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System Analysis and Design of Biofilters for the Three Components of Macrobrachium Production

S.L. Klemetson, D. Cohen

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SYSTEM ANALYSIS AND DESIGN OF BIOFILTERS FOR THE
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Names of Investigators:

Principal Investigator: Dr. Stanley L. Klemetson

Cooperating Investigator(s): Dr. Dan Cohen

Name and Address of Affiliated Institutions:

Principal Institution: The Brigham Young University, Provo, UT 84602, USA

Cooperating Institution(s): The Hebrew University of Jerusalem,
Life Science Institute, Jerusalem, ISRAEL

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Signatures:

Principal Investigator

Stanley L. Klemetson

Institution's Authorizing Official

Neal E. Lambert

Neal E. Lambert
Associate Academic Vice President

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B. ABSTRACT

Water quality maintenance is a primary technical factor limiting the intensification of closed system aquaculture. Allowing for gradual replacement, good water quality can be maintained by passage through biological filters where bacterial polycultures oxidize the toxic wastes, principally ammonia and organic substances, rendering the water fit for reuse. At the practical level, however, not enough was known about the basic design of biological filters to permit straightforward specification of appropriate filters to serve particular aquacultural purposes.

This research was a joint effort between Dr. Stanley Klemetson of The Brigham Young University in the United States and Dr. Dan Cohen of The Hebrew University of Jerusalem in Israel to study the water quality and site development requirements for the production of the Macrobrachium rosenbergii prawn. The work in the United States focused on biofilter design engineering and site development evaluation methods, and the work in Israel focused on the water quality requirements of the Macrobrachium rosenbergii using a stabilized biofilter system. The biofilter types studied were: Rotating Biological Contactor, Biodrum, Trickling Filter, and Submerged Anaerobic Filter.

The final report contains data on water quality requirements, biofilter design methods, site evaluation criteria, and development economics.

C. DISCUSSION OF THE RESEARCH PROJECT

Title of Research

Systems Analysis and Design of Biofilters for the Three Components of Macrobrachium Production

Introduction

Increased levels of aquaculture pond stocking and growout densities are necessary to improve the production rates of aquatic animals. Model aquaculture systems can provide valuable data concerning environmental control for full-scale aquaculture facilities. Information related to stocking density, feeding requirements, temperature control, water use and reuse, and waste removal for intensive aquatic farming on a commercial scale will depend on the technology developed in the laboratory using model systems. If aquaculture is to make a substantial contribution to needed food protein, data will need to be gathered concerning life histories of aquatic animals, growth studies, selective breeding, water quality needs, nutritional requirements, high technology schemes, and related non-technical problems (i.e., education of fish farmers, economics, and marketing).

The chosen aquaculture species for this study was the Malaysian fresh water prawn, Macrobrachium rosenbergii. All three components of Macrobrachium production (larvae, juvenile, and adult) were considered. This study was a joint effort between the Dr. Stanley L. Klemetson of Brigham Young University (formerly at Colorado State University) and Dr. Dan Cohen of the Hebrew University of Jerusalem. In general, the work in the United States involved the engineering and economic analysis of the biofilter wastewater treatment systems and site evaluations, and the work in Israel concentrated on the effects of the water quality on the factors of animal production. The results of each organization were exchanged regularly and used to modify the subsequent direction of the work. The work present in this portion of the final report will focus on the research conducted in the United States. The work in Israel is also presented in summary form using the annual reports.

Scope of Study

The study was broken into eight sections. The first section present a brief review of the literature. This section covers the water quality requirements, the animal characteristics, biofilter design, temperature control methods, and site selection criteria.

The second section presents the computer model used to evaluate the pond temperatures. The Maintenance of Aquaculture Pond Temperatures computer model (MAPT) was developed by the Klemetson previously and applied to this study. This study dealt with the evaluation of pond temperature under various climatic and site conditions. Included are a discussion of pond temperature maintenance methods.

The third section presents the procedures and results of the study of laboratory scale rotating biological contactors. The fourth section presents the procedures and results of a laboratory scale study conducted utilizing four types of biofilters: Rotating Biological Contactor, Biodrum, Trickling Filter, and Anaerobic Submerged Filter.

The fifth section presents research conducted on ammonia removal and nitrification. It includes a study of inhibition methods necessary for analysis of biofilter performance.

The sixth section of the study analyzed data on the modeling and design of biological filters. While extensive data has been gathered, only a summary of the data will be presented.

The seventh section presents the engineering criteria for aquaculture site development. Included are the physical, chemical, biological, economic, legal, and social factors in site development.

The eighth section presents a summary of the work conducted in Israel. The annual reports and other data exchanged were the source of the information presented.

Section 1. Literature Review

Macrobrachium rosenbergii

There are over 100 species of the genus Macrobrachium distributed over the tropical and semi-tropical waters of the world. Prawns are catadramous; spending most of their lives in freshwater, but returning to brackish water to spawn (Hanson and Goodwin, 1977). Most are limited to waters that maintain annual temperatures of 22 to 30 °C. The most popular species for culture is the Malaysian prawn, Macrobrachium rosenbergii, because of its growth rate, tolerance of a wide temperature range and salinity changes, short larval period, and less cannibalistic nature than other Macrobrachium species. The Malaysian prawn is now found in many areas of the world although it is native to Southeast Asia, Indonesia, Northern Australia, and the Palu Islands (Kawaratani, 1978).

Adult prawns are capable of living in freshwater and may be either intensively or extensively cultured. Intensive culture means rearing in tanks or raceways at high stocking density, while extensive culture consists of growth in earthen ponds at a lower density. Marketable size prawns may be harvested in five to six months under optimal conditions. An overview of freshwater prawn culture was prepared by Glude (1978). Descriptions of rearing techniques were presented by Dugan et al. (1975) and Smith et al. (1974).

The development of prawn larvae to metamorphosis requires temperatures between 24 and 32 °C. The preferred temperatures are 28 to 30 °C. These temperatures also apply for commercial growth of adults in ponds. Adults and broodstock may be maintained easily in either freshwater or brackish water. The larvae require brackish water of between 8 and 17 ppt salinity. Juveniles require salinities of about 5 to 8 ppt decreasing to freshwater as they grow into adults.

The respiration rate of Malaysian prawns is not dependent on the oxygen concentration if the concentration is above the tolerance level (Sharp, 1976). While the critical level of oxygen was shown to increase with temperature, a 0.2 g prawn, at 28 °C, has a critical level of 2.9 mg/l dissolved oxygen.

The problem of ideal stocking density has not been completely resolved. Growth rates and survival are significantly less at higher densities (Kawaratani, 1978). Ling suggests stocking density between 5,000 and 15,000 juveniles/ha depending on pond fertility, quality of feeds, and water quality (Ling, 1976). In Hawaii between 50,000 and 70,000 post larvae are stocked annually per acre of pond (Hanson and Goodwin, 1977). The factors involved in determining best stocking density include pond conditions, water quality, territorialistic behavior of males, cannibalism, quality of feed, and water temperature.

Water Quality Management

Ammonia production by fish and invertebrates has been well studied though much of the physiology of excretion is complex and still largely uncertain. In freshwater prawns a series of gills attached to each of the thoracic appendages functions both in respiration and excretion of wastes. Additionally, a large portion of ammonia is removed via fecal material. Antennal glands also function in excretion (McLaughlin, 1980).

Ammonia production rates and Biochemical Oxygen Demand (BOD) production rates of prawns in larval, juvenile, and adult stages were determined by several investigators (Armstrong, et al., 1978; Nelson, et al., 1977; Rogers and Klemetson, 1981). The major concern for application of design data in hatchery and aquaculture biofilter systems is the high water recirculation rates in the hatchery and nursery tanks. A fixed media filter must be capable of handling dilute organic and ammonia loads but at high hydraulic loading. There is little written in the literature concerning treatment of such a waste. Management of the filter is an important consideration in obtaining nitrification and BOD removal from either fresh or saline aquaculture wastewater.

The toxicity of ammonia to aquatic life has been well documented. Wuhrmann, et al. (1947) provided evidence that the unionized fraction of ammonia was toxic to fish. They also showed the unionized portion was largely dependent on the pH of the water (European Inland Fisheries Commission, 1973). Mortality and morbidity to fish is the result of gill hyperplasia, resulting in inadequate transfer of gases and wastes between the capillaries and the aqueous environment (Roberts, 1978).

Although the EPA criterion of un-ionized ammonia for fishes and aquatic life is 0.02 mg/l (US EPA, 1976), various species of fish exhibit varying degrees of susceptibility. The 144 hour LC_{50} (concentration of un-ionized ammonia that is lethal to 50% of the organisms tested under given conditions for the specified time period) for *Macrobrachium rosenbergii* was determined by Armstrong et al. (1978), to be 0.80 mg/l ammonia at a pH of 7.6. Ranges of toxic ammonia concentrations from 0.02 to 2.0 mg/l un-ionized ammonia have been reported for freshwater aquatic life (US EPA, 1976). Additional work is necessary to verify and establish these toxic limits of un-ionized ammonia especially for the Malaysian prawn.

Nitrite has also been shown to be toxic to fishes and invertebrates. Smith and Williams (1974) found Salmonids to be extremely sensitive to nitrite. In their study, gross examination of gill tissue and blood provided evidence that the fish suffered from methemoglobinemia involving the oxidation of hemoglobin to methemoglobin by nitrite. The fish die from anoxia, since methemoglobin is unable to transport oxygen. The criterion for nitrite was not considered in Water Quality Criteria by the US EPA, since levels toxic to fishes are not usually found in natural waters. However, levels of nitrite accumulation should be considered in a reuse facility for production of fish and invertebrates. An LC_{50} value of 5 mg/l nitrite during a 168-hour exposure was found for freshwater prawns (Kawaratani, 1978). The maximum level of nitrite tested with no deaths ranged from 9.7 mg/l for 24 hours to 1.8 mg/l at 168 hours.

Table 1 presents water quality criteria that should be considered in evaluating a water supply for aquaculture development. The suggested level for each parameter is based on evaluation of water quality levels listed in five publications (US EPA, 1976; Roberts, 1978; Klontz, 1979; Nightingale, 1976; and Environment Canada, 1979). The most critical parameters for aquaculture development are ammonia, carbon dioxide, dissolved oxygen, nitrite, pH, temperature, and heavy metals. Continual monitoring of those critical parameters throughout operation of the fish culture facility would be costly. As a result, water quality monitoring during production has, in most extensive culture facilities, been limited to routine analysis of pH, dissolved oxygen, and suspended solids. In recirculating intensive culture systems, many additional water quality parameters need to be evaluated since productivity and mortality would be adversely affected by unacceptable levels of toxic organic and inorganic compounds in the aquatic environment.

Biofilters

Several investigators have described water recirculating systems using biofilters for intensive cultivation of salmon smolts (Burrows, 1964; Meade; Risa and Skjervold, 1975), catfish (Brousard and Simco, 1976), trout (Harris, 1977; Fyock, 1977; Mayo, 1976; and Speece, 1973), shrimp (McSweeney, 1977; Mock, et al., 1975; Siddal, 1974), tilapia (Otte and Rosenthal, 1979; Allison, et al., 1980), polyculture (Van Gorder, 1980), carp (Meske, 1976; Bohl, 1977), mariculture of clams and oysters (Forster, 1974), and the combination of fish culture with hydroponics in a single recirculation system (Lewis, et al., 1978). Few of the authors, however, discussed the basis for their choice of biofilter design parameters. There is little indication that the designs conform either to economic or to resource minima for their declared purposes. Microbial population activity in the filter is essentially ubiquitous in distribution, spontaneously colonizing new filters, although inoculation can be expedited by addition of gravel from preexisting filters (Risa and Skjervold, 1975). The bacterial species that predominate at various depths of the filter are presumably self-selected, in response to environmental factors, such as the nutrient content, hydraulic flow, and aeration of the filter bed, although their respective numbers are known to fluctuate until an equilibrium is established (Kawaii, et al., 1964). Rapid start-up of biofilters is possible by seeding with nitrifiers (Carmignani and Bennett, 1977).

Few approaches to analysis of design standards for biofilters in aquacultural applications are given in the literature. Hirayama reported on filter carrying capacity for a 300 liter marine aquarium containing one or two sea breams, Chrysophrys major (Hirayama, 1966). Spotte (1970) cautioned that Hirayama's results need verification before accepting validity in freshwater, but there is reason to exercise a certain amount of skepticism in generalizing the results even for marine aquaria. One should keep in mind the small numbers of fish on which the results are based. Harris considered use of biological filters in hatchery water reuse systems. Submerged filters were tested and found satisfactory in control of fish wastes. The basis for design of the biofilters used was presented by Speece (1973) for calculating substrate volume based on ammonia production rate divided by the nitrification rate (Harris, 1977).

Table 1. Water Quality Criteria for Aquaculture

Parameter	US EPA	Roberts	Klontz	Nightingale	Canada	Suggested Level
Aluminum (mg/l)				0.2	0.1	<0.1
Ammonia (mg/l)	0.02	0.02	0.012	0.1	0.02	<0.02
Cadmium (mg/l)	1.2-12	0.4-3.0		0.05	0.2	varied
Calcium (mg/l)			52			>52
Carbon Dioxide (mg/l)			2.0			<2.0
Chromium (mg/l)	0.1	0.05		0.5	0.04	<0.1
Coliform (#/100ml)	14					<14
Color Units	75					<75
Copper (mg/l)	1.0	0.01	0.006	0.02	0.005	<0.01
Dissolved Oxygen (mg/l)			5.0		4.0	>4.0
Iron (mg/l)	1.0		1.0	0.5	0.3	<0.5
Hardness (mg/l)				300		<300
Lead (mg/l)		0.03		0.1	0.03	<0.03
Magnesium (mg/l)						no data
Mercury (mg/l)		0.05			0.1	<0.1
Manganese (mg/l)	100					<100
Nitrate (mg/l)						no data
Nitrite (mg/l)		0.1	0.55			<0.1
pH (pH units)	6.5-9	6.5-8.5	6.7-9	6.5-8	6.5-9	6.5-9
Sulfate (mg/l)						no data
Sulfide (mg/l)	0.002	0.002	0.002		0.002	<0.002
Specific Conductance						no data
Temperature (C)				50-90		varies
Total Alkalinity (mg/l)	20		20-200		20	20-200
Total Dissolved Gas		110%	110%	105%		<105%
Total Dissolved Solids	250		400			<400
Total Suspended Solids		8	80		25	<25
Turbidity (mg/l)				60		<60
Zinc (mg/l)			0.04	0.1	0.03	<0.03

References: US EPA, 1976; Roberts, 1978; Klontz, et al., 1979; Nightingale, 1976; and Environment Canada, 1979

The Rotating Biological Contactor (RBC) has gained increased popularity over the past several years. This popularity is due to low power consumption and ease of operation. The transferability of design standards to aquacultural biofilters is limited, however, since aquaculture deals with a portion of the pollution spectrum far removed from domestic wastes. The effluent of treated sewage may contain as much as 5 mg/l ammonia, some ten-fold or more above the toxic limit for aquatic crops, such as shrimp (Cohen, et al., 1976).

The wastewater undergoes progressively increasing degrees of treatment as it flows from stage to stage in the contactor. Organic constituents of the wastewater are in high concentrations in the initial stages of the RBC. Cultures of filamentous and nonfilamentous bacteria develop that degrade to organic compounds. The concentration of organics decreases, in the later stages, and higher life forms, including nitrifying bacteria appear, along with various types of rotifers and other predators (Antonie, 1976).

There are many reviews of biofilter use in the sanitary engineering profession. The annual literature review in the Journal of the Water Pollution Control Federation presented current thinking related to biofilters in environmental problems. The review of literature for 1981 represents a doubling in the knowledge of RBC technology, the result of an RBC conference in Pennsylvania in 1980 (Smith, et al., 1980).

In another study, Antonie et al. (1974) stated that nitrification in a rotating biological contactor begins when the BOD is below 30 mg/l. Nitrifying organisms are able to compete at this concentration with the more rapidly growing heterotrophic organisms and establish themselves in the process. Nitrification is allowed to proceed rapidly, with the establishment of nitrifying organisms, until a BOD concentration of approximately 10 mg/l is obtained and nitrification is complete.

Weng and Molof (1974) found that the chemical oxygen demand (COD) must be below 50 mg/l for nitrification to occur. An artificial substrate with 50 mg/l COD corresponded to a BOD concentration of approximately 14 mg/l. This is less than half the concentration stated by Antonie et al. (1974) for nitrification.

