTS AND DISCUSSION

#### Wastewater Characterization Study

On three different dates (June 29, 1984; July 27, 1984; and August 17, 1984), 24-hour composite samples were collected from the effluent being discharged from the fish culture tanks at the RTF. The samples were analyzed to determine the nutrient production (excreted metabolites) of the catfishgrowing operation. The results of the three analyses are presented in table In general, the results show that the nutrient production for most parameters increased between the first and second sampling periods. The increase in the parameter concentrations was due to the increase in the fish biomass corresponding to the respective periods. Total fish weights within the specific salinity groups corresponding to the water sampling dates are shown in table 4. Between the first two sampling periods total fish weights increased by an average of 30 percent. Between the second and third sampling periods the flow rate of water through the culture tanks was reduced from 5.7 to 3.8 1/min and the total weight of fish was reduced by approximately one half. The flow was reduced to conserve water usage and energy required to heat the water, therefore the fish densities were reduced to accomodate the change in the hydraulic flush rate. Following this change, the concentration of several of the water quality parameters also decreased due to the reduction in fish biomass. In general, the concentrations of all water quality parameters were low due to the high hydraulic flush rate which acted to dilute nutrient accumulation. This result was anticipated because the flow rate was purposely set at a relatively high rate to prevent the accumulation of ammonia thereby avoiding toxicity to the fish. The final  $\mathrm{NH}_3$ -N concentrations for the study ranged between 0.2-0.9 mg/l and averaged 0.4 mg/l. Under the test conditions

Table 3

Characteristics of water discharged from catfish culture tanks at salinity range 1000-9000 mg/l TDS

Parameter	Concentration Range of Parameter (mg/1) at Specified TDS <sup>5</sup>				
	1000	Total Di 3000	ssolved Solid	ls, mg/l 7000	9000
COD	X-20-X	X-31-62	X-24-62	X-8-62	X-12-64
BOD <sub>5</sub>	28-10-X	22-8-6	28-7-7	20-16-4	30-7-2
P0 <sub>4</sub> -P	0.1-1.1-X	0.2-1.1-0.1	0.1-0.6-0.3	0.1-0.7-0.3	0.1-0.2-0.2
Organic-P	0.1-0.1-X	0.7-0.1-X	0.4-0.4-XO	0.5-0.2-X	0.2-0.2-X
NH <sub>3</sub> -N	0.4-0.6-X	0.4-0.9-0.2	0.7-0.6-0.2	0.4-0.6-0.3	0.4-0.3-0.2
N0 <sub>3</sub> -N	0.1-X-X	0.1-0.2-0.5	0.2-0.2-0.3	0.1-0.3-0.2	0.1-0.2-0.2
Organic-N	1.3-1.6-X	0.7-0.8-3.4	0.4-1.1-0.3	<0.1-1.9-2.2	0.7-1.4-2.0
SS	27-24-X	27-49-14	18-19-11	19-17-25	13-22-8
VSS	16-13-X	18-32-7	4-13-6	6-14-16	5-14-6

<sup>&</sup>lt;sup>a</sup>Concentrations of 24 hr composite samples arranged in the following chronological order: 6/29/84-7/27/84-8/17/84, X indicates data not available.

Table 4

Total weight of catfish in culture tanks at time of water quality analysis

Total Dissolved	Total Fish Weight (kg) at Specified Date			
Solids, mg/l	6/29/84	7/27/84	8/17/84 <sup>a</sup>	
1000	59.04	75.33	32.74	
3000	57.71	74.19	31.48	
5000	59.10	76.72	31.42	
7000	52.90	72.12	26.00	
9000	33.50	42.13	37.47	

<sup>&</sup>lt;sup>a</sup>Water flow rate to culture tanks reduced from 5.7 l/min to 3.8 and total fish weight reduced by approximately one-half from previous sampling date.

the unionized  $\mathrm{NH_3}\text{-N}$  concentration was consistently less than 0.01 mg/l which is well below the level recognized to effect the growth rate of catfish (Piper et. al. 1982).

Combining the results of the water quality characterization study with fish weights at the times of sampling generates unit nutrient pro-The average nutrient production by the catfish cultured duction data. under the varying salinities in this study is presented in table 5 and figures 1 and 2. Although the data is somewhat variable, the results indicate that salinity influenced nutrient production. In general, with the exception of  $BOD_5$ ,  $PO_{\Lambda}-P$ ,  $N_2-N$ , and Organic-N, the following trend in nutrient production was observed. Between salinities 1000-3000 mg/l TDS, nutrient production increased to a maximum and thereafter decreased or remained constant. The increase in nutrient production between the salinity range 1000-3000 mg/l TDS indicated that catfish growth was normal or slightly stimulated while the decrease in nutrient production indicated that the growth of the catfish in the salinity range above 3000 mg/l TDS was adversely effected. The results of the catfish growth study confirmed these indications. At salinity levels of 7000 mg/l TDS and higher the growth of the catfish was found to be significantly less than at salinities ranging between 1000-5000 mg/l TDS (Turner 1985). As the growth rate decreased, the fish generated less metabolic wastes resulting in a decrease in nutrient production.

The scatter in the nutrient production data was somewhat expected because of the levels at which the concentrations of the contaminants were allowed to accumulate. As previously noted, nutrient concentrations were purposely diluted to prevent ammonia toxicity to the catfish. As a result of the concentrations being dilute, sensitivity in the water quality analysis was decreased resulting in a scatter of the water quality data.