Section 2. Aquaculture Pond Temperature Modeling

The principle purpose of this study was to determine the mechanisms that affect aquaculture pond heat loss and its effect on water quality requirements. An evaluation of factors affecting pond water temperatures and subsequently productivity was carried out using a model developed by the author and entitled, Maintenance of Aquaculture Pond Temperatures (MAPT).

Computer Model Development

The temperature of the water is determined by local climatic factors on the basis of the following energy budget equation (Velz, 1970):

$$H = H_e + H_c + H_r - H_s - H_a$$

where:

H = Net Heat Loss

H_e = Heat Loss By Evaporation

H_c = Heat Loss By Convection

H_r = Heat Loss By Radiation

H_s = Heat Gain By Solar Radiation

H_a = Heat Transfer By Advection

Evaporative Heat Losses - H_e

Using latent heat of vaporization, H_v , for a given water temperature, the evaporative heat losses, H_e , in Btu per hour per square foot of water surface are determined by:

$$H_e = 0.00722 H_v C_1 (1 + 0.1 W)(V_w - V_a)$$

where:

H_v = Heat of Vaporization

W = Mean Wind Speed (MPH @ 25 FT)

V_w = Vapor Pressure of Water (Inches of Hg)

V_a = Vapor Pressure of Air (Inches of Hg)

C_1 = Empirical Constant

10 For Large Deep Lakes

14 For Moderate Flowing Streams

15 For Shallow Ponds

Convective Heat Losses - H_c

Convection is a major source of heat loss within the pond. It is dependent upon the wind velocity, the mixing of the water within the pond, and temperature gradient between the pond and ambient air.

$$H_c = (C_3 + C_2 (W/2))(T_w - T_a)$$

where:

H_c = Convective Heat Loss (BTU/Hr sq ft)

C_2 = Constant

0.16 For Quiescent Water Body

0.24 For Most Water Bodies

0.32 For Flowing Stream

C_3 = Convective Losses From Flat Surface

0.5 BTU/hr sq ft if $T_w - T_a$ Few Degrees F

1.0 BTU/hr sq ft if $T_w - T_a$ 50°F

W = Surface Wind Speed (MPH)

T_w = Surface Water Temperature (°F)

T_a = Ambient Air Temperature (°F)

Radiation Heat Losses - H_r

The pond water acts as a warm body which radiates heat to the colder atmosphere during most of the year. A simplified equation for this loss is:

$$H_r = (T_w - T_a)$$

where:

T_w = temperature water, (°F)

T_a = temperature air, (°F)

Solar Radiation Heat Gains - H_s

While all of the other factors considered have removed heat from the water body, the energy from the sun adds heat to the water.

$$H_s = SR \times 0.1535 \times f$$

where:

H_s = solar radiation gain, Btu/hr-sq ft

SR = Solar radiation, langley/day

f = absorption coefficient at surface 0.95

Advection Heat Transfer - H_a

The water movement into and out of the pond from the surrounding soil also carries heat with it. However, this heat transfer is considered insignificant and therefore has been neglected.

Evaporation Losses - I

Evaporation from the water surface will cause a loss of heat from the water body to a depth of about ten feet. This evaporation will occur naturally whether the ponds are used for aquaculture or not; it also gives a measure of the water balance from the system. The evaporation equation is:

$$I = C_1 (1 + 0.1 W)(V_w - V_a)$$

where:

I = Evaporation (Inches/Mth)

W = Mean Wind Speed (Mph @ 25 Ft.)

V_w = Vapor Pressure of Water (Inches of Hg)

V_a = Vapor Pressure of Air (Inches of Hg)

C_1 = Empirical Constant

10 For Large Deep Lakes

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The amount of geothermal well water required will depend upon the heat balance within the pond and the quantity of water flowing in the system. Assuming that the ponds are full and that the body stays at a constant elevation, the water entering from the well must equal the water flowing out of the ponds and the water evaporating from the ponds. Rainfall and snowmelt have been neglected in this analysis, but they would improve the water balance if they were included.

The heat balance relates the total heat capacity of the well water minus the heat capacity of the water leaving the ponds to the net heat losses computed in the previous section. Therefore, the well production rate can be computed directly when the desired level of net heat losses are known. The total heat capacity of the water is equated to one Btu/lb-°F, and the computations are based on the total heat capacity within the system.

The water from a geothermal well can be exchanged directly with the water in the aquaculture ponds or it can be passed through a heat exchange unit to extract its heat. The direct exchange of water is more efficient, uses less water, and provides more heat to the ponds.

Direct Exchange Water Required

The net capacity of the water coming into the system via the geothermal well and the loss of heat capacity from the water leaving the pond at a given pond temperature can be calculated on the basis of flow rate and changes in water temperatures as shown below:

$$q = Q \gamma (T_{gw} - T_{pw})$$

where:

- q = maximum available heat energy (Btu/hr)
- Q = flow rate of geothermal waters (cfs)
- γ = average specific weight of water (61.38 lb/ft)
- T_{gw} = temperature of geothermal water, ($^{\circ}$ F)
- T_{pw} = temperature of pond water, ($^{\circ}$ F)

Heat Exchange Unit Water Required

The use of a heat exchanger for the transfer of heat from the geothermal well water is less efficient than the direct exchange of water, but it does permit the use of lower quality water. Water is still evaporated from the pond system and will have to be made up from other sources. It is assumed that this system would not have a discharge or that the quantity of water discharged would be reduced and a treatment process would be used on the recycled water. Assuming that the heat exchange unit operates at a 100 percent efficiency and has a log-mean temperature, the following relationship has been derived:

$$q = Q \gamma \frac{(T_{gw} - T_{pw}) - (T_{pw+10} - T_{pw})}{\ln \frac{T_{gw} - T_{pw}}{T_{pw+10} - T_{pw}}}$$

where:

- q = available heat energy (mil Btu/hr)
- Q = flow rate of geothermal water (cfs)
- γ = average specific weight of water (61.70 lb/ft)
- T_{gw} = initial geothermal water temperature (140 $^{\circ}$ F)

T_{pw+10} = final geothermal water temperature ($^{\circ}\text{F}$)

T_{pw} = pond water temperature ($^{\circ}\text{F}$)

Model Input

The data input for each site includes: well flow rate, well temperature, pond depth, pond area, initial pond temperature, and name of well location. Any specific conditions can be evaluated by changing one of these program cards. The model will sequentially evaluate a number of different sites during the same computer run.

The principal factors that affect the heat loss calculations are the wind speed, ambient air temperature, relative humidity, and solar radiation. These are site specific factors that must be obtained for each site, or at least in the general locale.

A number of factors must be computed for use in the heat loss equations. Two of these are vapor pressure and heat of vaporization. Since both of these factors are temperature dependent, it was necessary to develop an approximation equation for each rather than putting the entire tables of data in computer memory.

Plotting of the data for the latent heat of vaporization, H_v , a straight line plot was obtained which yielded the equation:

$$H_v = 1,004 - 0.57 (^{\circ}\text{F})$$

The plot of the saturated vapor pressure data versus temperature yielded a higher level exponential curve. The simplified form of the equation is:

$$\text{VP} = 0.0498 [\exp (^{\circ}\text{F})]^{0.0375}$$

Another factor, C_3 , which is related to the convective losses from flat surfaces was entered as input data but can be computed using the equation:

$$C_3 = 0.5 + 0.01 (^{\circ}\text{F}) \quad \text{Valid range: } (0^{\circ}\text{F to } 50^{\circ}\text{F})$$

Above 50°F , the value is set at $C_3 = 1.0$ and below 0°F , the value is set at $C_3 = 0.5$.

The final temperature of the pond water is determined by iteration using the net heat losses of the pond calculated from climatic conditions and the net heat input from well water. Since this model is using monthly average data, and the water in the ponds can be exchanged completely in a few days, an equilibrium condition can be achieved by iterating 15 times to reach the equilibrium point. Once equilibrium is reached between the heat losses and gains, this temperature is used to initialize the pond temperature for the next month.

Model Output

The output (Table 2) of the model includes the temperature profile of the pond at a specific site throughout the year and for different pond sizes. In addition it also indicates the percent of total annual production available at each site on the basis of twelve months of optimum production rate.

The second output (Table 3) is the productivity profile for the same site on the basis of 100 percent productivity in any given month. Also provided is the annual evaporation rate in inches per year. These values were computed on the basis of the temperature data in the previous output. Also shown in this output is the total productivity on the basis of a year's production potential.

The third output (Table 4) presents the pond surface area in acres that can be supported at a given site for the indicated productivity rate. It will be used to calculate areas that can be used in a management system aquaculture production. Sample runs have been made for sites around the world.

While the basic purpose of the model was to evaluate the heat balance of the aquaculture pond system on the basis of geothermal temperature and flow rate, pond size, and climatic conditions, optional portions of the model can determine the animal production rate and the area of pond surface that can be supported each month at selected production rates. It appears from our studies, that only a narrow strip of the earth between 15°N latitude and 15°S latitude would provide natural pond water temperatures for production of warmwater aquatic animals like prawns. Addition of supplemental heat from geothermal or power plant waste heat would be required in outlying areas.

Application to Heat Loss Reduction

In order to conserve energy and improve productivity, several heat loss reduction mechanisms were evaluated. There are three main classes of evaporation suppression (Magin & Randall, 1960): (1) Wind shear fences and shelterbelts, (2) surface covers and films, and (3) greenhouses.

A review of the literature related to fences and shelterbelts (Von Eimern, 1964) showed that they can reduce wind shear and evaporative losses. Wind speeds may be reduced to 20 percent of ambient winds on the leeward side of a fence for a distance of ten times the height of the barrier. Evaporation rate may be reduced by 20 percent to ten times the height of the fence downwind.

Surface covers and films include aquatic plants, floating plastic covers, floating balls, and monomolecular films. Monomolecular films (cetylalcohol) could be used if an adequate grid system were constructed for protection from wind and wave action (Nicholaichuk, 1978). The chemical film would not affect growth of animals though submerged aeration and feeding may be required.

Table 2. Computer Prediction of Aquaculture Pond Temperatures

Quaculture Pond Temperature Profile Summary for Direct Exchange of Water
Poncha Hot Springs Area

Normal Wind and Normal Humidity				Productivity for Varied Pond Parameters													
Flow cfs	Temp F	Depth Fl	Area Ac	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	Prod Mths	Total Prod Percent
.53	141.00	3.50	.5	84.06	84.30	83.72	85.06	88.04	90.81	94.44	94.44	91.63	88.43	85.96	84.43	12	100.00
.53	141.00	3.50	1.0	70.81	71.56	72.07	74.63	78.58	82.18	86.39	86.18	82.38	77.60	73.54	70.95	12	74.01
.53	141.00	3.50	5.0	50.06	49.58	53.16	58.30	64.55	69.75	74.89	74.35	68.66	60.55	52.77	47.29	5	14.03
.53	141.00	3.50	10.0	46.25	45.03	49.18	55.02	61.81	67.39	72.72	72.14	66.05	61.17	48.47	42.17	4	13.47
.53	141.00	3.50	25.0	43.78	41.87	46.44	52.79	59.36	65.82	71.29	70.67	64.32	54.91	45.55	38.65	4	11.44
.53	141.00	3.50	50.0	42.93	40.74	45.46	52.00	59.31	65.26	70.78	70.16	63.71	54.11	44.52	37.40	4	10.54
.53	141.00	3.50	100.0	42.50	40.17	44.96	51.59	58.97	64.98	70.52	69.90	63.40	53.70	43.99	36.75	4	10.15

Table 3. Computer Prediction of Animal Productivity

Agriculture Pond Productivity Profile Summary for Direct Exchange of Water																	
Flow cfs	Temp F	Depth Ft	Area Ac	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Evap In/yr	Total Prod
.53	141.00	3.50	.5	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	283.26	1200.00
.53	141.00	3.50	1.0	47.42	50.93	53.35	64.89	83.95	100.00	100.00	100.00	100.00	79.30	60.24	48.09	272.43	898.17
.53	141.00	3.50	5.0	0.00	0.00	0.00	0.00	17.99	42.42	66.58	64.05	37.32	0.00	0.00	0.00	262.75	228.37
.53	141.00	3.50	10.0	0.00	0.00	0.00	0.00	0.00	31.33	56.40	53.64	25.04	0.00	0.00	0.00	261.57	166.42
.53	141.00	3.50	25.0	0.00	0.00	0.00	0.00	0.00	23.95	49.65	46.76	16.90	0.00	0.00	0.00	260.88	137.25
.53	141.00	3.50	50.0	0.00	0.00	0.00	0.00	0.00	21.34	47.27	44.34	14.04	0.00	0.00	0.00	260.65	126.99
.53	141.00	3.50	100.0	0.00	0.00	0.00	0.00	0.00	20.01	46.05	43.11	12.58	0.00	0.00	0.00	160.54	121.75

Table 4. Computer Prediction of Pond Surface Area Required for Selected Animal Productivity

Pond Size in Acres That Will Support Various Monthly Productivities												
<u>Flow</u>	<u>Temp</u>	<u>Depth</u>	<u>Prod</u>	<u>Jan</u>	<u>Feb</u>	<u>Mar</u>	<u>Apr</u>	<u>May</u>	<u>Jun</u>	<u>Jul</u>	<u>Aug</u>	
.53	141.00	3.50	25.00	1.29	1.38	1.50	1.99	3.93	25.05	999.99	999.99	
.53	141.00	3.50	50.00	.96	1.01	1.05	1.27	1.94	3.68	28.78	14.58	
.53	141.00	3.50	75.00	.73	.75	.75	.86	1.17	1.73	3.47	3.11	
.53	141.00	3.50	100.00	.56	.57	.55	.61	.77	1.02	1.59	1.59	

Greenhouses would provide the best alternative for maintenance of pond water temperatures, although they can be quite expensive (Walker & Duncan, 1975). Low cost shelters can also be constructed. Conventional heating systems could provide back up for extending the growing season and increasing productivity. Greenhouses should find special application in hatchery and nursery facilities generally smaller than one acre in size. They could also be used in multiple-use facilities for other cash crops like tomatoes or mushrooms in addition to prawn production.

The wind shear fences reduced the wind speed to a minimum of about 20 percent of the prevailing wind speed and evaporation rates by about 20 percent. Either rigid or fluid surface covers can be used on the ponds. The inflatable plastic structures and the floating plastic covers reduce wind speeds to zero and increase humidity to near 100 percent. The monomolecular films provide about 25 percent reduction in evaporation, and the floating ball systems reduce heat losses about 75 percent and liquid losses about 87 percent.

A structural shelter or greenhouse, like the inflatable plastic structures, reduce wind speeds to zero and increase the humidity to near 100 percent. Although there was insufficient information available, it was assumed that the insulating characteristics and the interior temperature gradients above the water surface would significantly reduce these losses.

Application of the computer model to data for two sites are presented in Figures 1 through 4. Figure 1 gives the results of wind speed reduction for a one acre Aquaculture pond with geothermal flow of 1.0 cfs and temperature 100 °F. The pond water temperature range of a one acre pond, 3.5 feet in depth would be approximately 5 to 6 °F for each month of the year. Similar analysis for monomolecular films assuming 25% reduction of evaporation (Figure 2) suggests that the water temperature may be increased by about 3 to 5 °F each month. Figure 3 presents the expected pond water temperature for each month of the year under the same conditions with or without a green house. The green house could increase the pond water temperature as much as 6 °F. Greenhouses, fences, and monomolecular films were compared in Figure 4. The greenhouse would be the best alternative for reducing heat losses.

Conclusions

The use of heat loss reduction methods can be worth considering for development of any site. The following conclusions were reached in this evaluation:

- (1) Wind shear fences and monomolecular films can achieve the same 6 °F increase in pond temperature (Figures 1 and 2).
- (2) Monomolecular films require some wind and wave protection.
- (3) Structural shelters, inflatable shelters, and greenhouses are feasible methods of covering the ponds. Further study is needed to determine the total degree of effectiveness.

- (4) Fixed film or plastic surface covers should be considered as a viable solution to both the heat loss and water loss problem. Subsurface aeration may be required.
- (5) Floating ball systems are questionable until more study is completed concerning their effects on the aquatic animals.

Figure 1. Effect of Wind Speed Reduction on Temperature Profile of One-Acre Aquaculture Pond (Geothermal 1.0 cfs, 100°F, 3.5 ft. depth)

Figure 2. Comparison of Pond Temperatures with and without Monomolecular Film on Surface of One-Acre Pond (assume 25 percent reduction in evaporation rate, Geothermal 1.0 cfs, 100°F, 3.5 ft. depth).