Table 5

Average nutrient production by catfish cultured under salinity range 1000-9000 mg/l TDS

Parameter	Nutrient Pro	duction (	gm/day/kg :	fish) at	Specified	TDS
	Total Dissolved Solids, mg/l					
	1000	3000	5000	7000	9000	
COD	2.18	7.05	6.65	6.95	5.80	
BOD <sub>5</sub>	2.50	1.67	1.97	1.90	3.00	
PO <sub>4</sub> -P	0.06	0.05	0.04	0.05	0.03	
Organic-P	0.01	0.06	0.05	0.05	0.05	
NH <sub>3</sub> -N	0.07	0.06	0.06	0.06	0.06	
NO <sub>3</sub> -N	0.01	0.04	0.03	0.03	0.03	
Organic-N	0.20	0.26	0.22	0.24	0.27	
SS	3.15	3.87	2.15	3.33	2.90	
VSS	1.80	2.43	1.00	1.97	1.60	

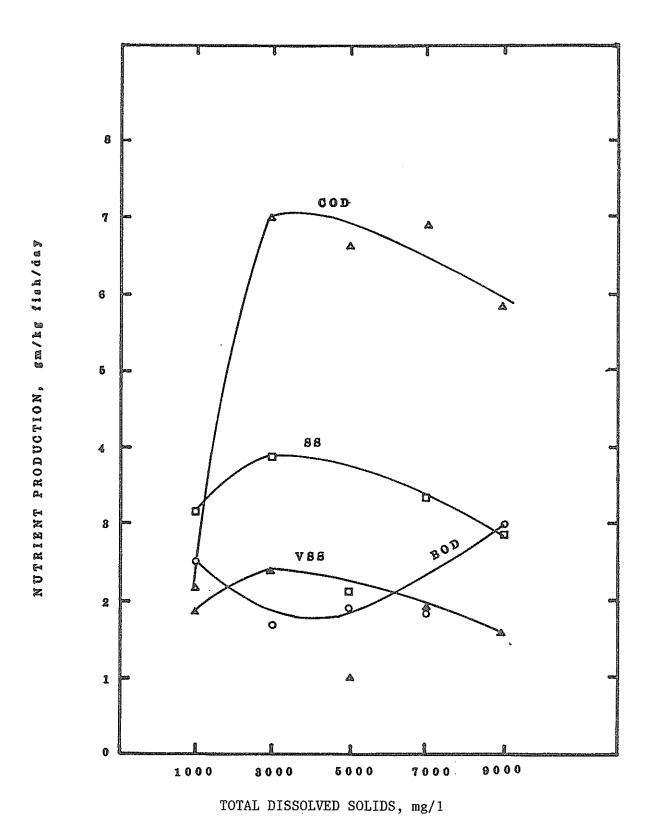


Figure 1. Nutrient production by catfish cultured under salinity range 1000-9000 mg/1 TDS.

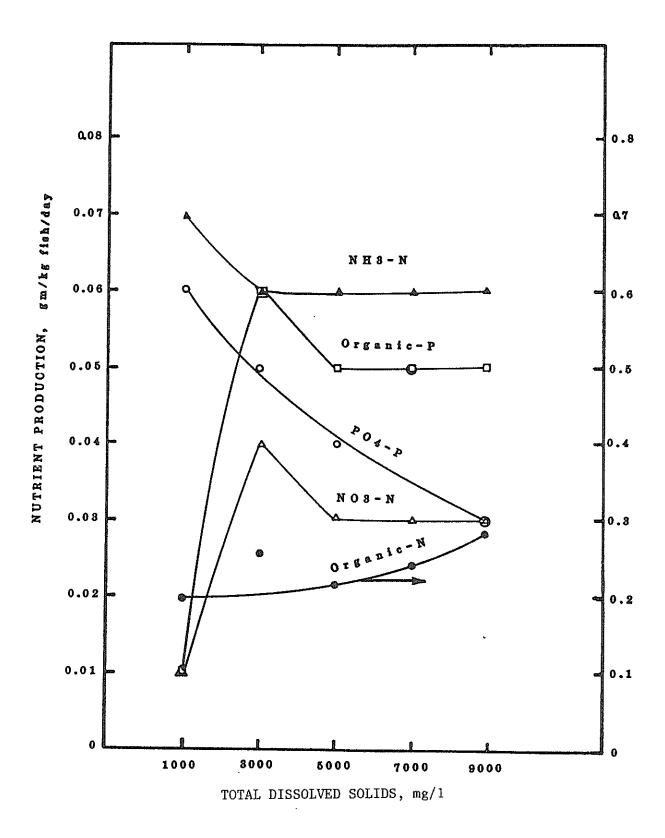


Figure 2. Nutrient production by catfish cultured under a salinity range 1000-9000 mg/1 TDS.

A final summary of the wastewater characterization study is presented in table 6. From this data it is observed that the average concentrations of each water quality parameter were consistently low due to the high dilution rate. With respect to ammonia levels it is clear that the goal of minimizing toxicity was accomplished. Under the experimental conditions the average unionized ammonia concentration was less than 0.004 mg/l and never exceeded 0.01 mg/l. These concentrations are far below the levels reported to significantly decrease the growth rate of catfish (Piper et. al. 1982). It can therefore be concluded that the results obtained during the catfish growth study (Turner 1985) were not influenced by the concentration of ammonia. It was also observed that the majority of the average nutrient production rates obtained during this study were considerably lower than average rates reported in the literature. Of particular interest is the result that significantly less  $\mathrm{NH}_3\text{-}\mathrm{N}$  was produced under the saline conditions. The literature based data was obtained from studies where catfish were grown in fresh water where the TDS concentrations were consistently less than 500 mg/l. The difference between the literature based and present study rates was not uniformly consistent but the findings of the current study averaged around 57 percent less than those obtained from the literature. This result supports the previous conclusion that the high salinity water decreased nutrient production due to reduced fish metabolic activity and waste generation. This finding is significant because with less nutrient production (specifically ammonia), a reduced water rate could be used to culture the catfish. In the growth study conducted by Turner (1985), it was concluded that no problems should be encountered in culturing channel catfish in waters having a TDS concentration as high as 5000 mg/l although a slightly longer time would be required for the fish to reach market size. In a state like

Table 6

Summary of water quality characteristics and nutrient production for saline water catfish culture

		Nutrient Production, gm/kg fish/day		
Parameter	Average Concentration mg/l	Average for Present Study	Literature Based <sup>a</sup>	
COD	38	5.73	1.94	
BOD <sub>5</sub>	14	2.21	2.84	
PO <sub>4</sub> -P	0.4	0.05	0.04	
Organic-P	0.3	0.04	0.16	
NH <sub>3</sub> -N	0.4	0.06	0.36	
no <sub>3</sub> -n	0.2	0.03	0.26	
Organic-N	1.4	0.20	0.05	
SS	21	3.08	3.41	
VSS	12	1.76	5.23	

<sup>&</sup>lt;sup>a</sup>Source: Gordon 1974, Murphy and Lipper 1970, Page and Andrews 1974, Ruane et al. 1975.

New Mexico where saline water is three times more abundant than fresh water, the culture of an aquatic species like catfish could be beneficial from an effective water use standpoint as well as a water conservation standpoint. Culturing catfish in a 3000-5000 mg/l TDS saline water would produce a marketable food product from an otherwise unuseable resource while at the same time conserving what is probably the state's most precious resource, water.

### Nitrification Study

Seeding of the filters was found to be a slow process. In total, 90 days were required to effectively seed the five filters, 30 days using the enrichment medium and 60 days using the final synthetic wastewater containing 100  $\mbox{mg/l NH}_{\mbox{\scriptsize Q}}\mbox{-N}.$  After beginning the recycle of the synthetic wastewater, it was believed that seeding was occuring rapidly because a white material was accumulating quickly in the filters yet the rate of nitrification was very slow. Nitrate production was steady but slow, indicating limited biological activity. On several occasions nitrite-nitrogen  $(NO_2-N)$  was qualitatively checked to determine if the culture may have been deficient in nitrite oxidizing bacteria, Nitrobacter. NO2-N was not detectable. After noticing that a similar white material was accumulating in the feed containers used to recycle the synthetic waste it was discovered that the white material was soluble in dilute hydrochloric acid. The solubility in the acid suggested that this material was an inorganic chemical precipitate rather than biological growth. This suggestion was to be confirmed later in the study when the contents of the filter were analyzed for their volatile solids content. It was also observed that the amount of the precipitate accumulating in the filters increased significantly as the salinity of the water was increased. The recycle was continued until the nitrifier population and its biological activity, as measured by nitrate production, was well established. At this point the experimental nitrification program was initiated. The following sections present the findings of the study.

Experiment No. 1. During this experiment the synthetic waste containing 1000-9000 mg/l TDS and 15 mg/l NH<sub>3</sub>-N was continuously recycled through the filters for a period of eight days. The water was cycled through the filters at a hydraulic loading rate of 4.7 m/day. Samples were collected once per day to determine the NO<sub>3</sub>-N concentration. The results of this experiment are presented in figure 3. The filters operating under salinity levels of 1000 and 3000 mg/l TDS consistently produced twice as much NO<sub>3</sub>-N as the filters operating under salinity levels of 5000, 7000, and 9000 mg/l TDS. The data suggests three possibilities: (1) as previously noted the filters operating under the higher salinities were accumulating a chemical precipitate and were therefore limited in their biological activity as compared to the two lower salinity levels, (2) an increase in salinity from 1000 to 3000 mg/l TDS appears to stimulate nitrification and, (3) the salinity levels 5000, 7000, 9000 mg/l were inhibiting nitrification.

Experiment No. 2. During this experiment the synthetic wastewater containing 15 mg/l NH $_3$ -N was continuously recycled through the filters at hydraulic loading rates ranging between 4.7 to 37.9 m/day. The water was recycled for a period of 24 hours at which time samples were collected and analyzed for their NO $_3$ -N concentration. The results of this experiment are shown in figure 4. Three observations can be made from this data: (1) under all hydraulic loading conditions the filters operating at salinity levels of 1000 and 3000 mg/l TDS produced an average of 80 percent more NO $_3$ -N that the filters operating at salinity levels 5000-9000 mg/l TDS; (2) the filters operating at hydraulic loading rates of 18.9 to 37.9 m/day produced an average of six times more NO $_3$ -N than the filters operating at hydraulic loading rates of 4.7 and 9.5