Figure 3. Comparison of Pond Temperatures with and without Greenhouse Cover (no wind, 100 percent humidity with greenhouse, geothermal 1.0 cfs, 100 °F, 3.5 ft. depth).

Figure 4. Projected Pond Temperature Profiles as a Function of Heat Loss Reduction Mechanisms (geothermal 1.0 cfs, 100 °F, one acre)

Section 3. Rotating Biological Contactor Study

Introduction

The pilot study was designed to determine if a rotating biological contactor (RBC) could effectively treat the saline wastewater generated in a closed system aquaculture facility for the production of Macrobrachium rosenbergii (prawns). In a recirculating aquaculture facility, water quality parameters must be kept within prescribed limits to provide optimum conditions for prawn growth and development. The most significant parameters included dissolved oxygen, temperature, pH, unionized ammonia, and organic substances. The rotating biological contactor was evaluated for its ability to maintain these parameters at levels allowing maximum prawn development.

Methodology

Description of the rotating biological contactor. The rotating biological contactor used for the study was a relatively small four-stage unit having a capacity of 0.92 ft^3 (0.026 m^3) with discs in place. Each stage contained five discs having a total surface area of 26.40 ft^2 (2.45 m^2), and a specific surface area of $28.7 \text{ ft}^2/\text{ft}^3$ ($94.37 \text{ m}^2/\text{m}^3$).

The tank was fabricated from a section of 12 inch (30.5 cm) PVC pipe. The tank ends, staging baffles, and discs were all fabricated from plexiglass. The discs have been placed perpendicular to the flow with 36 percent of the discs submerged. The discs were rotated using a 1/4 horsepower Dayton gear reduced electric motor. The discs rotational speed was 14 revolutions per minute yielding a peripheral velocity of 40.8 ft/min (12.4 m/min). A diagram of the rotating biological contactor may be seen as Figure 5.

Description of the RBC System. The RBC was fed a uniform flow from a 1.01 ft^3 (0.03 m^3) holding basin which was kept at a constant head using an overflow weir and two 4.0 ft^3 (0.11 m^3) storage tanks. A schematic diagram of the RBC system may be seen as Figure 6.

The two storage tanks were used to hold the saline synthetic wastewater to be treated by the system. The synthetic sea water was produced using instant ocean sea salts obtained from Aquarium Systems of Eastlake, Ohio. The system was fed a balanced minimal media identical to that used by Stover et al. (1976), in their evaluation of rotating biological contactor performance. A carbon source sucrose, served as the growth limiting nutrient (Stover et al., 1976). The composition of the synthetic feedstock is shown in Table 5. Compressed air was introduced to insure adequate mixing within the two storage tanks.

Figure 5. Simplified Diagram of Rotating Biological Contactor.

Figure 6. Simplified Diagram of Rotating Biological Contactor Flow Scheme.

TABLE 5
Composition of Synthetic Feedstock

<u>Constituent</u>	<u>Concentration</u>
Sucrose	100 mg/l
$(\text{NH}_4)_2 \text{SO}_4$	25 mg/l
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	10 mg/l
K_2HPO_4	6 mg/l
$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	1 mg/l
CaCl_2	0.76 mg/l
$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	0.05 mg/l

The flow through the RBC system averaged approximately 155 liters/day and 134 liters/day for freshwater and saltwater testing respectively. At these flow rates the holding tanks had to be filled on the average of once every ten hours. This frequency was required to insure uniform flow from the constant head tank. All preparation of synthetic feedstock took place in the two holding tanks. The constituents were kept in a concentrated form so that 1 ml of constituent was added for each liter of feedstock solution to be produced. Once the various constituents were added to a holding tank tap water was used to fill the tank to its desired level. Again, compressed air was utilized to insure proper mixing before the new feedstock was allowed to enter the RBC.

Tests Performed on the RBC System. The series of tests performed on the rotating biological contactor in both fresh and saline waters were as follows: Ammonia, chemical oxygen demand, specific conductivity, dissolved oxygen, pH, salinity, and temperature. All tests with the exception of ammonia were performed in accordance with Standard Methods for the Examination of Water and Wastewater, Fourteenth Edition (APHA et al., 1976). Ammonia concentrations were determined using two methods: (1) Orion Specific Ion Electrode, Model 95-10 for ammonia was used on the freshwater samples, and (2) the Nesslerization Method 418B of Standard Methods for Examination of Water and Wastewater, Fourteenth Edition, (APHA et al., 1976) was used on the saline samples.

Results

The freshwater segment of the testing was undertaken to show that the rotating biological contactor could successfully remove ammonia from a freshwater simulated waste, and to achieve loading rate-removal efficiency relationships to compare to those of saline wastewaters. The 10 percent sea water saline segment was undertaken to determine the loading rate-removal efficiency for a wastewater similar to those generated in a aquaculture facility.

The data obtained during the freshwater segment of the study showed the removal efficiency of the contactor to be dependent upon the applied ammonia load. The data showed that the ammonia removal tended to decrease as the applied ammonia loading increased, with a maximum ammonia removal efficiency of 62 percent which occurred at an ammonia loading of 50 grams/100 m²/day.

After completion of the freshwater data collection, the RBC was allowed to acclimate to 10 percent sea water salt concentration. As anticipated, the system's ability to remove ammonia was altered by the increased chloride concentration. The data showed that an increasing percentage of ammonia is removed as the ammonia loading is increased. Although there is an increase in percentage of ammonia removed as the influent ammonia load was increased, the removal efficiencies were lower than those observed during the freshwater segment. From the data obtained the estimated optimal ammonia loading is approximately 60 grams/100 m²/day which yielded an ammonia removal efficiency of 56 percent. Along with ammonia removal, the percentage of unionized ammonia decreased as the ionic strength of the solution increased.

Assuming the increase of ionic strength causes the percentage of unionized ammonia to decrease at a relatively constant rate, the decrease in the percentage of unionized ammonia caused by increasing the salinity from zero to 3.50/00 would be approximately 2 percent. In the previous example it would change the percentage of unionized ammonia in the sample from 1.24 percent to 1.22 percent. This difference can be assumed negligible, and the concentration of un-ionized ammonia can be calculated using freshwater percentage of unionize ammonia tables.

The ammonia loading was the only parameter varied throughout the monitoring program. The artificial substrate yielded an influent COD of approximately 115 mg/l. The total chemical oxygen demand reduction was virtually the same for both the freshwater and 10 percent sea water segments of the study. The weak wastewater experienced a 70 percent average reduction in chemical oxygen demand while being treated in the RBC. The average COD data for each stage were recorded during the study. The chemical oxygen demand in the effluent of Stages II, III, and IV were found to be 33, 32, and 31 mg/l respectively. Based on the method of analysis used these three values are identical. From this it can be concluded that after the second stage of the RBC very little, if any organic removal takes place.

The 10 percent sea water concentration yielded a salinity of 3.5⁰/₀₀ (grams/kilogram). This corresponds to a chloride concentration just under 2,000 mg/l (1,920 mg/l). This chloride concentration is considerably lower than others used in previous saltwater testing. The 10 percent sea water salt concentration was used in this study because it is within the range of salt concentrations required by Macrobrachium rosenbergii.

The specific conductance of the 10 percent sea water increased slightly as it passed through the contactor. The influent specific conductance was 5,500 umho/cm @ 20°C. It increased to 5,800 umho/cm @ 20°C in the first stage and remained constant through the remaining three stages. This increase of 300 umhos/cm represents an increase of dissolved ionic matter of approximately four percent based on the assumption that dissolved ionic matter in mg/l is equal to the specific conductance multiplied by an empirical factor of 0.8 (APHA et al., 1976).

The dissolved oxygen and temperature were also monitored within the contactor during saltwater testing. The influent dissolved oxygen was 0 mg/l and increased to a concentration of 7.6 mg/l in the effluent of the fourth stage. This increase represents a reoxygenation rate of 1.6 mg/l/hour. The temperature of the wastewater decreased as it passed through the contactor. The influent temperature ranged between 28° and 30°C (82° and 86°F) and dropped to between 18° and 15°C (64° and 59°F) in the influent of the fourth stage.

Section 4. Biofilter Studies

Introduction

This research studied the operating characteristics and removal efficiencies of four types of biological treatment units treating both freshwater and saline water wastes. The units studied including a rotating biological contactor (RBC) a biodrum (BD), a trickling filter (TF) and a down flow submerged filter (DSF).

Methodology

The research included the design and construction of the four biofilters. The size and design of these treatment units will be a determining factor in the effective operation of larger scale systems. For example, the height of a trickling filter and downflow submerged filter influences the total surface area and contact time between the wastewater and the biological film. Influential factors for the biodrum and RBC are basin length, width and height, media type, and the percent of submergence.

The research plan also included the design and construction of other components of a wastewater supply system. Once constructed, the biofilters were operated and tested. The synthetic wastewater used as an influent for these biofilters was designed to be representative of waters from a prawn production facility. Complete testing of the water quality parameters was accomplished using EPA approved analytical testing procedures (APHA, 1981). The biofilters were tested at various flowrates to define removal efficiencies for ammonia and biochemical oxygen demand (BOD). From these relationships the optimum biofilter can be designed.

Biofilter Design and Construction

The RBC was obtained from the Clow Corporation of Beacon, New York, as a donation to the project. The basin was constructed of galvanized sheet metal and was 16 1/2 inches long, 5 inches tall, and 12 inches wide as shown in the diagram in Figure 7. The total water volume was approximately 0.41 ft³ (11.5L). Sixteen 10-inch diameter disks were attached to an axle and rotated clockwise, perpendicular to the direction of wastewater flow. The basin was divided into four bays with four discs in each. The total media surface area was 17.9 ft².

The Biodrum consisted of a plexiglas basin, 23 1/2 inches wide, 7 inches high, and 28 inches long, having a total volume of 2.1 ft³ (58.7L). The drum was constructed using two plexiglass discs, 12 inches in diameter, as end pieces which attached to a covered copper shaft. One and one-quarter inch diameter PVC pipe cut one-half inch long served as media. This media was enclosed in a plastic mesh fastened to the end discs, completing the drum form. There were 2000 one-half inch pieces of PVC pipe enclosed in the drum, resulting in 42.6 ft² of surface area. The area of the mesh and end discs were not included as part of the surface area. Water flow was parallel to the direction of the rotation which was in a clockwise manner. The basin was

divided into three compartments. The first compartment was divided from the second by a sectional divider 7 inches high. The influent wastewater flowed into the first compartment, then over the top of this sectional divider into the larger second compartment (where the biodrum is located). From there it traveled across another sectional divider 6-5/8 inch in height into the final compartment, and from there drained out as effluent to be wasted. (See diagram in Figure 8.)

The trickling filter was constructed from a 3 ft. piece of 10 inch diameter PVC pipe. Four inches from the bottom a perforated plexiglass disc was installed to hold the filter media in place, resulting in an under-drain. The freeboard was 1 1/2 inches, leaving an overall filter height of 30 1/2 inches and a 1.38 ft³ volume. The filter media was 1-1 1/2 inch slag having a specific surface of 60 ft²/ft³ (Clark et al., 1977), resulting in a total media surface area of 83.2 ft². Operation of the filter consisted of water flowing in a continuous stream over the filter slag media to the underdrain. Two intermediate sampling ports, located at the 1/3 and 2/3 points along the height of the filter, were constructed to relate filter height to removal efficiency. An additional sampling port was located at the underdrain to sample the final effluent.

The downflow submerged filter was constructed exactly the same as the trickling filter except with a 2 inch freeboard. The height was 30 inches, volume 1.36 ft³, and the media surface area was 81.8 ft². All of the media was completely submerged with wastewater during operation. Operation consisted of wastewater flowing down through the filter rising through a tube to a small basin which was used to regulate the water height in the filter by the principle of equal hydraulic head. Drawings of the downflow submerged filter and the trickling filter are shown in Figure 9. The specifications are summarized in Table 6 in metric units.

System Design and Operation

Tanks, pumps and other related equipment were required to mix, store, and transport the wastewater to and from the biofilters. A plexiglass tank was constructed to house and deliver the synthetic wastewater to all four biofilters through gravity flow. In addition, a wastewater mixing tank, wastewater storage tank, several pumps, a constant temperature room (to keep the ambient air temperature within 28-30°C), and tubing were required. The plexiglass tank was called a constant head tank due to a stand pipe being installed to drain extra inflowing wastewater, thus maintaining a constant depth of water, enabling the flowrate to each biofilter to be controlled. A diagram of the system is shown in Figure 10.

When the water in the mixing tank reached room temperature, synthetic wastewater was mixed. From the storage tank, the wastewater was pumped to the constant head tank at a rate exceeding the outflow. The overflow from the stand pipe returned to the wastewater storage tank. From the constant head tank the wastewater flowed into each biofilter. Flow was regulated by the height of the wastewater in the constant head tank and the height and diameter of the discharge nozzle. The nozzles were disposable hypodermic needles available in a variety of gauges. From the biofilters, wastewater flowed as effluent into a drain in the constant temperature room.

Scale: 1/4
Note: All dims are in inches.

Figure 7. Diagram of the Rotating Biological Contactor (RBC)

Figure 8. Diagram of the Biodrum.

Figure 9. Diagram of Trickling and Downflow Submerged Filter.

Table 6. Specifications of Four Laboratory Scale Biofilters.

Biofilter	Volume Container	Support Media	Biofilm Surface Area
RBC	0.006 m ³	16-25.4 cm discs	1.66 m ²
Biodrum	0.057 m ³	2000-2.5 cm rings	3.96 m ²
Submerged	0.039 m ³	2.5-3.8 cm slag	7.60 m ²
Trickling	0.039 m ³	2.5-3.8 cm slag	7.73 m ²

m³ x 35.31 = ft³; m² x 10.76 = ft²; cm x 0.394 = in.

Figure 10. Overall Wastewater Treatment Scheme.

A synthetic wastewater mix was designed to be representative of actual prawn wastes. The mix is shown in Table 7 and the water quality analysis of the feed is presented in Table 8.

The continuous mix system consisted of several components: a wastewater concentrate storage and constant head tanks, a water supply constant head tank, and a mixing chamber. The wastewater concentrate storage and constant head tanks were placed in the adjacent constant temperature room, kept at between 1-2°C to inhibit microbial growth. The wastewater concentrate was mixed continuously with the water supply in the mixing chamber by an air stone and pumped to the constant head tank supplying wastewater to each biofilter. A diagram of the continuous mixing system is shown in Figure 11.

In order to maintain a consistent hydraulic loading rate, each biofilter influent flow rate was checked three times a day, and the nozzles replaced twice daily.

It was determined that 2 1/2 days were required for each biofilter to reach a new treatment efficiency equilibrium after a change in flowrate. Therefore the biofilters were sampled once a day from Monday through Friday with an adjustment period from Friday to the following Monday morning.

After the biofilters had been in operation, it was notice that there was no biofilm growth on the outside of the PVC rings used as media in the biodrum. There must have been a protective coating which was not conducive to microbiological growth on the outside of the pipe.² This reduced the effective surface area of the biofilter from 42.6 to 23.9 ft². Before this was realized, the hydraulic loading rate was advanced too fast, causing a severe shock load. It took several weeks for the biodrum to recover, resulting in a limited set of results.

Water Quality Analysis

Daily samples were collected from the influent and effluent of each biofilter as well as periodic quality control samples from the mixing chamber. The flowrates entering the mixing chamber were checked twice daily. Tests were performed daily for the water quality parameters considered major. The remaining parameters were tested once a week and assumed to be constant over the five day period.

The major parameters tested with each sample were: ammonia, biocarbonate alkalinity, carbonate alkalinity, COD, dissolved oxygen, nitrite, nitrate, pH, temperature, and total alkalinity.

The parameters not considered as essential were: calcium, chloride, total hardness, magnesium, total nitrogen, specific conductance, sulfate, suspended solids, and total dissolved solids. At the beginning of operation, these parameters were tested every day. The results were consistent enough to be considered constant by testing one sample per week.

Table 7. Substrate Characteristics

Dextrose	100 mg/L
Yeast Extract	10 mg/L
Urea	5 mg/L
Ammonium Chloride (NH_4Cl)	14 mg/L
Na_2HPO_4	40 mg/L
NaHCO_3	125 mg/L

Table 8. Characteristics of Synthetic Feedstock Solution.