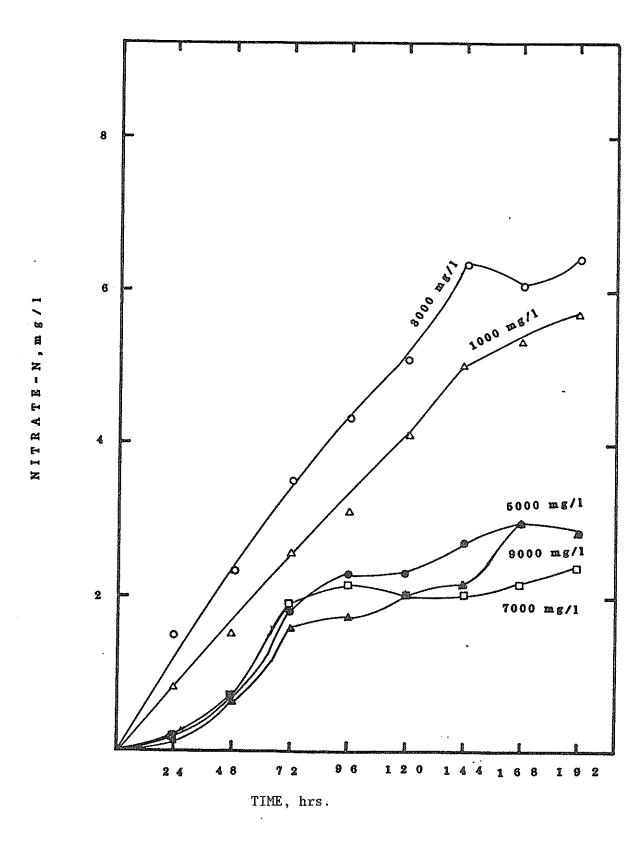
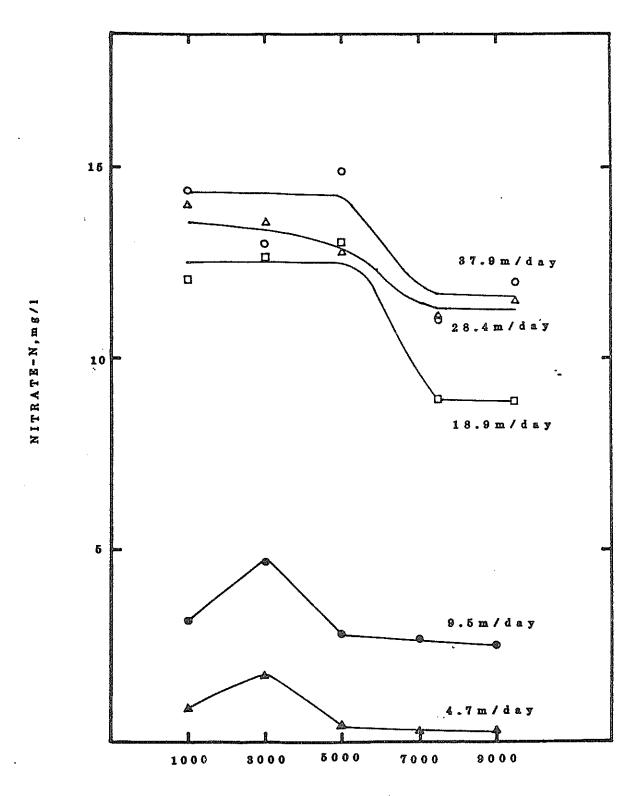


Figure 3. Influence of salinity on nitrate production in the submerged filters at a hydraulic loading rate of 4.7 m/day.



TOTAL DISSOLVED SOLIDS, mg/1

Figure 4. Influence of hydraulic loading rate and salinity on nitrate production in the submerged filters.

m/day; and (3) at the two lower hydraulic loading rates the filter operating at a salinity level of 3000 mg/l TDS produced optimum nitrification while at the higher loading rates an optimum range of 1000-5000 mg/l TDS was identified. The results of this experiment tend to confirm the suggestions from experiment no. 1 that salinity levels 7000-9000 mg/l TDS were inhibitory to nitrification and that a salinity near 3000 mg/l TDS was optimum for nitrification. The results also confirm the previous hypothesis that biological activity was reduced due to accumulation of chemical precipitate in the filters receiving the higher salinity water. As the amount of precipitate increased within the filter, void space available for bacterial growth was decreased and the growth of the nitrifiers was directly inhibited by the minerals contained in the precipitate.

As seen in figure 4, progressively increasing the hydraulic loading rate increased the degree to which nitrification was achieved. The maximum achievable NO<sub>3</sub>-N concentration was 15 mg/l. At hydraulic loading rates of 37.9, 28.4, and 18.9 m/day the maximum NO<sub>3</sub>-N concentrations (NO<sub>3</sub>-N concentrations at 1000-5000 mg/l TDS) averaged around 14.2, 13.3, and 12.9 mg/l, respectively. These concentrations represent 86-95 percent completion of nitrification. At hydraulic loading rates of 4.7 to 9.5 m/day the maximum average NO<sub>3</sub>-N concentrations were 1.7 to 4.3 mg/l, respectively. These concentrations represent 11-29 percent completion of nitrification. Considering the significant difference in nitrification completion between the two ranges of hydraulic loading rates it can be concluded that the higher loading rates would be more appropriate operational parameters for a nitrification system. The filters operating at the higher loading rates were more effective in completing nitrification because the mass transfer of substrate (NH<sub>3</sub>-N) and dissolved oxygen was greater. Under the higher loading conditions the nitrifying bacteria

could oxidize more  $\mathrm{NH_3}$ -N because more oxygen was available. Under the lower loading conditions oxygen became the limiting factor. At the higher loading rates the water cycled through the filters more times (see table 2) and therefore cycled through the feed container more times also. The wastewater was aerated with compressed air in the feed container. In cycling through the feed container oxygen was replenished in the water and was then returned to the filter. The higher hydraulic loading rates provided more mass of oxygen therefore more  $\mathrm{NH_3}$ -N was oxidized to  $\mathrm{NO_3}$ -N.

Experiment No. 3. During this experiment the synthetic wastewater continuously recycled through three filters. Only salinity levels of 3000, 5000, and 7000 mg/l TDS were tested. Following the results of experiment no. 2 filters receiving TDS concentrations of 1000 and 9000 mg/l were eliminated from the testing program because their performance was a duplication of other The filters were loaded at a hydraulic loading rate of 18.9 m/day. This loading rate was selected based on the results obtained during experiment no. 2 and was considered to be capable of achieving a reasonable level of efficiency in an actual nitrification system. The filters were operated in this mode for a period of 48 hours. Samples were collected and analyzed for their NO3-N concentration every six hours. The results from this experiment are presented in figure 5. A comparison of the three nitrate production curves shows that the filter receiving synthetic wastewater containing 5000 mg/l TDS produced nitrate at a much faster rate than the filters receiving 3000 and 7000 mg/l. Nitrification rates (day 1, base 10) for each filter system were -0.54, -1.76, and -0.42 for TDS levels 3000, 5000, and 7000 mg/1, respectively. The filter treating 5000 mg/l TDS also reached its maximum nitrate production 12 hours before the filter treating 3000 mg/l and approximately 24 hours before the filter treating 7000 mg/l. These results suggested that a TDS