Bicarbonated alkalinity	272 mg/l
Biochemical Oxygen Demand	56.3 mg/l
Calcium	51 mg/l
Carbonate Alkalinity	0.0 mg/l
Chemical Oxygen Demand	96.3 mg/l
Chloride	< 1.0 mg/l
Dissolved Oxygen	4.3 mg/l
Hardness	173 mg/l
Kjeldahl Nitrogen	7.0 mg/l
Magnesium	11 mg/l
Nitrate	0.35 mg/l
Nitrite	0.007 mg/l
Sulfate	19 mg/l
Total Suspended Solids	10 mg/l
Total Alkalinity	225 mg/l
Total Dissolved Solids	301 mg/l
Temperature	29.4 °C
pH	7.5

Figure 11. Diagram of the Continuous Synthetic Wastewater Mixing System.

The BOD test was run once a week because of the time involved. A correlation was found between the BOD and COD test results to use for computing the BOD values for the other four days of the week. No BOD tests were completed during the first three test periods, therefore average correlations derived from the results of all correlations were used for this period.

The analytical chemical water quality testing was accomplished utilizing EPA approved procedures in the 15th edition of Standard Methods (APHA, 1981). Table 9 shows a list of methods used.

Table 9. Water Quality Parameters and Methods of Analysis

Ammonia mg/L	Ammonia Selective Electrode Method
Biocarbonate Alkalinity mg/L as CaCO_3	Standard
Biochemical Oxygen Demand mg/L	Standard
Calcium mg/L	EDTA Titrimetric Method
Carbonate Alkalinity mg/L as CO_3	Standard
Chemical Oxygen Demand mg/L	Dichromate Reflux Method
Chloride mg/L	Argentometric Method
Dissolved Oxygen mg/L	Membrane Electrode Method
Hardness (mg/L as CaCO_3)	EDTA Titrimetric Method
Magnesium mg/L	By Calculation
Total Nitrogen	Macro-Kjeldahl Method
Nitrate mg/L	Cadmium Reduction Method
Nitrite mg/L	Standard Colorimetric
pH	Standard Probe
Specific Conductance	Standard
Sulfate mg/L	Turbidimetric Method
Suspended Solids mg/L	Standard
Temperature	Standard
Total Alkalinity mg/L	Standard
Total Dissolved Solids mg/L	Standards

Results

Major parameters of water quality testing were Ammonia, BOD, and COD. These results were reported as removal efficiencies. The results are presented as average values for the period when the hydraulic loading rate was held constant, which in most cases was at least one week. The average influent values for fresh and salt waters are shown Table 10.

In general, the biofilters which aerated the wastewater had the highest removal efficiency. A representative sample of the ammonia removal, nitrite production, and nitrate production for various hydraulic loadings are presented in Table 11. Figure 12 shows the relationship of ammonia removal to hydraulic load for the three biofilters that removed ammonia. The RBC ammonia removal efficiencies ranged from 66.2% to 100%. Note that the RBC removed over 90% of the ammonia at hydraulic loads up to 0.05 cu m/sq m/d (1.2 gal/sq ft/d). The biodrum ammonia removal efficiencies ranged from 86% to 96% and falls below 90% at 0.024 cu m/sq m/d (0.6 gal/sq ft/d). Peak BOD and COD removals 0.016 cu m/sq m/d (0.40 gpd/ft²) and were 98.1% and 97.6%, respectively. Nitrification in the trickling filter is the least efficient, with ammonia removals less than 50% at hydraulic loads above 0.012 cu m/sq m/d (0.3 gal/sq ft/d). BOD and COD removal efficiencies also had their highest values at 0.008 cu m/sq m/d (0.2 gpd/ft²) and were 78% and 77%, respectively. The downflow submerged filter hydraulic loading rates ranged from 0.016 to 0.049 cu m/sq m/d (0.4 to 1.2 gpd/ft²). Ammonia removal efficiencies ranged from -32% to -5%, optimum ammonia addition occurred at a hydraulic loading rate of 0.024 cu m/sq m/d (0.60 gpd/ft²). BOD and COD removal efficiencies also peaked at a flow rate of 0.024 cu m/sq m/d (0.6 gpd/ft) and were 52 and 62 percent, respectively. From a removal standpoint, the RBC seems to be the best process since high removals are observed at a wide range of hydraulic loads.

For the biodrum the two sets of seawater, 4% and 17%, the ammonia removal efficiency was 92% and 90%, respectively; for BOD, 97% and 97%, respectively; for COD 94% and 92%, respectively. For the RBC the two sets of seawater had ammonia removal efficiency which were 94% and 94%; for BOD 97% and 92%; for COD 92% and 90%, respectively. For the trickling filter the two sets of seawater, 4% and 17%, the ammonia removal efficiency was 59% and 54%; for BOD, 79% and 63%; and for COD, 74% and 69%, respectively. For the downflow submerged filter the two sets of seawater, 4% and 17%, the ammonia removal efficiency was -9% and -68%; the BOD, 32% and 14%; and COD was 46% and 40%, respectively.

Figure 13 presents the levels of un-ionized ammonia concentration for the four biofilters at varied hydraulic loadings. The data have been standardized for warmwater aquaculture. It was assumed that in all cases, the water temperature and pH were 30 °C and 8, respectively. The results show that the RBC would provide adequate water quality for warmwater aquaculture (less than 0.02 mg unionized ammonia/l) at hydraulic loading rates up to 0.04 cu m/sq m/d (1.2 gpd/sq ft). The Biodrum could be utilized up to a hydraulic load of 0.016 cu m/sq m/d (0.4 gpd/sq ft). The other processes did not maintain un-ionized ammonia levels below the 0.02 mg/l required for warmwater aquaculture.

Table 10. Influent Water Quality

Parameter	Freshwater Average Value	4% Sea Water Average Value	17% Sea Water Average Value
Ammonia, mg/l	3.6 ± 0.2	3.2 ± 0.2	1.8 ± 2.9
BOD, mg/l	60 ± 16	57 ± 6	65 ± 8
COD, mg/l	96 ± 24	116 ± 3	84 ± 1
Dissolved Oxygen, mg/l	3.7 ± 1.4	4.9 ± -	4.3 ± 0.9
pH	7.5 ± 0.4	7.5 ± 0.5	7.4 ± 0.5
Bicarbonate Alkalinity, mg/l	276 ± 13	262 ± 27	268 ± 11
Nitrite, mg/l	0.013 ± 0.009	0.006 ± 0.004	0.037 ± 0.030
Nitrate, mg/l	0.12 ± 0.009	0.25 ± 0.15	0.15 ± 0.05
Total Nitrogen, mg/l	7.5 ± 1.2	7.1 ± 1.1	7.2 ± 2.2
Temperature, °C	29.5 ± 1.5	28.6 ± 2.1	29.9 ± 0.6
Suspended Solids, mg/l	10 ± 5	10 ± 5	10 ± 5
Hardness, mg/l	172 ± 11	348 ± -	1010 ± 83
Calcium, mg/l	44 ± 9	54 ± -	96 ± 16
Magnesium, mg/l	15 ± 4	50 ± -	187 ± 11
Chloride, mg/l	1 ± 1	712 ± 0.1	3195 ± 61
Sulfate, mg/l	18 ± 6	119 ± -	484 ± 98
Total Dissolved Solids, mg/l	322 ± 29	1657 ± -	6495 ± 146
Specific Conductance, umhos/cm	592 ± 46	3166 ± -	11290 ± 424

Table 11. Percent Ammonia Removal and Nitrate/Nitrite Production at Various Hydraulic Loadings

Date	Biodrum				RBC				Trickling				Submerged			
	NH ₃	NO ₂	NO ₃	Flow	NH ₃	NO ₂	NO ₃	Flow	NH ₃	NO ₂	NO ₃	Flow	NH ₃	NO ₂	NO ₃	Flow
5/17	90	94	97	.020	99	57	95	.021	38	57	63	.017	-53	-33	-30	.016
5/31	95	88	99	.017	98	93	97	.019	42	-33	25	.024	-20	25	53	.025
6/7	82	99	99	.033	97	99	98	.032	39	-90	-40	.032	-3	-75	-85	.032
6/29	82	96	96	.017	84	97	89	.041	41	63	92	.016	-8	-13	-79	.049
7/6	92	93	93	.017	96	78	76	.049	49	-83	39	.009	-22	-67	-77	.017
7/12	84	97	90	.026	93	50	85	.063	56	-87	-27	.009	-28	-70	-63	.024
7/19	96	82	84	.016	69	90	81	.073	-	-	-	-	-28	-83	-79	.033
7/26	92	85	85	.016	92	67	89	.040	45	96	28	.008	-14	-100	-68	.025
8/2	90	75	71	.017	93	74	51	.040	61	76	66	.008	-44	-93	-92	.025

percent ammonia removal
percent nitrate production
percent nitrite production
flow rate in cu m/sq m/d
note: cu m/sq m/d X 24.545 = gpd/sq ft

Figure 12. Relationship Between Ammonia Removal and Hydraulic Loading.

The results clearly show that the RBC and biodrum are the most efficient treatment units from the standpoint of removal efficiency. The trickling filter was reasonably effective. The downflow submerged filter was actually accomplishing denitrification while reducing BOD and COD. The RBC and biodrum aerated the wastewater which allowed aerobic bacteria to metabolize the soluble BOD and COD at a slower rate because of its anaerobic nature, limiting bacterial populations to facultative and strictly anaerobic microorganisms. Anaerobic metabolism has a rate limiting step which does not allow the BOD or COD to be removed as quickly. The trickling filter did not aerate fast enough to replace all of the dissolved oxygen used in the removal of soluble substrate, hence the removal efficiencies are lower.

Figure 13. Comparison of Four Fixed-Film Processes for Maintenance of Un-Ionized Ammonia Levels Required for Fish Culture.

Conclusions

The major conclusions found from this study are the following:

1. The biofilter design parameters should be ammonia removal efficiency, hydraulic loading rate, and their relationship which can be found from empirical data. Ammonia removal efficiency is the most important water quality parameter and when the ammonia effluent concentrations are acceptable, BOD, COD, and dissolved oxygen will generally be within acceptable ranges. However, a check is required to determine if the design will provide acceptable un-ionized ammonia and BOD effluent concentrations.
2. The rotating biological contactor (RBC), biodrum, and trickling filter provided biological nitrification which was effective in removing ammonia to acceptable levels. This is true for the entire range of hydraulic loading rates and ammonia removal efficiencies which were a) RBC, 0.019 cu m/sq m/d (0.46 gpd/ft²) with an ammonia removal efficiency of 98% to 0.11 cu m/sq m/d (2.59 gpd/ft²) with an ammonia removal efficiency of 66%; b) biodrum, 0.016 cu m/sq m/d (0.40 gpd/ft²) with an ammonia removal efficiency of 96% to 0.032 cu m/sq m/d (0.80 gpd/ft²), with an ammonia removal efficiency of 86%; and c) trickling filter, 0.0085 cu m/sq m/d (0.21 gpd/ft²) with an ammonia removal efficiency of 58% to 0.032 cu m/sq m/d (0.79 gpd/ft²), with an ammonia removal efficiency of 38%.
3. The RBC was the most effective biofilter overall in terms of ammonia, BOD, and COD removal efficiencies. The peak ammonia removal efficiency was 100% and at the maximum hydraulic loading rate, it was 66%, the peak BOD removal efficiency was 97%, at the maximum hydraulic loading rate it was 94%, and the peak COD removal efficiency was 97% and at the maximum hydraulic loading rate it was 87%.
4. The trickling filter experienced the lowest heat loss, averaging 1.1° C. Therefore, the trickling filter may be the most economical biofilter if heating costs are high.
5. The downflow submerged filter reduced the concentrations of nitrate and nitrite while increasing the ammonia concentration. Therefore, this biofilter could be used to control nitrate buildup and buffer the pH, but not to perform nitrification.
6. The treatment efficiency of the trickling filter may be improved by increasing the filter height and the aeration capacity through ventilation.
7. Saline waters did not adversely affect the removal efficiencies when the biofilters were tested up to a salinity of 5.7 ppt (17% seawater).
8. Lower BOD influent may enhance nitrification through less competition between the nitrifier and carbon oxidizing microorganisms.
9. Additional heat is necessary to recover the evaporative heat loss in the biofilters.

Section 5. Nitrification Studies

Introduction

In most biofilter studies, nitrification in mixed cultures has been modeled using data from chemical analysis of inorganic nitrogen compounds. In fact, much of the modeling work for fixed-film treatment processes has been based on data with pure cultures of nitrifying organisms which can only be used to study the transformation of ammonia to nitrite and nitrite to nitrate (US EPA, 1975). The populations of microorganisms involved in the nitrogen cycle are complex and difficult to study with available microbiological techniques. A black box approach has been applied to the analysis of these mixed populations considering only what enters and leaves the system. The rates of ammonia oxidation and nitrate production have been studied by varying certain environmental and chemical parameters.

The purpose of this study was to examine individual populations of nitrifying organisms in a complex culture system. The removal of ammonia in this case was considered critical to fish and prawn production. Techniques (most probable number, fluorescent microscopy, and electron microscopy) for determining the relative populations of organisms involved in the nitrification process are cumbersome and of questionable validity. Consequently, it was necessary to modify existing methods and develop new procedures to study microbial populations and kinetics in aquaculture biofilters. Two inhibitors of nitrifier populations were tested in an effort to develop a simple procedure that could give scientists and engineers more information concerning the relative activity of nitrifier populations.

Specifically, the following objectives were considered in this study:

- 1) To determine, by altering environmental and hydraulic conditions, whether fixed-film biological processes could be applied efficiently to completely recycled aquaculture wastewaters where ammonia and nitrite removal is critical for production of warmwater species of fish and invertebrates.
- 2) To utilize available techniques (most probable number, fluorescent microscopy, nitrite and nitrate production, and electron microscopy) for determining relative populations and kinetics of nitrification.
- 3) To use selective inhibitors to gain a more thorough understanding of the biological nitrification process.
- 4) To develop a simple procedure that could aid in evaluating the relative activity of nitrifiers in mixed populations and samples.
- 5) To determine kinetic constants for ammonia oxidizer bacterial populations in aquaculture water reuse biofilters.

Scanning Electron Microscopy

Samples of biofilm were taken from each of the four biofilters and dehydrated by successive exposure for ten minutes each in 30%, 50%, 70%, and 95% ethanol. After three changes of 10 minutes each in 100% ethanol, the samples were placed in acetone. The samples were critically point dried to lessen the chance of distortions caused by surface tension of the evaporating fluid. A Polaron Critical Point Apparatus was used to remove the acetone from the samples. Six cycles at 800 psi (three minutes with carbon dioxide gas and five minutes equilibrating without gas) were followed by raising the pressure to 1250 psi at which point the surface tension approaches zero. The elimination of surface tension results in evaporated samples with little or no surface distortions. The biofilter samples were then coated with gold film. The coated specimens were observed by scanning electron microscopy and photographs taken of typical sections of the samples.

Fluorescence Microscopy

Biofilm smears were made from biomass taken from each of the four biofilters. The air dried microscope slides were sent to Dr. E. L. Schmidt at the University of Minnesota for staining and observation. Parts of each slide were stained separately with 3 "cocktails" of Fluorescent Antibody (FA). The Nitrosomonas cocktail was comprised of FA to Nitrosomonas europaea and strains Nitrosomonas europaea "tara" and Nitrosomonas europaea "sewage". A Nitrosolobus cocktail was comprised of FA to the 3 soil isolates Nitrosolobus AV, Nitrosolobus Fargo, and Nitrosolobus Bearden. The third cocktail was Nitrosospira, made up of Nitrosospira AV, Nitrosospira Spitzbergen 30, and Nitrosospira Spitzbergen. The slides were observed by fluorescence microscopy for evidence of cross reactivity to the three cocktails.

Most Probable Number Study

The most probable number (MPN) medium composition is shown Table 12. It was dispensed at 5 ml per tube then autoclaved at 121 °C for 15 minutes. The inoculation scheme for each of the four biofilter samples involved multiple tube dilutions (five tubes at each dilution) using 0.0002, 0.00002, and 0.000002 ml of sample. Because growth rates of nitrifiers is slow, the tubes were incubated at 27 °C for 3 months. Phenol red was added to the medium to detect the production of acid resulting from the oxidation of ammonia. The medium in positive tubes turned yellow due to the drop in pH.