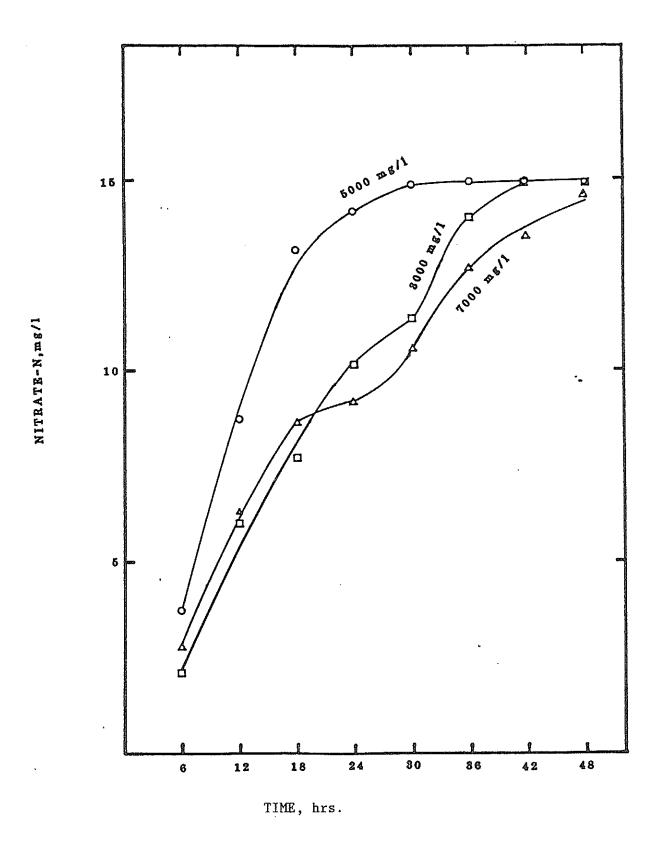


Figure 5. Influence of salinity on nitrate production in the submerged filters at a hydraulic loading rate of 18.9 m/day.

concentration of 7000 mg/l was slightly toxic because it reduced the rate of nitrification but it did not completely inhibit the process. Although a TDS concentration of 3000 mg/l appeared to reduce the rate of nitrification this result was contradictory to the results obtained in the previous two experiments. Based on the results of this and the previous two experiments the decision was made to eliminate the filter treating a salinity of 7000 mg/l TDS in the remaining experiment. This decision was made in an effort to optimize nitrification by an upflow submerged filter treating high salinity water.

Experiment No. 4. During this experiment two filters receiving synthetic wastewater containing NH $_3$ -N concentrations at 15 mg/l and TDS levels of 3000 and 5000 mg/l were operated in a continuous mode with varying degrees of recycle. The ratios (Qr/Q) which were evaluated included 0.33, 1.0 and 3.0. Each filter was operated at an overall hydraulic loading rate of 18.9 m/day. The final effluent from each filter was sampled once per day and analyzed for its NO $_3$ -N concentration. The results of this experiment are shown in figures 6 and 7.

As shown in figure 4 the filter receiving a TDS concentration of 3000 mg/l consistently produced an average of 15 percent more NO<sub>3</sub>-N than the filter receiving 5000 mg/l. Although this result suggests that a salinity level of 3000 mg/l TDS was optimum for nitrification the difference between the two salinity levels was not considered to be significant. In fact, a review of all previous filter data indicates that the difference in the degree of nitrification between salinity levels of 1000, 3000, and 5000 mg/l TDS was not significant. In general, the results indicate that under the salinity range 1000-5000 mg/l TDS, achievement of 75-100 percent of maximum nitrification was possible. This finding is compatible with the results obtained by Jones and Hood (1980). In their study it was determined that for a freshwater

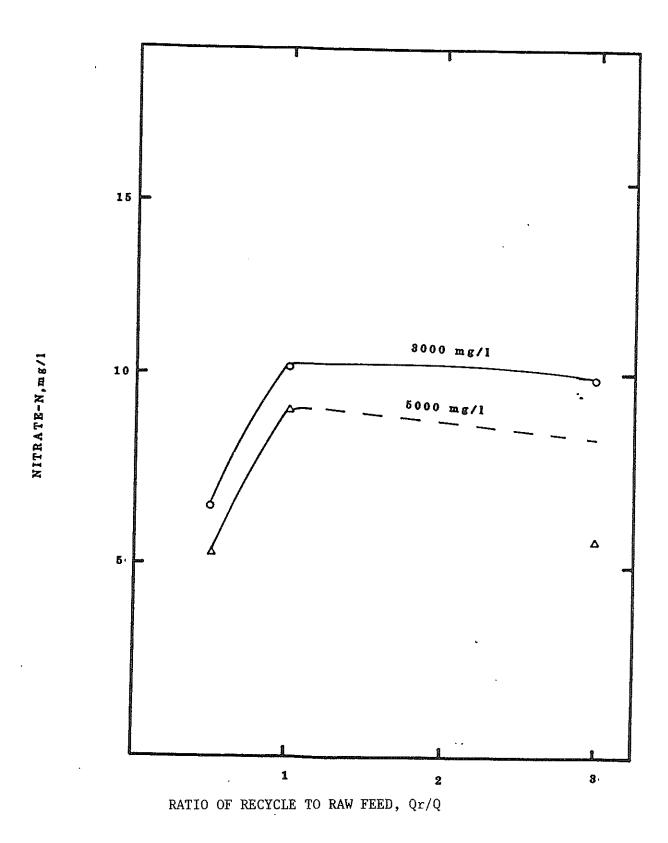
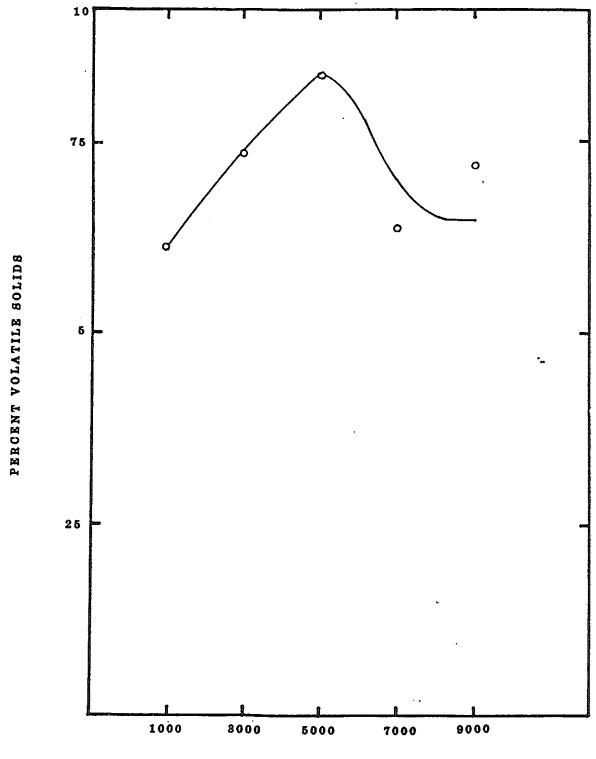