Nitrification Assay

The assay procedure used was a modification of that given by Belser and Mays (1982) and Hall and Murphy (1980). Bottles with 100 ml of culture medium (Table 13) were inoculated either with aseptic scrapings from biofilter support media or well mixed solutions of biomass from pure cultures and biofilter samples (Figure 14). Biofilter samples were obtained from three national fish hatcheries that employ water reuse for rearing salmonids. Table 14 presents a summary of the biofilter support media and description of the biomass. The

Table 12. Medium Used in Most Probable Number Determination

$(\text{NH}_4)_2\text{SO}_4$	1.0	g/l
KH_2PO_4	0.2	g/l
CaCl_2	0.04	g/l
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.04	g/l
Chelated Fe	0.5	mg/l
Phenol red	0.5	mg/l

pH adjusted to 7.5-8.0 by addition of 5% sodium carbonate

Table 13. Nitrification Inhibition Assay Medium^a

Ammonium Sulfate	68	mg/l
Potassium Phosphate Dibasic	136	mg/l
Magnesium Sulfate	50	mg/l
Calcium Chloride	4	mg/l
Trace Elements Solution ^b	0.5	ml/l

^aAdjust pH to 8.0-8.2 with 5% Na_2CO_3

^bTrace elements: Na_2MoO_4 0.1 g/l; MnCl_2 0.2 g/l;
 CoCl_2 0.002 g/l; ZnSO_4 0.1 g/l; CuSO_4 0.02 g/l.

Table 14. Samples Utilized in the Chlorate Inhibition Assay

Sample	Support Medium	Description
Spring Creek	5.0-7.6 cm rock	rust color growth
NFH	5.0 cm oyster shell	green growth on shell
Lahonton	2.5 cm flexring	yellow-brown biofilm
NFH		
Kooskia	1.3-2.5 cm rock	rust colored biofilm
NFH		
RBC	25.4 cm discs	yellow-brown biofilm
Biodrum	2.5 cm PVC rings	light brown biofilm
Trickling Filter	2.5-3.8 cm slag	yellow-brown biofilm
Submerged Filter	2.5-3.8 cm slag	white to grey biomass
Undergravel Filter	0.64 cm aquarium gravel	brown biofilm

Note: cm X 0.394 = inches

samples from Spring Creek National Fish Hatchery (Underwood, Washington) and Kooskia National Fish Hatchery (Kooskia, Idaho) were sent in sample containers with water from the filters. The biofilter sample from Lahonton National Fish Hatchery (Gardnerville, Nevada) was sent in demineralized water. The samples represent several kinds of biofilter support media and varied biomass. The kinds of support media included rock, oyster shell, flexring, plexiglass discs, slag, and gravel.

Figure 14 presents a summary of the assay procedure. The specific inhibitor, chlorate, was added to three culture bottles as was the nitrapyrin. Several other culture bottles provided both positive and negative controls. The samples were incubated at 26 °C on a shaker (200 rpm). Samples of 5 ml volume were taken at varied time intervals for nitrite analysis. The samples were filtered then diluted to 50 ml prior to analysis using the Griess-Ilosvay reagents sulfanilamide and N-(1 naphthyl) ethylene diamine dihydrochloride (NED). The diazotized samples were compared to standards by measuring absorbance at 543 nm using a Bausch and Lomb Spectronic 70 spectrophotometer.

^aMedium defined in Table 7.

^b0.5 ml 1M NaClO₃

^c5 µl 20% Nitrapyrin

Figure 14. Inoculation Scheme for Second Stage Nitrification Inhibition.

Nitrifier Activity Calculations

The relative activities of nitrifiers in a mixed population may be determined based on Monod kinetics and controlled experimental conditions. Since chlorate inhibits the formation of nitrate, it may be assumed that the ammonia is stoichiometrically converted to nitrite. The rate of nitrite production from ammonia is related directly to the number of cells (Neidhardt and Boyd, 1965). The specific growth rate μ may be calculated using the change in nitrite concentration for various time intervals (Belser and Schmidt, 1980) according to the following relation:

$$\mu = \frac{\ln ([\text{Nitrite}]_a / [\text{Nitrite}]_b)}{t_a - t_b}$$

where μ is the specific growth rate and the concentrations of nitrite are determined at an initial time b and final time a .

It can be shown that the generation time is related to the specific growth rate (Neidhardt and Boyd, 1965) by the relationship:

$$\text{G.T.} = \ln 2 / \mu$$

where the generation time is inversely related to the specific growth rate, μ . Activity in terms of mmoles nitrite produced/g total biofilter biomass/hr was calculated from nitrite data and the concentration of cells (volatile non filtrable residue or volatile suspended solids).

Results

Scanning Electron Microscopy. Scanning Electron Microscopy (SEM) in and of itself is of little value in the evaluation of nitrification processes. However, coupled with other data, it may be useful in the qualitative determination of nitrifier populations. The resolution of SEM enables resolution of microbial particles and can aid in the identification of certain morphological groups of bacteria. Coupled with the data from fluorescence microscopy and inhibition with chlorate, the SEM micrographs may be used to qualitatively identify nitrifiers since most nitrifiers are rod shaped and about a micron in length.

Fluorescence Microscopy. The information received from Dr. E. L. Schmidt for fluorochrome stained biofilter preparations is presented in Table 6. The submerged filter specimens were apparently minus nitrifiers (at least for the genera listed). Nitrifiers were present in both the RBC and Biodrum samples. The nitrifier population of the Biodrum appeared to be quite diverse. The data suggested that any or all three of the genera of nitrifiers were present. The RBC on the other hand, was limited to Nitrosomonas and Nitrospira.

Table 15. Presence or Absence of Three Genera of Ammonia Oxidizers in Biofilter Samples as Determined by Fluorescence Microscopy.

sample	Nitrosolobus	Nitrospira	Nitrosomonas
SF 1	+/-	-	+/-
SF 2	-	-	-
SF 3	-	-	-
SF 4	+/-	-	-
RBC 1	-	+/-	-
RBC 2	-	+/-	+
BD 1	ND	ND	ND
BD 2	-	+/-	+/-
BD 3	+/-	-	+/-
BD 4	-	+	+
TF 1	ND	ND	ND
INF 1	ND	ND	ND
INF 2	ND	ND	ND
TW 1	ND	ND	ND

A Nitrosolobus cocktail was comprised of FA to the three soil isolates N1. "AV", N1. "Fargo", and N1. "Bearden".

A Nitrospira cocktail was comprised of Nitrospira "AV", Np. "Spitzbergen 30", and Np. "Spitzbergen".

A Nitrosomonas cocktail was comprised of FA to N. europaea, N. "tara", and N. "sewage".

Trickling filter (TF), Submerged filter (SF), Rotating Biological Contactor (RBC), Biodrum (BD), Influent (INF), and Tap water (TW)

ND--Not Done

Most Probable Number Results. The results of the MPN test for each of the four laboratory scale biofilters indicated that the populations of nitrifiers for the three aerobic processes ranged from 4,000 bacteria/ml in the Trickling filter to 24,500/ml in the RBC. No nitrification activity was detected in the Submerged filter sample throughout the study period. These observations are consistent with the results of fluorescence microscopy and SEM data.

Nitrifier Activity and Kinetics. It is difficult to determine accurately, the kinetic constants for nitrification for fixed film systems. However, assuming that steady state conditions were achieved at each of the separate hydraulic loadings to the filters, calculations were made to determine the kinetic constants of nitrification for the four laboratory scale filters. It can be shown that the data for the RBC does fit Monod kinetics. The maximum specific growth rate was 0.72 (1/day) and the half saturation constant was 0.2 mg/l. The Biodrum data provided poor fit to the Monod model. The specific growth constant for the Biodrum was 0.04 / day and the half saturation constant was 0.08 mg/l. The Trickling Filter and Submerged Filter data did not fit the model.

Several samples were used in the chlorate inhibition assay for evaluation of nitrification. Table 14 presented a list of the samples and a description of the support medium and biomass characteristics. Figures 15 through 22 are plots of the data obtained by use of chlorate inhibition for the filters listed in Table 14 and a pure culture of *Nitrosomonas europaea* obtained from Dr. E.L. Schmidt at the University of Minnesota. Each sample appeared unique in relation to nitrite production under similar environmental culture conditions.

Figures 15 through 17 present the results of chlorate and nitrapyrin inhibition on biofilter samples from the three national fish hatcheries. The results for Kooskia and Lahonton hatcheries were similar in that both chlorate and nitrapyrin inhibited the production of nitrite. In the case of the biofilter sample from the Lahonton hatchery, chlorate was as inhibitive as nitrapyrin. The samples from Lahonton and Kooskia were unlike any of the other samples in that nitrite production was less in the presence of the inhibitor chlorate. Figure 15 shows the effects of the two inhibitors on growth of the biomass from the Lahonton National Fish Hatchery sample. There was a long lag period especially when compared to the other samples. Both chlorate and nitrapyrin caused nearly complete inhibition of nitrite production. Figure 16 shows a similar pattern for the sample from Kooskia. Again, there was a long lag period. The only difference in this case was the fact that chlorate reduced nitrite production by about half, rather than nearly 100% as observed with the Lahonton sample.

Results of the analysis of the samples for the remaining biofilters and the pure culture (Figures 18 to 22) are more consistent with the results of other researchers who have used this technique. The curve of production of nitrite by the sample without inhibitors and the curve of nitrite production by sample plus chlorate are essentially the same. Levels of nitrite production were observed to be higher in most cases with chlorate inhibition. Figure 17 shows growth of biomass from the National Fish Hatchery at Spring Creek,

Figure 15. Nitrification Assay With Specific Inhibitors Chlorate and Nitrapyrin Using Biomass From Lahonton National Fish Hatchery Biofilters.

Figure 16. Nitrification Assay With Specific Inhibitors Chlorate and Nitrapyrin Using Biomass From Kooskia National Fish Hatchery.

Underwood, Washington. The production of nitrite is typical, with chlorate causing very little reduction in nitrite levels. There was a lag period observed that was much less than the lag period for either the Lahonton or Kooskia samples. Figure 18 gives the growth curve for the RBC. The production of nitrite in the presence of the inhibitor was essentially linear. Like the Biodrum sample (Figure 19), the lag period observed in the other data was absent. The growth curve of the Biodrum sample was unique because nitrite production increased again, after leveling off, late in the cycle. Figure 20 presents the nitrite production data for the trickling filter biomass. The levels of nitrite production were essentially the same for either the presence or absence of chlorate. The data for the undergravel filter (Figure 21) again showed the trend of earlier graphs. Like the results for the pure culture of *N. europaea*, in Figure 22, the production of nitrite was higher in the presence of chlorate. In all cases, when nitrapyrin was added to the samples, there was nearly complete inhibition of nitrite production, indicating ammonia oxidation was due to autotrophic microorganisms.

The data relating nitrite production with time were used to determine the activity and specific growth rates of nitrifiers in the varied samples using calculations presented earlier. The values of generation time ranged from a low of 8.5 hr for the pure culture to 103.3 hr for the sample received from Lahonton National Fish Hatchery. Calculating the generation time for the Lahonton sample using nitrite accumulation data in the absence of chlorate, a generation time of 21.3 hr was obtained. This value may provide a more accurate estimate of ammonia oxidation since, unlike the other samples, the ammonia oxidizing biomass of the Lahonton biofilter was susceptible to chlorate inhibition. The ranges of activity were from 0.0003 mmole nitrite produced/g total biofilter biomass/hr to 0.114 mmole nitrite/g total biofilter biomass/hr for the Lahonton National Fish Hatchery and RBC samples, respectively.

Discussion

The use of the MPN technique to determine nitrifier populations may not provide an accurate understanding of the population dynamics of the biofilter. Even though this technique is the most common one used in nitrification studies, it can in many cases underestimate the true population sizes in the environment. Belser and Mays (1982) used MPN and fluorescence microscopy data to show that the MPN counts may be from 0.1 to 5% of the actual population in soils and sediments. Since the population may be grossly underestimated, they suggested that the potential activity of nitrification be calculated based on nitrite accumulation with specific inhibitors to index the counting efficiency. This is done with some difficulty, because the dominant nitrifier species must be known, since the counting efficiencies vary from one species to another.

Figure 17. Nitrification Assay With Specific Inhibitors Chlorate and Nitrapyrin Using Biomass From Spring Creek National Fish Hatchery Biofilter

Figure 18. Nitrification Assay With Specific Inhibitors Chlorate and Nitrapyrin Using Biomass From the Rotating Biological Contactors.

Figure 19. Nitrification Assay With Specific Inhibitors Chlorate and Nitrapyrin Using Biomass From the Biodrum.

Figure 20. Nitrification Assay With Specific Inhibitors Chlorate and Nitrapyrin Using Biomass From the Trickling Filter.

Figure 21. Nitrification Assay With Specific Inhibitors Chlorate and Nitrapyrin Using Biomass From an Undergravel Filter in Prawn Aquarium.

Figure 22. Nitrification Assay With Specific Inhibitor Chlorate Using Biomass From a Pure Culture of N. europaea.

The data collected in this study may be used to conclude that nitrification in the systems studied, is the result of autotrophic oxidation of ammonia. In nearly all cases, nitrite production was reduced 95 to 99% by nitrapyrin, which selectively inhibits autotrophic nitrification (Shattuck and Alexander, 1963).

Kinetics of Nitrification. It was shown in this study that the four biological processes operated somewhat independently, even though operational conditions were similar. The kinetic constants for specific growth rate and half saturation were different for the processes studied. This indicated that the kind and numbers of nitrifier organisms selectively isolated on the separate biofilters varied greatly. One may wonder why it would be that four biofilters could be so different when all were provided with the same seed, feedstock, and environmental conditions during start-up. One observation from the scanning electron microscopy work was that there was a greater diversity of morphological bacterial types associated with the processes that provided the best ammonia removal efficiency. The trickling filter biomass was fairly homogeneous as opposed to the heterogeneous biomass of the RBC and Biodrum. It seems that in the process of start-up, rotation and resulting aeration of the RBC and Biodrum caused selective isolation of a diverse nitrifier population that could adapt to changes in loadings and conditions. Additionally, with the motion of the rotating filters, there was more sloughing of biomass than observed in the effluent of the Trickling filter. The nitrifying populations of the RBC and Biodrum adapted best for ammonia removal.

The use of specific inhibitors for the evaluation of nitrifier activity and populations is gaining popularity. Belser and Schmidt (personal communication with Dr. E.L. Schmidt) now use the technique routinely, to the exclusion of certain other methods of study. Hall and Murphy (1980) applied the procedure to the study of nitrification kinetics in wastewater treatment (laboratory scale extended aeration). The technique involved chlorate inhibition of second stage nitrification. The procedure provided a simple means for determining kinetic constants and quantitating nitrifier activity.

Samples of biomass from the biofilters studied have distinct characteristics for oxidation of ammonia. Samples obtained from the national fish hatcheries may very well be comprised of different species of nitrifying bacteria, since the generation times ranged widely from 19.9 hr (Kooskia sample) to 103.3 hr (Lahonton sample). There was even a wide variation resulting from calculations for the laboratory scale biofilters, undergravel filter, and the pure culture, where the ranges were 8.5 hr for the pure culture to 82.9 hr for the biodrum.

The diversity of activity was probably the result of different strains of nitrifiers selectively favored by different environmental conditions. Belser and Schmidt (1980) also suggested that the lower activities were the result of smaller biomass. Such may very well have been the case in the present study since the concentration of biomass on the flexrings of the Lahonton biofilter was barely visible while all the other samples were more concentrated.

The use of nitrifier activity based on the inhibition of nitrate formation by chlorate offers a potentially useful technique for the study of nitrification though there are still questions that need to be answered. How do the activities in pure culture compare to those in nature? Do the kinetic constants determined by nitrite accumulation give a true index of what is actually the case for nitrification in soil or wastewater environments?

In any event, the technique has merit in that it is easily applied to aqueous, soil, and sediment systems. With more research, information could be gained that would enable biofilters to be indexed according to nitrification rates. This could aid in the identification of biofiltration systems that would be ideally suited to aquaculture and even possibly provide for stock cultures of nitrifier strains which may be used for rapid start-up of operations and more efficient removal of the toxic inorganic nitrogen compounds.

Summary and Conclusions

Fixed film biological filters are capable of providing adequate removal of nitrogen compounds from aquaculture wastewaters. They should be considered for facilities where there is partial or complete reuse of the water supply. The present study has examined four biofiltration processes from the standpoint of ammonia removal and the process of nitrification as it relates to the concentration of ammonium and nitrite in treated water. The major findings and conclusions of the project are given below.

- 1) A most probable number method was used to enumerate ammonia oxidizer populations in the biofilter samples. The population of nitrifiers in the RBC was 24,500 per ml. The Biodrum and Trickling Filter populations were 8,500 and 4,000 bacteria per ml, respectively. No nitrifiers were found in the Submerged Filter sample.
- 2) Several genera of ammonia oxidizers may coexist in each biofilter sample. Fluorescent microscopy suggested the presence of both Nitrosomonas and Nitrospira in the RBC biomass. Three genera of nitrifiers (Nitrosomonas, Nitrospira, and Nitrosolobus) might have been present in the Biodrum sample. The Submerged Filter was shown not to contain nitrifiers.
- 3) Scanning electron micrographs showed bacterial cells (possibly nitrifiers) of about 1 μ m in length in each of the RBC, Biodrum and Trickling filter samples. The Submerged filter biomass consisted mostly of a filamentous organism several microns in length which was probably Beggiatoa.
- 4) Selective inhibitors of nitrification were used to evaluate biofilter performance. Chlorate, which inhibits the conversion of nitrite to nitrate, and nitrapyrin, which inhibits autotrophic nitrification, were used to index relative nitrifying activity of the biofilters.

- 5) The removal of ammonia was primarily due to autotrophic nitrification as evidenced by 95-99% reduction in nitrite formation when nitrapyrin was added to the biofilter samples.
- 6) Chlorate inhibition of nitrite conversion to nitrate was used to determine the specific growth rates and generation times of biomass from biofilter samples which are either in use or are to be used in aquaculture reuse systems.
- 7) The specific growth rate constants ranged from 0.007 (1/hr) for a biomass sample obtained from Lahonton National Fish Hatchery to 0.082 (1/hr) for a pure culture of Nitrosomonas europaea.
- 8) Generation times calculated for ammonia oxidation ranged from 8.5 hr for the pure culture of Nitrosomonas europaea to 103 hr for a biomass sample from Lahonton National Fish Hatchery. Most generation times for the other biofilter samples ranged from 13 hr to 40 hr.
- 9) Nitrifying activity calculated for the biofilter samples ranged from 0.0003 mmole nitrite produced/g total biomass/hr for the Lahonton sample and to 0.114 mmole nitrite/g total biomass/hr for the RBC sample.
- 10) Ammonia oxidation rates varied widely for the biological filters depending on the amount of total biomass and the strains of nitrifiers environmentally selected in the separate biofilters.

Section 6. Modeling and Design of Biofilters

Introduction

A design procedure was developed which could be used by an aquaculturist to design a reasonably efficient biofilter based on the results of this research project. The major design parameters chosen were ammonia removal efficiency, un-ionized ammonia remaining, and hydraulic loading rate.

Ammonia removal efficiency was chosen due to the results being fairly reliable and consistent, and ammonia is the principle excretory product from aquatic animals. Ammonia is also the most important pollutant level indicator. Ammonia production is greater than BOD or COD production and generally when the ammonia concentrations are within acceptable levels, so is the BOD and COD concentration. Hydraulic loading rate is a uniform parameter which can easily be applied to all three biofilters. Un-ionized ammonia is used as a check to assure the recommended toxic limit will not be exceeded. It is not used as a major design parameter because the pH and temperature influence its concentration, making reliable estimated prior to operation more difficult. The curves which will be used relating ammonia removal efficiencies to the hydraulic loading rate are estimated curves; however, they do lie fairly close to the data points. The BOD and COD production rates are very small; however, estimates are made to assess whether the BOD and COD concentrations will reduce the dissolved oxygen concentration below critical levels. The BOD and COD removal efficiencies are great enough to meet effluent water quality standards. It appears at this time the most crucial item is ammonia removal.

Biofilter Design Procedure

A determination of preliminary data must be made. Some suggestions are:

1. basin dimensions
2. prawn density
3. average prawn weight
4. average ammonia production rate which is $0.021 \text{ gNH}_4/100\text{g-Animal-Day}$ (Klemetson and Rogers, 1981)
5. allowable total ammonia level of 3.5 mg/L
6. allowable un-ionized ammonia concentration which is $0.02 \text{ mg/l NH}_3 \text{ as NH}_3$ (EPA, 1976).

The specific steps to complete the design are:

1. Compute the total basin water volume and surface area.
2. Using the Prawn Culturing Density and average prawn weight, compute the total mass of prawns. This is accomplished by finding the number of animals per square foot of surface area, the total number of animals and the total weight of animals.
3. Find the total ammonia production in grams/day by multiplying the production rate by the total grams of animal.
4. Find the total production in mg/l by dividing the ammonia production in grams/day by the total volume, using the appropriate conversion factors.
5. Choose a hydraulic loading rate and corresponding ammonia removal efficiency from the curve.
6. Compute the amount of ammonia which can be produced by the prawns by multiplying the allowable ammonia concentration by the removal efficiency. This is the amount which will be removed.
7. Compute the number of days it will take for the prawns to produce the removable ammonia by dividing the removable ammonia in mg/L by the ammonia production in mg/L-day.
8. Find the flowrate into the filter by dividing the basin volume in gallons by the number of days computed in step #7.
9. The required filter surface area then is found by dividing the flowrate in gallons/day by the hydraulic loading rate.
10. Follow the above procedure using the range of hydraulic loading rates to find the minimum surface area required. The minimum surface area flow rate should then be checked to ensure that the un-ionized ammonia is low enough to meet the acceptable requirements.
11. Compute the relationship between total cost and unit cost at the optimum hydraulic loading rate for freshwater and the two seawater concentrations.
12. At this point it is assumed that data would be available for the unit cost for each type of biofilter so that a determination could be made of the most economical filter.

Biofilter Design Example

Basin Size

4' wide, 35' high, 48' long

4-Basin Volume

$$4' \times 3.5' \times 48' \times 4 \text{ basins} = 2,688 \text{ ft}^3$$

Total number of animals per square foot of surface area. Assume one animal every 6" of height per square foot of surface area (reasonable if basin equipped with artificial substrates).

$$\frac{3.5' \text{ height}}{0.5} = 7 \text{ animals/ft}^2 \text{ of surface area}$$

Total number of animals

Surface area of one basin $48' \times 4' = 192 \text{ ft}^2$

$$192 \text{ ft}^2 \times 7/\text{ft}^2 \times 4 = 5,376 \text{ animals}$$

Assume an average animal weight of 25 g

$$5,376 \text{ animals} \times \frac{25 \text{ g}}{\text{animal}} = 134,400 \text{ g of animal}$$

Ammonia production rate is $0.021 \text{ g NH}_4/100 \text{ g animal-day}$ (Klemetson and Rogers, 1981).

Total ammonia production then is

$$\frac{0.021 \text{ g NH}_4}{100 \text{ g animal-day}} \times 134,400 \text{ g animal} = 28.22 \text{ g/day}$$

Production rate as

mg/L - day is

$$\frac{28.22 \text{ g}}{\text{day}} \times \frac{1}{2688 \text{ ft}^3} \times \frac{1000 \text{ mg}}{1 \text{ g}} \times \frac{1 \text{ ft}^3}{7.48 \text{ gal}} \times \frac{1 \text{ gal}}{3.785 \text{ L}} =$$

0.3709 mg/L-day

RBC Design

Choose flowrate of 1.0 gpd/ft²

Treatment efficiency is 96.5%

Maximum ammonia concentration allowed is 3.5 mg/L.

Amount of ammonia which can be produced by prawns which will be

$$\text{removed: } (3.5 \text{ mg/L}) (.965) = 3.38 \text{ mg/L}$$

Number of days to reach ammonia conc. which will be removed:

$$(3.38 \text{ mg/L} / .3709 \text{ mg/L-days}) = 9.11 \text{ days}$$

Flowrate into filter then is:

$$\frac{2688 \text{ ft}^2}{9.11 \text{ days}} \times \frac{7.48 \text{ gal}}{1 \text{ ft}^3} = \frac{2,207.97 \text{ gal}}{\text{day}}$$

The required filter area then is:

$$(\text{day ft}^2 / 1.0 \text{ gal}) \times (2,207.97 \text{ gal/day}) = 2,207.97 \text{ ft}^2 \quad 2,208 \text{ ft}^2$$

By following this procedure for the range of ammonia removal efficiencies and hydraulic loading rates, a curve can be defined relating the required filter surface area to the hydraulic loading rate. Table 16 shows the results of this analysis for all the biofilters.

It is evident from Table 16 that the RBC and the trickling filter reach a minimum filter area. This point is computed in greater detail in Table 17 for the RBC unit and in Table 18 for the Trickling Filter unit. The value of 2.5 gpd/ft² was chosen as optimum hydraulic loading rate for the RBC unit because the curve is too steep to compute more accurate values (see Figure 23). The optimum hydraulic loading rate for the trickling filter is determined in Table 18 as 0.81 gpd/ft². Curves showing the data from Table 16 are plotted in Figures 23, 24, and 25.

Table 16. Required Biological Filter Areas versus Hydraulic Loading Rates

RBC			Biodrum			Trickling Filter		
Flow gpd/ft ²	Eff. %	Filter Area	Flow gpd/ft ²	Eff. %	Filter Area	Flow gpd/ft ²	Eff. %	Filter Area
0.1	99.5	21,414	0.1	98.6	21,609	0.2	58	18,368
0.2	99.3	10,729	0.15	98.3	14,450	0.25	54.5	15,638
0.3	99.0	7,174	0.20	97.7	10,904	0.30	52	13,658
0.4	98.7	5,397	0.25	97.3	8,759	0.35	50	12,175
0.5	98.5	4,326	0.30	96.7	7,345	0.40	48.2	11,051
0.6	98.0	3,624	0.35	96	6,341	0.45	47	10,074
0.7	97.7	3,115	0.40	95.4	5,584	0.50	45.5	9,366
0.8	97.3	2,737	0.45	94.5	5,010	0.55	44	8,804
0.9	97	2,441	0.50	93.7	4,548	0.60	43.3	8,201
1.0	96.5	2,208	0.55	92.7	4,179	0.65	42.2	7,768
1.1	96	2,018	0.60	91.7	3,873	0.70	40.7	7,479
1.2	95.5	1,859	0.65	90.3	3,614	0.75	39	7,284
1.3	94.7	1,731	0.70	89.4	3,405	0.80	37	7,198
1.4	94	1,619	0.75	88	3,228	0.85	34.5	7,266
1.5	93.2	1,524	0.80	86.7	3,072			
1.6	92	1,447	0.85	85.2	2,942			
1.7	90.5	1,385						
1.8	88.6	1,336						
1.9	86.6	1,295						
2.0	84.5	1,261						
2.1	82.3	1,233						
2.2	80	1,211						
2.3	77.5	1,195						
2.4	74.5	1,192						
2.5	70.5	1,209						
4% Seawater			4% Seawater			4% Seawater		
1.0	94	2,267	.4	92	5,790	0.2	59	18,037
17% Seawater			17% Seawater			17% Seawater		
1.0	94	2,267	.4	90	5,919	0.2	54	19,729

Table 17. RBC Minimum Point

gpd/ft ²	Eff.	Filter Area
2.5	70.5	1,192
2.4	74.5	1,209
2.45	72.3	
2.43	73.2	1,198

Table 18. Trickling Filter Minimum Point

gpd/ft ²	Eff.	Filter Area
0.80	37	7,198
0.85	34.5	7,266
0.81	36.7	7,168
0.82	36	7,218
0.83	35.6	7,211
0.84	35	7,247

The unit cost is computed as total cost relationship for freshwater at the optimum hydraulic loading rate, as well as for both seawater concentrations. RBC minimum area is 1,192 ft². Since this will be a linear function, only two points are needed to plot the curve:

Point one is 11,192

Point two is 10,11,920

Biodrum minimum is 2,942 ft².

Point one is 1,2,942

Point two is 10,29,420

Figure 23. RBC Design Surface Area as a Function of Hydraulic Loading Rate

Figure 24. Biodrum Design Surface Area as a Function of Hydraulic Loading Rate

Figure 25. Trickling Filter Design Surface Area as a Function of Hydraulic Loading Rate

Trickling filter minimum area is 7,162 ft².

Point one is 1,7,162

Point two is 10,71,620

For the 4% seawater influent

RBC is 2,267 ft²

Point one is 1,2,276

Point two is 10,22,760

Biodrum is 5,790 ft²

Point one is 1,5,790

Point two is 10,57,900

Trickling filter is 18,057 ft²

Point one is 1,18,057

Point two is 10,180,570

For the 17% seawater influent

RBC is 2,262 ft²

Point one is 1,2,276

Point two is 10,22,760

Biodrum is 5,919 ft²

Point one is 5,919

Point two is 10,59,190

Trickling filter is 19,729 ft²

Point one is 1,19,729

Point two is 10,197,290

These three curves are shown in Figures 26, 27, and 28 and can be used to choose the most economical biofilter.

Figure 26. RBC, Biodrum, and Trickling Filter Total Cost as Fuction of Unit Cost at Minimum Surface Areas for Freshwater Influent.

Figure 27. RBC, Biodrum, and Trickling Filter Total Cost as Fuction of Unit Cost at Minimum Surface Areas for 4% Seawater Influent.

Figure 28. RBC, Biodrum, and Trickling Filter Total Cost as Function of Unit Cost at Minimum Surface Areas for 17% Seawater Influent.

Section 7. Site Selection Criteria & Economics

Introduction

Potential aquaculture sites must be evaluated on the basis of a set of criteria that has been determined by the aquaculture industry. Then the costs of local site and facility development must be determined. Finally, all of the economic facets must be considered before a final decision about possible development is made. As an example of the process, many of the engineering and economic factors of proposed geothermal/aquaculture sites in Southern Colorado, USA, were considered. Many of these data were obtained by personal contact with individuals and firms. Some of the values were obtained by direct evaluation of local costs. A similar approach would be required for evaluation of any other site.

Criteria for Site Selections

A long list of factors must be considered in selection of a suitable site for an aquaculture production facility. While not all of these will be essential to determine the economic feasibility of the project, much of the data must to be collected sooner or later anyway. This list is summarized in Table 19. Each of these factors will be discussed briefly in this section and some will be presented later in more detail in separate sections.

Soil Properties. While all of the parameters listed may be important, from a construction cost standpoint, soil type, percolation rate, topography, and angle of reponse are critical factors. On flat land with clay soils, the ponds can be formed by pushing the soil into berms at a very low cost. In sandy soil, clays must be imported to make the basins watertight.

Water Properties. In most cases the quality of the geothermal well water is quite adequate unless it is extremely hard. The dissolved gases in the well waters can generally be removed by aeration prior to entering the aquaculture ponds. The biggest concern for geothermal water in Southern Colorado was its flowrate and temperature. Additional wells with larger flowrates and higher temperatures would need to be drilled to provide the needed heat to maintain large commercial scale aquaculture ponds. It was for this reason that this study centered on methods of conserving heat and maximizing the existing well capacities.

Climatological Factors. This data must be collected to determine the temperature profiles within the ponds. Using the Maintenance of Aquaculture Pond Temperatures (MAPT) computer model developed by the authors, it was possible to estimate many of the factors that will determine the economic viability of proposed projects based on the environmental conditions. Among these were heat inputs required, the resulting pond temperatures, and animal productivity.

Biological Factors. The biological factors have a significant effect on the economics of the aquaculture system. Algae can be used as a food source for the prawns and therefore is allowed to grow in the ponds. One of the significant costs of operation is feed. If local feeds, such as discarded

chicken mash, are available at lower costs, significant savings can be realized. Another large operating cost is prawn larvae for stocking. If these are purchased, the costs are high. It would be better to develop a hatchery and nursery building to supply the local needs.

Economic Factors. Initially, some of the necessary economic factors such as land, water, and labor, will not be fully covered since the size of the development will be small. A farmer who owns his land, has an existing well, has some unused buildings, and has full-time help that can operate the ponds, can receive a good return on even a small scale aquaculture project. It does not appear feasible to buy everything to start a small aquaculture project of only a few acres, unless they have already paid some of the development costs.

Social Factors. Any project will have some affect on the local social conditions. In the case of many areas, it may be that these factors will be positive as new jobs are created by these projects.

Legal Factors. All legal costs and requirements must be considered and entered into the time frame for the project as well as its proposed costs.