Figure 6. Influence of recycle ratio and salinity on nitrate production in the submerged filter at a hydraulic loading rate of 18.9 m/day.



TOTAL DISSOLVED SOLIDS, mg/1

Figure 7. Variation of percent volatile solids in the submerged filters at a salinity range 10000- 9000 mg/1 TDS.

isolate of a <u>Nitrosomonas</u> species optimum nitrification occurred at a salinity range of 3000-5000 mg/l TDS. In the salinity range 1000-5000 mg/l nitrification reached 80-100 percent of maximum activity. At a salinity range of 10,000-30,000 mg/l TDS, nitrification progressively decreased from 55-5 percent of maximum level. In the current filtration study the original inoculum was enriched from secondary sewage effluent and soil, and therefore, was considered to be a mixture of freshwater species.

Speculation as to which specific ions inhibited nitrification at a TDS level above 5000 mg/l was difficult because of the complex makeup of the saline water (see table 1). A summary of the concentrations of the major ions contained in the synthetic wastewaters used in this study is presented in table 7. In table 8 are shown concentrations of cations (studied as a chloride form) which have been determined to produce complete or substantial inhibition of oxygen uptake in Nitrosomonas species (Meiklejohn 1954). A comparison of the two tables shows that the concentrations of the inhibiting ions (table 8) far exceed those contained in the synthetic wastewaters (table 7). Therefore, it can be concluded that the cations shown in table 8 (as well as chloride) did not contribute to the inhibition of nitrification. Chloride ion was also eliminated as a potential inhibitor because the maximum concentration associated with the ions shown in table 7 (Cl = 17,750 mg/l) far exceeded the concentrations contained in the waters used for the study (see table 7). Two elements which showed potential to act in a toxic manner were the heavy metals cadmium (Cd) and selenium (Se) but information specific to the concentrations required to effect nitrification or nitrifier species was not available in the literature. In general, it is very likely that the inhibition resulted from an overall effect of all the ions combined together due to increased osmotic pressure. As salinity was increased the osmotic pressure of the solution was

Table 7

Chemical composition of synthetic wastewaters used during the submerged filter nitrification study

Constituent		Total Disso	lved Solids	, mg/l	
	1000	3000	5000	7000	9000
Sodium, Na	312	937	1562	2187	2812
Potassium, K	2	5	8	11	15
Calcium, Ca	37	111	184	258	332
Magnesium, Mg	11	33	55	77	99
Chloride, Cl	488	1464	2440	3415	4391
Sulfate, SO <sub>4</sub>	102	305	508	712	915
Zinc, Zn	0.03	0.10	0.17	0.24	0.30
Silver, Ag	0.001	0.003	0.01	0.01	0.01
Cadmium, Cd	0.002	0.01	0.01	0.01	0.02
Selenium, Se	0.18	0.53	0.88	1.23	1.58

Table 8

Concentrations of metal ions (chloride form) which produce complete or substantial inhibition of oxygen uptake in Nitrosomonas bacteria

Metal Ion	Concentration
Na, K, Mg	0.5 M (11,500; 19,550; 12,150 mg/l, respectively)
Ca, Sr, Ba	0.2M (8000; 17,500; 27,500 mg/l, respectively)
Fe, Al, Cu	0.01M (560, 270, 630 mg/l, respectively)
Zn, Pb, Mn	0.01M (650, 2070, 550 mg/l, respectively)
Ag	$2.5 \times 10^{-6} \text{ M } (0.27 \text{ mg/l})$

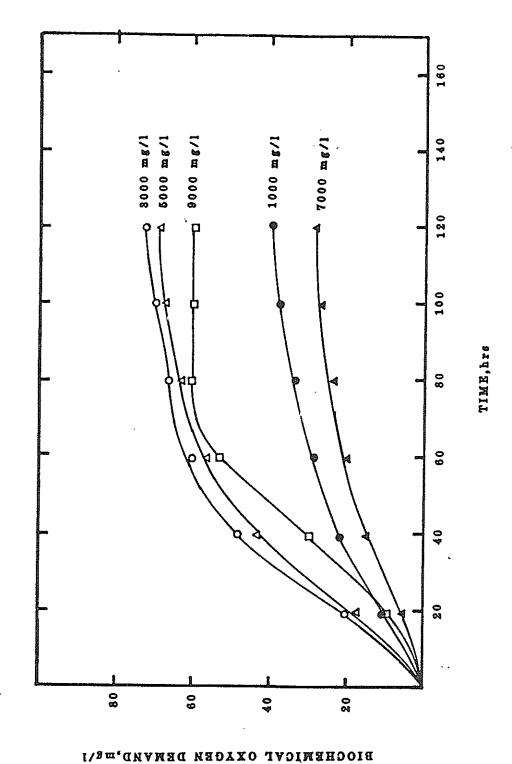
<sup>a</sup>Source: Meiklejohn, 1954

also increased. Since the seed culture was obtained from a fresh water source it was not able to function properly at the higher osmotic pressures created by the higher salinity levels.