Table 19. Criteria for Site Selection

- A. Soil Properties
  - 1. Soil type
  - 2. Percolation rate
  - 3. Topography
  - 4. Particle size and shape
  - 5. Angle of repose
  - 6. Fertility
  - 7. Microbial population
  - 8. Absence of toxins
  - 9. Color - absorption light
  - 10. pH 6.5
  - 11. Low CaCO_3
  - 12. Windbreaks
- B. Water Properties
  - 1. Temperature - geothermal and cold groundwater
    - a. reservoir temperature ($^{\circ}\text{F}$)
    - b. surface temperature ($^{\circ}\text{F}$)
  - 2. Water quality (chemical analysis; see criteria)
  - 3. Solutes
    - a. nutrients
    - b. gases
    - c. toxic compounds
  - 4. Bacteriology of water
  - 5. Watershed characteristics
  - 6. Groundwater supply
  - 7. Flow rate of well
- C. Climatological Factors
  - 1. Wind
  - 2. Solar radiation
  - 3. Ambient temperature
  - 4. Relative humidity
  - 5. Precipitation
- D. Biological Factors
  - 1. Photosynthetic activity
  - 2. Trophic levels - eutrophication
  - 3. Natural feed available
  - 4. Availability of prawns for stocking
  - 5. Density of stocked prawns
  - 6. Feed availability and storage
  - 7. Harvesting

Table 19 (continued). Criteria for Site Selection

E. Economic Factors

1. Land
  - a. costs
  - b. restrictions on ownership
  - c. zoning regulations
2. Water
  - a. availability - accessibility
  - b. water rights
3. Labor
  - a. wages
  - b. availability of labor and management and engineers
  - c. union rules
  - d. liability
  - e. bookkeeping
4. Transportation
  - a. accessibility to facilities
  - b. transport to market (time required)
  - c. transport and handling facilities
5. Materials and Services
  - a. feed
  - b. fuel
  - c. equipment - pumps, aerators, etc.
  - d. service
  - e. maintenance
  - f. harvesting and packaging equipment
  - g. laboratory
  - h. boat
6. Construction
  - a. earth moving
  - b. piping, valves, pumps, etc.
  - c. wells
  - d. buildings
  - e. roads
7. Communication
  - a. telephone, telex
  - b. cable
8. Power
  - a. public gas and electric
  - b. private generators
9. Markets
10. Taxes
11. Depreciation

Table 19 (continued). Criteria for Site Selection

- F. Social Factors
  - 1. Community services
    - a. schools
    - b. medical care
    - c. housing
    - d. protection
    - e. cultural resources
  - 2. Local customs - common property
  - 3. Environmental impact
    - a. adverse effects of development
    - b. waste disposal
- G. Legal Factors
  - 1. Local, state, and federal permit requirements
  - 2. Limitations
  - 3. Water rights
  - 4. Incentives to investors

System Economics

Roberts and Bauer (1978) discussed the economics of Macrobrachium production for grow-out ponds in South Carolina. The net returns were small but it was too early to predict feasibility. Reference was given to studies in Hawaii which have proven economically feasible. Additional work is needed to determine the variability of results and risks and returns for prawns grown in temperature areas.

Godfriaux et al., (1978) investigated the potential for culturing both Macrobrachium and Rainbow trout in power plant wastewater. Using high density intensive culture in raceways, the production of trout appeared "very attractive." Under their conditions, a commercial production of freshwater prawns would not bring much of a return on investment until the organism could be reared at higher densities. The project has since dropped Macrobrachium production at the PSE & G Mercer Generating Station in New Jersey (Godfriaux, personal communication, July 1980).

Freshwater prawn farming in a tropical area, such as Hawaii, have been shown profitable by Shang and Fujimura (1977). Their analysis of the economics of production suggest that there is a great potential for culturing Macrobrachium. The authors give costs and returns estimated for ponds ranging from one acre to 100 acres.

A study on the feasibility of prawn culture by Bloomstein et al., (1977) was presented as a guideline for investment shrimp farming. Since the market price is variable, costs of production were calculated, but not returns. It was suggested that juveniles could be hatched and grown for \$7.00 to \$8.00 per thousand and that adults could be produced for \$3.80/kg to \$4.94/kg.

Allen and Johnston (1976) developed an extensive model to predict feasibility of lobster culture. The model was applied to data from prawn farms in Hawaii with poor success. The "mystique" of the "pond" or poor understanding of processes occurring in the ponds was given for the inability to use the computer model for freshwater prawn production (Dr. Johnston, personal communication; July 1980).

Walker and Baaker-Arkema (1975) and Madewell (1971) have considered economics for catfish production. The techniques and parameters discussed are applicable to prawn production in grow-out ponds.

Computer simulation models have been developed for estimating prawn numbers (Huang, Wang, and Fujimura, 1976) modeling prawn production (Morita, 1977) and in management economics for shrimp farming (Stamp, 1978). The application of computer studies to aquaculture have distinct advantages in design of aquaculture facilities that will benefit management, reduce operational costs, and eliminate trial-and-error pond management (Orth, 1980).

The Maintenance of Aquaculture Pond Temperatures (MAPT) model developed by Klemetson and Rogers (1981) provided information on the relationship between climatic conditions, heat inputs, and prawn productivity potential.

The general procedure for discussing the economics of production is similar though each study is unique and based on criteria for that geographical location. The economic feasibility of the studies vary since some are year-round culture systems while the growing season in temperate areas may be around six months. In addition, different methods of harvesting, different market prices, different feed conversion ratios, and other factors may vary from location to location. Figure 29 is a chart which lists parameters needed for aquaculture development.

Economic Size of Ponds

Little data exists for an economic pond size. One may infer from the works of others that an ideal pond size will bring a return on its investment. Roberts and Bauer (1978) suggest that the net revenue for South Carolina is approximately \$763 per ha or \$308 per acre. This was calculated from returns from one acre ponds. The cost of juveniles was not included in the computation for production costs. One may speculate that in South Carolina, where labor costs are kept minimal, a break even pond size would be about five acres.

In Hawaii and other tropical areas, the economy is much different than that of prawn production in temperate areas. Shang and Fujimura (1977) found that smaller farms are profitable when operated as a family venture. Freshwater prawn farming is profitable for farms of ten acres or larger, and having an annual production of 3000 lb/Ac and sale price of about \$4.00/lb.

A desired economic size of an aquaculture operation would be one hundred (100) acres, however, ponds as small as one quarter ($1/4$) acre could be used for a proposed aquaculture system. An outside investor who purchased or leased rights to land and water, and had to pay for all development costs, could not expect a near future return on less than about 10 acres. However, if the site is developed by the owner, with well and land costs already paid, a net return could be possible on as little as $1/4$ acre. While the returns would be small, it could provide a starting point for larger developments.

Site Development Costs

Detailed studies of site costs have been presented in the literature. Godfriaux, et al., (1977) discussed the costs for intensive polyculture of trout and prawns in waste thermal water from power plants. Johnson developed a model for determining the feasibility of lobster culture. The parameters discussed are applicable to prawn production.

Roberts and Bauer (1978) and Shang and Fujimura (1977) have presented pond construction costs for South Carolina and Hawaii, respectively. However, since pond construction costs are very site specific, the costs for Colorado have been determined by communication with individuals within the State.

Figure 29. Economic Factors of Aquaculture

In the following sections, the costs of construction, operation and maintenance, and other items will be presented for a one-acre pond. Following that a section will be presented on the costs of several heat loss reduction methods. Most of the costs have been approximately adjusted to current prices by a factor for inflation. True cost should be obtained by bid with specific pond requirements.

Land Costs. Land costs are quite variable and depend upon the availability of water rights. In Southern Colorado they vary from \$750/Ac to about \$1000/Ac.

Construction Costs. Since each site will have different construction costs, the costs of a 10-acre pond on a per acre basis will be tabulated. Some of the costs would be the same whether a one-acre pond or a 100-acre pond was built.

- 1) Earth moving costs calculated on the basis of \$1.50/yd in a sandy area and \$2.00/yd in a rocky area. Shrinkage due to compaction has not been included.
  - a) Complete excavation:
(1 Ac)(5ft deep) = 8067 yd @ \$1.50/yd = \$12,100/Ac
  - b) Berm around 1-Ac ponds by moving surface materials into berm
Volume of berm = 840 LF x 80 ft = 2489 yd (5ft. eff.)
Berm built with 4 foot height, 12 foot wide top and 1:2 slopes
Since soil will not be hauled away, a earth moving cost of \$0.50/yd was estimated.
Excavation costs = 2489 yd @ \$0.50 = \$1245/Ac
- 2) Clay liner to seal ponds. Cost computed on basis of \$4/yd to import clay and install in two 4-inch lifts.
(1 Ac)(8" depth) = 1076 yd @ \$4/yd = \$4304/Ac
- 3) Concrete liner. Costs computed at \$90/yd installed and 3 inch thickness.
1 Ac(3" thick) = 403 yd @ \$90/yd = \$36,300/Ac
- 4) Plastic Liners. Costs for 10 mil and 20 mil PVC liners
10 mil - (1 Ac) @ \$0.26/ft = \$11,325/Ac installed
20 mil - (1 Ac) @ \$0.31/ft = \$13,504/Ac installed
Use of PVC also required 12 inches of soil cover
(Personal communications, Watersaver Company, Denver)
- 5) Piping, valves, etc. Facilities may be required for draindown for prawn harvesting. Costs estimated at \$2897/Ac
(Roberts and Bauer, 1978, costs inflated 36%)
- 6) Feed Storage Bin. Estimated costs = \$1746
(Roberts and Bauer, 1978, costs inflated 36%)
- 7) Pumps and Assembly. Estimated costs = \$3376
(Roberts and Bauer, 1978, costs inflated 36%)
- 8) Aerators. Estimated costs = \$476
(Roberts and Bauer, 1978, costs inflated 36%)

Equipment Costs. Several pieces of equipment are required for the ponds. These are total costs rather than costs per acre.

- 1) Lab Instruments. A large facility can justify a greater investment in lab instruments than could a 1-acre pond. The parameters generally measured on a regular basis are temperature, pH, dissolved oxygen, and conductivity. Inexpensive equipment could be obtained for about \$500 or the more expensive Digital 4041 from Hydrolab Corp (Austin, Texas) could be obtained at \$3500.
- 2) Row boat. Costs assumed at \$449 (Roberts and Bauer, 1978, cost inflated 36%)
- 3) Harvesting and Packaging Equipment. The equipment needs for harvesting and packaging will be the same for a few acres as it will for one acre. The harvesting seine (net) cost approximately \$544 and a small quick-freeze freezer would be available for around \$500. A larger operation would require better freezer capacity. (Roberts and Bauer, 1978, costs inflated 36%)
- 4) Shop and Maintenance Equipment. Most of the small facilities that would be built in southern Colorado would already have some shop and maintenance facilities available, therefore, a \$500 limit on miscellaneous equipment should be adequate.

Operations Costs. Most of these costs are highly site dependent. The costs were determined on the basis of expected local costs for the southern Colorado area. Much of it was obtained by personal communications with responsible parties in that region.

- 1) Feed. It is desirable use locally available waste products for feed, however, if these are not available Marine ration Ralston Purina can be purchased at \$320/ton and a feeding ratio of 2:1 used. On the basis of a production rate of 600 kg/Ac or 0.66 tons/Ac, 1.32 tons of feed would be used at a costs of \$420/Ac. Production rates and feed costs may be higher.
- 2) Imported Stock. The purchase of post larvae can be a major cost in stocking a pond with a density of about 65,000/Ac. If purchased from Aquatic Farms in Kaneohe, Hawaii, the cost is \$30/1000 pl's or \$1950/Ac. Assuming that sufficient demand is created in southern Colorado or surrounding states to justify the construction of hatchery and nursery, the cost can be reduced to less than one quarter of this amount.

- 3) Labor. Labor costs are quite variable since very little extra work is required to handle several acres of ponds rather than one acre. Labor costs were determined at \$4.50/hr.

			Entire Season
			hrs cost
Stocking, 1/2 day	4	\$18	
Feeding, 1 hr/day	200	900	
Management & Testing, 3 hr/day	600	2700	
Harvesting, 2 days	16	72	
Transport to market	-	100	
		\$3,790	

Many of these costs could be reduced on a family farm basis, or where the ponds are a part of some other production unit.

- 4) Marketing. The costs of marketing could vary from nothing to about 10 percent of total revenues. A higher value could be expected at the beginning, however, several of the contacts in project region indicated that all of the prawns would be sold locally in the beginning.
- 5) Communications. The minimum expenses for telephone and mail are expected.
- 6) Power. Electricity costs are approximately \$0.04/kilowatt-hour. That rate will vary with location and amount of use.
- 7) Taxes. The property taxes are based on 30 percent of the assessed value times a mill levy of 11.2. (Alamosa County Assessor).
- 8) Depreciation. Depreciation was figured at 12 percent of capital costs and will vary with each unit of the facility.
- 9) Bookkeeping and Administration Costs. These costs may be included under the labor costs of item 3 or as a separate item equal to 1 to 3 percent of gross receipts. They will vary considerably depending upon pond management methods.

Income. Depending upon the overall economic organization of the pond system, either a profit or a loss can be obtained. A poorly run or inadequately managed system may produce only 1000 lb of animals per acre per year, while a well run system with optimal temperatures could yield over 3000 lbs per acre per year.

The market value of the prawn is somewhat dependent upon its size, with the average sizes bringing about \$5/lb and larger sizes yielding \$8 to \$10/lb. Of course, production rate would be sacrificed to achieve the larger sizes.

Heat Loss Reduction Method Costs

The construction costs associated with the heat loss reduction methods are considered a separate additional cost to the normal pond system. However, temperature climates require that these methods be used if an extended growing season is desired.

The three types of methods compared were wind shear fences, surface covers, and structural shelters. The costs of each will be presented below.

Wind Shear Fences. It was assumed that fences would be placed in parallel rows about 10 fence-heights apart. For a 4-foot fence, a 24 foot wide by 1815 foot long pond would be constructed (or its equivalent) for each acre of pond required. The cheapest construction would use 98 inch snow fence (slats 3/8" x 1 1/2", openings 2 1/4") which sells for about \$35 for 50 feet. Steel poles would be placed every 20 feet and cost \$1 each. The total cost for one acre would be \$1361 for materials only.

A taller, eight-foot cedar fence (50% permeable) could be purchased at about \$8/LF. The fence spacing would be 80 feet apart allowing a 64 foot by 681 foot pond to be installed. The total cost for one acre would be \$5448 for materials. The choice of this fence is unlikely.

Surface Covers. Several types of surface covers were considered. The floating, UV resistant, plastic covers were approximately \$1.50/ft installed (Tom Snow, Personal communications, Staff Industries). Using cheaper, 4 mil, non-UV resistant, PVC film on a one year disposable cost basis, the cost was \$0.02/ft or about \$1045/Ac plus about \$100 in labor for installation. This cover would be discarded at the end of each season.

A monomolecular film could be purchased with an applicator for about \$150/Ac. However, some type of grid system would be required on the surface to keep the thin film from blowing away. This cost could run as high as \$2500/Ac.

Inflatable surface covers or inexpensive greenhouses are less expensive than greenhouses and more expensive than a plastic cover placed directly on the surface of the water. Depending upon the width of the ponds and the type of cover used, the costs could range from a low of \$0.10 to \$1.00/ft. Detailed costs should be prepared for a specific site.

Structural Covers. Roberts (1975) presented material on greenhouse structures. Among the factors considered in the design of greenhouse are the latitude, orientation, roof slope, covering materials, structural support, and available sizes of covering materials. Buildings can have wooden frames, steel arches, steel trusses, and post and cable systems. The latter method is not recommended for snow load areas since it is air inflated and may collapse under high snow loads. Aldrich (1975) has presented information on the engineering characteristics of glass and other types of covering materials.

The basic costs of a green house can be computed from the data presented by Roberts (1975) and herein listed in Table 20. Since the data is 9 years old, all of the costs should be doubled. The current construction costs for a glass greenhouse is about \$6.52/ft , for a plexiglas it is \$6.14/ft , and for a temporary plastic cover on a rigid frame it is about \$2.52/ft .

All of the economic data have been summarized in Table 21 for Poncha Hot Springs, Colorado. Shown are the base case costs and the effect of heat loss reduction methods on costs.

Summary

The previous materials have been presented as a source of information on the cost associated with the construction and operation of proposed aquaculture facilities in southern Colorado. A true cost analysis would require the selection of an actual site and the evaluation of all of the factors for its development. Actual site drawings and other details would need to be determined to complete the economic analysis.

Table 20. Estimated annual fixed costs for greenhouse operation in midwest, 1973 data. (Roberts, 1975, Table 3; double all costs to bring to current costs).