Previously it was indicated that during the seeding process an inorganic chemical precipitate began to accumulate in the filters. At the end of the nitrification study the contents from each filter was removed and analyzed for percent volatile solids. Volatile solids was selected as an indication of biological mass. As shown in figure 7, the percent volatile solids of the filter contents was extremely low ranging between 6-9 percent. Volatile solids in most biological filters ranges between 75-85 percent. The low percentage figures confirm the previously stated hypothesis that accumulation of inorganic solids reduced the level of biological activity within the filters and may have been a contributing factor to the inhibition of nitrification at the higher salinity levels. Although the data suggests that for salinity levels of 1000 to 5000 mg/l TDS, the biological activity was steadily increasing towards an optimum and thereafter decreasing, the overall difference in percent volatile solids was not significant. Therefore it is difficult to reach a definite conclusion from this particular data regarding salinity and its influence on the growth of nitrifying bacteria.

# Respirometer Study

In the respirometer study exertion of nitrogenous BOD at a salinity range of 1000 to 9000 mg/l TDS was continuously measured over a period of five days. Each reactor contained an NH $_3$ -N concentration of 15 mg/l. The results of this study are presented in figure 8. With the exception of salinity levels 1000 and 9000 mg/l TDS the results of the BOD test were similar to the results of the filtration experiments. The salinity level 3000 to 5000 mg/l TDS exerted the maximum BOD while the reactor containing a salinity of 7000 mg/l exerted



Exertion of nitrogenous biochemical oxygen demand at a salinity range  $1000-9000~\mathrm{mg/1\ TDS.}$ Figure 8 .

Table 9

Analysis of data from the respirometer study conducted at a salinity range  $1000-9000~\mathrm{mg/1~TDS}$ 

Total Dissolved $BOD_5$ Final $NO_3$ -N Solids, $mg/1$ $mg/1$ Concentration	BOD <sub>5</sub>	$BOD_5$ Final $NO_3$ -N Theoretical $mg/1$ Concentration, $mg/1$ $BOD_{\tt t}$ , $mg/1^a$	Theoretical BOD <sub>t</sub> , mg/1 <sup>a</sup>	Final Requirements, $ \text{mg } 0_2/\text{mg NH}_3\text{-N}^b $
1000	42	9.2	42	4.6
3000	74	14.7	89	5.0
2000	69	14.8	89	5.0
7000	29	13.7	63	2.1
0006	09	13.4	62	4.5
				all disperses in the disperse of the second

 $^{\rm a}$ Determined by multiplying final NO  $_{\rm 3}\text{-N}$  by 4.6

 $<sup>^{\</sup>mathrm{b}}\mathrm{Determined}$  by dividing  $\mathrm{BOD}_{\mathrm{5}}$  by final  $\mathrm{NO}_{\mathrm{3}}\text{-N}$ 

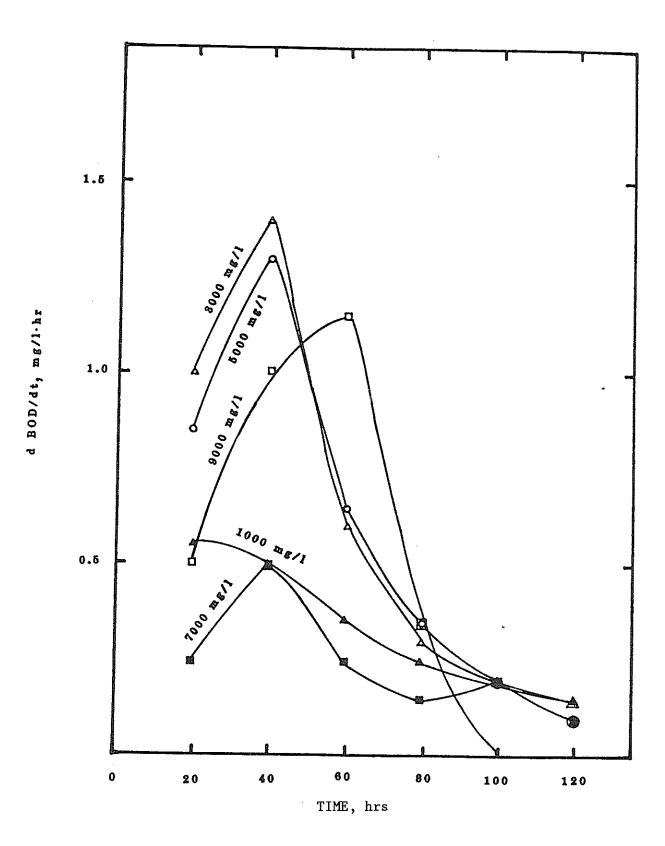


Figure 9. Incremental rate of exertion of nitrogenous biochemical oxygen demand at a salinity range 1000-9000 mg/l TDS.

the minimum BOD. The reversal in performance of the reactors containing salinity levels 1000 to 9000 mg/1 TDS could not be explained. On a stoichiometric basis the biochemical oxygen requirement for complete nitrification is 4.6 mg  $0_2$  per mg NH<sub>3</sub>-N (Gaudy and Gaudy 1984). Because each reactor contained 15 mg/1 NH<sub>3</sub>-N the maximum theoretical BOD which could be exerted was 69 mg/1. A summary of the final five-day BOD and the corresponding NO<sub>3</sub>-N concentrations are shown in table 9. Additional information presented in the table includes the theoretical BOD (BOD<sub>t</sub>) excepted based on a stoichiometric requirement of 4.6 mg  $0_2$ /mg NH<sub>3</sub>-N and the final NO<sub>3</sub>-N concentration and the calculated oxygen demand (mg  $0_2$ /mg NH<sub>3</sub>-N) exerted by the respirometer system. This analysis shows that only the reactor containing a salinity level of 7000 mg/1 TDS deviated significantly from stoichmetric requirements. Excluding a salinity level of 7000 mg/1 TDS the average oxygen demand was 4.7 mg  $0_2$ /mg NH<sub>3</sub>-N.

An estimate of the incremental rate at which the BOD was being exerted was determined by calculating the change in the exertion of nitrogenous BOD over 20 hour time increments (dBOD/dt, mg/l-hr). The results of this analysis are shown in figure 9. In general, over the first 40 hours of testing the change in the rate of BOD exertion steadily increased and reached a maximum. Thereafter the rate steadily decreased theoretically approaching zero. For each 20 hour increment salinity levels of 3000 and 5000 mg/l TDS exerted the maximum oxygen demand rate. Overall, the results of the respirometer study tend to verify the results of the filter evaluation study. Salinity levels of 3000 and 5000 mg/l TDS optimized nitrification while salinity levels greater than 5000 mg/l tended to inhibit nitrification.

## Final Summary

The results of this study have demonstrated that a salinity range of 1000 to 5000 mg/1 TDS was not inhibitory and may have even improved nitrification

while a salinity level greater than 5000 mg/l was clearly inhibitory to the nitrification process. This finding was compatible with the results of the catfish growth study (Turner 1985) which showed that catfish could not be feasibly cultured at a salinity level greater than 5000 mg/1 TDS. Therefore, the upper limit on salinity for the overall success of culturing channel catfish (from a standpoint of culture efficiency as well as treatment efficiency) using a saline groundwater from Roswell, NM, is 5000 mg/1 TDS. In future applications to treatment of wastes generated by saline water aquaculture, such as catfish culture, the submerged filter has good potential for being used to optimize water usage and minimize ammonia toxicity to the fish. A potential treatment system might include sedimentation to remove suspended solids and a submerged filter to achieve nitrification. Suspended solids removal would be essential as a pretreatment step to prevent filter clogging. Filter clogging is still a potential problem because the saline water used in this study produced a chemical precipitate which could eventually fill the filter void space and cause flow obstruction. Past experiences with submerged upflow nitrification filters indicate that clogging by bacterial growth is also a potential problem (Haug and McCarty 1972). Once in operation the filters could be hydraulically loaded at a rate ranging between 18.9 and 37.9 m/day. Although only a rate of 18.9 m/day was tested by this study under a continuous operation mode, the results suggest that a filter could function satisfactorily at loading rates up to 37.9 m/day. Under these loading conditions a filter could produce a treatment (nitrification) efficiency of 65-75 percent oxidation of ammonia. This treatment efficiency should consistently maintain the unionized ammonia concentration below the inhibitory level of 0.15 mg/1 (Piper et al. 1982) and allow the water to be recycled through the catfish culture system at a fairly extensive rate.

#### CONCLUSIONS

Based on the results of this investigation the following conclusions can be drawn:

# Wastewater Characterization Study

- 1. The results obtained by the catfish growth study conducted at the Roswell Test Facility (Turner 1985) were not influenced by ammonia toxicity. Because of the high hydraulic dilution rate, unionized ammonia concentrations (maximum 0.01  $\text{mg/l NH}_3$ ) did not approach a concentration toxic to channel catfish (0.15  $\text{mg/l NH}_3$ ).
- 2. Increasing the salinity level from 1000 to 3000 mg/l TDS stimulated nutrient production, while increasing the salinity from 3000 to 9000 mg/l TDS decreased the nutrient production rates of the channel catfish cultured at the Roswell Test Facility.
- 3. Nutrient production rates measured for channel catfish cultured at the Roswell Test Facility were approximately 57 percent less than nutrient production rates reported in the literature for catfish cultured in freshwater systems (TDS less than 500 mg/l).

### Nitrification Study

- 1. A salinity level of 3000-5000 mg/l TDS was found to be the optimum salinity range for nitrification in the submerged upflow filter.
- 2. Salinity levels greater than 5000 mg/l TDS were found to be inhibitory to nitrification in the submerged upflow filter.
- 3. A specific ion(s) in the saline water from the Roswell Test Facility was not identified as the source(s) of inhibition to nitrification. It is hypothesized that inhibition exerted by the saline water was caused by high osmo-

tic pressure exerted on the nitrifer species which were originally cultured from freshwater.

- 4. Hydraulic loading rates of 18.9 to 37.9 m/day were found to be acceptable operating rates for a submerged upflow filter being used to nitrify a high salinity wastewater.
- 5. At a hydraulic loading rate of 18.9 m/day a recycle ratio (recycle flow/ fresh feed flow) of 1.0 was found to optimize nitrification in the submerged upflow filter. Increasing the recycle ratio to 3.0 did not improve the performance of the filters.
- 6. The results of the respirometer study verified that a salinity range 3000 to 5000 mg/l TDS was optimum for nitrification under high salinity conditions.

### RECOMMENDATIONS

Based on the results of this investigation the following recommendations for future research are made:

- 1. Further investigations should be made to determine the composition of the chemical precipitate formed in the submerged upflow filters and an effort should be made to determine the problems the precipitate may cause to the performance of the filters.
- 2. Further studies to evaluate the influence of recycle during continuous operation of the nitrification filters should be conducted. Specifically the relationship between recycle ratio, salinity, and the level of dissolved oxygen required to achieve acceptable nitrification should be evaluated.
- 3. Ion exchange by clinoptilolite should be evaluated as an alternative to biological nitrification for treatment of saline wastewaters contaminated with ammonia. Specifically, the influence of salinity to improve or decrease the exchange capabilities of the resin should be investigated.

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