Type of Construction			
<u>Itemized fixed cost</u>	<u>Glass \$/acre</u>	<u>Fiberglass \$/acre</u>	<u>Temp. Plastic \$/acre</u>
Initial investment	142,106	133,580	54,945
Interest ^a	4,974	4,675	1,923
Taxes ^b	1,421	1,336	--
Insurance	696	848	344
Maintenance ^c	990	990	2,613
Depreciation ^d	7,105	13,358	10,987
Total Fixes Costs	15,186	21,207	15,869
5/ft ²	0.35	0.49	0.36

^aBased upon a 7% midpoint valuation.

^bBased upon 1% of an initial investment.

^cIncludes plastic replacement annually for temporary plastic construction.

^dDepreciation schedule of 20 years for glass, 10 years for fiberglass, and 5 years for the frame and structure on temporary plastic facilities.

Source: Glen II. Sullivan and J. I. Robertson. Production, Marketing, and Economic Trends in Greenhouse Tomato Industry, Research Bul. 908, Purdue University, June 1974.

Table 21. Cost comparison of various heat loss reduction methods and the family managed pond with the base case aquaculture pond for Poncha Hot Springs, Colorado (cost per acre for 10 acre pond system).

CAPITAL COSTS	BASE CASE	FENCES	SURFACE COVERS			GREENHOUSES		
			MONOFILM	PLASTIC	PLANTS	GLASS	PLEXIGLASS	PLASTIC
(1) Land	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
(2) Water	-	-	-	-	-	-	-	-
(3) Pond Construction								
(a) Earth moving	16,000	16,000	16,000	16,000	16,000	16,000	16,000	16,000
(b) Clay seal	4,500	4,500	4,500	4,500	4,500	4,500	4,500	4,500
(c) Piping, valves, etc.	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
(d) Wells	-	-	-	-	-	-	-	-
(e) Buildings	-	-	-	-	-	-	-	-
(f) Roads	-	-	-	-	-	-	-	-
(4) Equipment								
(a) Feed storage bin	1,500	1,500	1,500	1,500	1,500	1,500	1,500	1,500
(b) Pumps and assembly	3,000	3,000	3,000	3,000	3,000	3,000	3,000	3,000
(c) Aerators	400	400	500	500	-	400	400	400
(d) Lab and instruments	3,500	3,500	3,500	3,500	3,500	3,500	3,500	3,500
(e) Row Boat	400	400	400	400	400	400	400	400
(f) Harvesting and packing	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
(g) Shop and maintenance	500	500	500	500	500	500	500	500
(h) Auxiliary generators	-	-	-	-	-	-	-	-
(i) Vehicle	-	-	-	-	-	-	-	-
(5) Heat Loss Control Facilities	-	1,600 ^a	2,500 ^b	-	10,000 ^c	284,000 ^d	267,500 ^e	110,000 ^f
TOTAL CAPITAL COSTS	\$32,800	34,400	35,400	32,900	42,400	316,800	300,300	142,800
OPERATING COSTS								
(1) Feed	450	675	675	675	450	900	900	900
(2) Imported Stock	1,950	1,950	1,950	1,950	1,950	3,900	3,900	3,900
(3) Labor (\$4.50/hr)								
(a) Stocking	18	18	18	18	18	36	36	36
(b) Feeding	900	1,000	1,000	1,000	900	1,800	1,800	1,800
(c) Pond management	2,700	2,600	2,600	2,600	2,700	5,400	5,400	5,400
(d) Harvesting/packing	72	72	72	72	200	144	144	144
(e) Transport to market	100	100	100	100	100	200	200	200
(4) Marketing	-	-	-	-	-	-	-	-
(5) Communication								
(a) Phone	175	175	175	175	175	350	350	350
(b) Mail	50	50	50	50	50	100	100	100
(6) Power								
(a) Electric	.75	.75	.75	.75	.75	150	150	150
(b) Gas	-	-	-	-	-	-	-	-
(7) Taxes	220	220	220	220	220	440	440	440
(8) Amortization (12% Cap.Cost)	4,000	4,130	4,250	3,950	5,090	38,000	36,000	17,140
(9) Bookkeeping and Admin.	1,000	1,000	1,000	1,000	1,000	2,000	2,000	2,000
(10) Cover Replacement	-	-	150 ^g	1,145 ^h	-	-	-	-
TOTAL OPERATING COSTS	\$11,710	12,065	12,335	13,030	12,928	53,420	51,420	32,560
RETURNS								
(1) Production (lb)	1,320	2,000	2,000	2,000	1,320	3,000	3,000	3,000
(2) Sale of Byproducts (\$)	-	-	-	-	100 ⁱ	-	-	-
(3) Market value (\$5.00/lb)	\$6,600	10,000	10,000	10,000	6,700	15,000	15,000	15,000

^a4 ft. high snow fence, \$0.75/ft, 25 ft x 1742 ft pond, metal posts at 20 ft o.c., \$1.00 each, labor \$100.

^bFischer Cetylalcohol, \$100/Ac, applicator \$50, surface grid system, \$2500. Submerged aeration, more feed required, more pond surface possible.

^cPlants must be harvested, cost depends upon volume to be harvested, increased transpiration water use.

^dGlass greenhouse cost \$6.52/ft², two cycles of animal growth; feed, stock and labor cost doubled.

^ePlexiglass greenhouse \$6.14/ft².

^fTemporary plastic greenhouse \$2.52/ft². Annual replacement of plastic cover may be required.

^gFischer Cetylalcohol, same as time b.

^hPlastic cover to be placed directly on the surface of the pond. Annual replacement \$0.02/ft², \$100/Ac labor.

ⁱPlants, same as c.

Section 8. Summary of Israeli Research

Introduction

At the project's initiation, the biofilters that were used for aquaculture and Macrobrachium specifically, were primitive yet nevertheless effective systems. At that time, the most popular biofilter in use was submerged gravel bed filter. The polluted effluent would pass through the gravel bed and in this manner both mechanical and biological filtration would take place. Purified water (influent) would be returned to the production tanks via an air-water lift. This system operated effectively at a biofilter to production tank ratio of 1:2. However, during production cycles it would not be uncommon to encounter fluctuations in water quality. This would occur due to the accumulation of organic matter in the gravel and its subsequent decomposition. This build-up eventually required a backflush and in extreme cases a thorough stripping and wash of the biofilter. This procedure is a major operational constraint as it affects production schedules and the necessity to implement it is virtually unpredictable and can thus occur in the midst of a larval cycle resulting in mortalities and financial loss. As the "state of the art" was quite limited, it was clear that more efficient biofilters had to be designed that would eliminate most of the drawbacks of the submerged system.

The second year was directed to the selection of alternative filtration procedures. In this context, two rotating biodrums were evaluated. Each biodrum was connected to eight production tanks of 0.9m³. The systems were analyzed both quantitatively and qualitatively by assaying the reduction of applied concentrations of ammonia as well as the subsequent nitrification of nitrite. Our findings proved that the biodrum was capable of handling high levels of ammonia loading in a relatively short period of time. Nitrite was also rapidly oxidized to nitrate. Having analyzed the efficiency parameters, the systems were stocked with prawns. In both larviculture and in the nursery, the biodrums were highly effective maintaining optimal water quality throughout the production cycles.

The third year was directed to assimilating all the operational data and criteria that had been established during the two previous years and to designing a biofilter for the production requirements of Macrobrachium rosenbergii.

Description of System

The constraints of closed circulating systems often fall on the efficiency of the filtration procedures implemented, both mechanical and biological. The filtering system should fulfill the following requisites:

1. Maintain water quality as close to optimal levels as possible.
2. Economical, using little of the culturing area as possible.
3. Easily monitored vis a vis effluent and influent.
4. Flexible in order to carry out rapid structural changes of system.
5. Simple maintenance.

Rotating Biological Contractors (R.B.C.) used effectively in waste water management are capable of reducing BOD levels rapidly. When adapting such a technology to aquaculture the RBC should carry out essentially the same functions as it does in municipal waste treatment, i.e. reduction of BOD, oxidation of water and maximum contact of water to filter media and high degree of nitrification.

Two identical RBC's were obtained from Euromatic (Copenhagen). The filter media unlike conventional bio-discs are comprised of plastic balls contained in a spherical device, hence, the use of the commercial name "Biodrum". The dimensions of the Biodrum are: 625 x 680 mm. The total volume of plastic balls was 38 m³, the number of balls was 4,500 and the active surface area was 20.3 m². In addition, the Biodrum was fitted with 12 water lifts in order to provide a trickling effect. The rotational velocity of the Biodrum could be adjusted to either 8 or 16 RPM. Each Biodrum was integrated into a skid mounted assembly. Each system consisted of eight 940 liter tanks, one Biodrum (rotated in 700 liter tank) and one 300 liter sedimentation tank. Water was circulated at a rate of 10 exchanges per day. A flow diagram of a typical system is given in Fig. 30.

The first series of experiments evaluated the efficiency of the removal of toxic N compounds introduced artificially as a function of rotational velocity of the Biodrum (8 vs. 16 r.p.m.).

The second series of experiments evaluated the speed of adaptability of biofilter from freshwater to brackish water, in which larviculture activities of M. rosenbergii were carried out.

Figure 30. Flow diagram of closed recirculating system for the culture of *M. rosenbergii*; 1. Biodrum + tank; 2. Sedimentation tank; 3. Distribution & heating reservoir; 4. Centrifugal pump; 5. Header tank; 6. Return flow; 7. Culturing tank; 8. Drainage pipes; and 9. By-pass.

Methodology

Ammonia was introduced in to the systems chemically, using known amounts of NH_4Cl . Systems were stocked with post larvae for 90 days and afterwards a 150 day larviculture period was carried out. On a routine basis, ammonia, nitrite and nitrate were assayed spectrophotometrically.

2nd Year Results

1. At a concentration of 0.5 ppm, both dissolved ammonia and nitrite were removed rapidly (Table 22), with slight differences between high and low speeds. Nitrate levels, throughout the experiments remained relatively constant at 20.00 ppm, and will not be dealt with below. At additions of 1 ppm and 5 ppm, ammonia and nitrite removal efficiency was more rapid at the high speed by 13 and 34% respectively (Table 22 and Figure 31). The higher the ammonia load the greater the difference in the removal efficiency between high and low rotational velocities.

Upon adaptation of biofilter to 1 and 5 ppm respectively (2nd consecutive application) Table 1, c and e, the removal efficiencies of both ammonia and nitrite increased markedly.

The relative contribution of the Biodrum vs. the tank system (By pass - see Figure 29 flow diagram) is shown via a rapid disappearance of ammonia and nitrite in the two biodrum tanks (Table 22) as opposed to significantly lower ammonia removal rates of the two tank systems and a continued presence of nitrite.

The effect of a continuous infusion of ammonia is shown in Figures 32 and 33. In system 1 (slow speed) the amount of ammonia continued to rise until reaching a plateau, nitrite during the experiment increased slightly. In System 2 (high speed), ammonia increased over the first six hours and subsequently decreased. Nitrite levels began to increase slightly when ammonia concentrations declined. Figs. 34 and 35 show the effect of increased infusion rates of ammonia. The results indicate that at the low speed, nitrification of ammonia commenced only when the rate of ammonia input was regulated to 0.05 ppm/hr. On the other hand, in the high speed system, an input level of 0.175 ppm/hr did not affect the efficiency of nitrification. Oxidation of nitrite was only evident in the high speed system (Fig 34), while in the low speed system (Fig. 34) nitrite levels remained relatively constant.

2. The two systems were stocked with post-larvae at a density of 3-4/liter for a period of 90 days. Water quality remained optimal throughout the period of nursing (below 0.1 ppm for both ammonia and nitrite). During this period the prawns were fed a daily ration consisting of live adult artemia, daphnia, pelletized feed and ground fish fillet. With each application the feed administered was calculated according to 10% of weight of prawn population.

Table 22. Ammonia and Nitrate Removal Efficiency as a Function of Time and Ammonia Dosage (as NH_4Cl)

BIOFILTER SYSTEM I - LOW SPEED						BIOFILTER SYSTEM II - HIGH SPEED					
Time (hr)	Ammonia	$\Delta\%$	NO_3	NO_3	pH	Time (hr)	Ammonia	$\Delta\%$	NO_3	NO_3	pH
<u>Chemical 0.5 ppm Addition NH_4Cl</u>											
5	0.4100	-18.00	0.00625	20.22	8.94	5	0.3840	-23.00	0.00125	20.75	8.85
23	0.0410	-91.80	0.00875	26.72	8.85	23	0.0087	-98.00	0.00375	27.27	8.82
26	0.0125	-97.50	0.02000	25.00	8.91	26	0.0087	-98.00	0.00750	24.50	8.86
=====						=====					
<u>Chemical 1.00 ppm Addition NH_4Cl</u>											
2	0.6950	-10.50	0.0025	25.82	8.80	2	0.81875	-18.125	0.0	27.1	8.79
5	0.7980	-20.20	0.0125	26.10	8.84	5	0.75000	-25.000	0.02125	26.55	8.81
7	0.6890	-31.05	0.0713	24.47	8.88	7	0.60400	-39.625	0.07250	29.17	8.85
11.5	0.5175	-48.25	0.0125	27.90	8.86	11.5	0.38900	-61.125	0.00625	28.25	8.84
26.5	0.0400	-96.00	0.0225	25.55	8.80	26.5	0.0	-100.000	0.01250	25.92	8.80
29.0	0.0200	-98.00	0.0200	26.67	8.81	29.0	0.0	-100.000	0.00875	27.67	8.81
31.0	0.0075	-99.25	0.0	23.42	8.81	31.0	0.0	-100.000	0.0		
=====						=====					
<u>Chemical 1.00 ppm Addition NH_4Cl</u>											
0	0	0	0	18.37	8.64	0	0	0	0	19.1	8.68
1.5	0.8660	-13.50	0.0100	19.17	8.75	1.5	0.7212	-27.88	0.02	19.67	8.71
6.5	0.408	-59.20	0.0262	20.00	8.56	6.5	0.2300	-77.00	0.03	19.00	8.54
20.5	0.	-100.00	0.0150	19.50	8.45	20.5	0	-100.00	0.005	18.20	8.54
=====						=====					
<u>Chemical 5.00 ppm Addition NH_4Cl (A)</u>											
3	4.675	-6.5	0.0125	22.12	8.58	3	4.77500	-4.45	0.01000	22.90	8.62
5	4.525	-9.5	0.0100	22.82	8.61	5	4.67500	-6.50	0.01000	23.37	8.64
10	4.037	-19.25	0.0240	21.62	8.60	10	4.08800	-18.25	0.02170	23.15	8.62
24	3.753	-24.25	0.0510	23.52	8.53	24	3.55500	-28.90	0.04300	24.37	8.57
27	3.675	-26.50	0.0430	23.45	8.60	27	3.48700	-30.25	0.04800	24.32	8.61
48	2.053	-58.75	0.1710	22.10	8.74	48	1.41200	-71.75	0.23400	21.62	8.77
53	1.250	-75.00	0.2160	21.85	8.62	53	0.42500	-91.50	0.31700	22.30	8.58
57.5	1.051	-79.00	0.2700	22.60	8.56	57.5	0.34100	-93.18	0.35600	22.02	8.53
72	0.072	-98.50	0.3590	21.17	8.50	72	0.00250	-99.95	0.23900	20.70	8.58
75	0.022	-99.50	0.3780	22.00	8.61	75	0.0	-100.00	0.17600	21.00	8.65
96	0.0	-100.00	0.0950	23.00	8.61	96	-	-	0.01100	22.00	8.65
99	-	-	0.0780	23.87	8.70	99	-	-	-	-	-
=====						=====					
<u>Chemical 5.00 ppm Addition NH_4Cl (C)</u>											
2	4.916	-1.68	0.0166	22.12	8.55	2	4.925	-1.5	0.00300	21.82	8.60
24	2.183	-56.25	0.3040	20.20	8.41	24	0.513	-90.0	0.40100	21.07	8.39
32	0.788	-84.25	0.3700	20.62	8.54	32	0.0	-100.0	0.27100	20.87	8.60
48	0.000	-100.00	0.2360	21.42	8.56	48	-	-	0.04600	19.95	8.65
72	-	-	0.0220	20.70	8.70	72	-	-	0.0	19.90	8.73
=====						=====					
<u>Chemical 1.00 ppm Addition NH_4Cl By Pass</u>											
1.5	1.282	-	0.0250	21.00	8.70	1.5	1.1975	-	0.030	19.00	8.73
18.5	0.348	-65.00	0.1660	21.12	8.59	13.5	0.1390	-86.13	0.165	20.25	8.59
22.5	0.191	-81.00	0.2060	21.77	8.71	22.5	0.0420	-95.75	0.198	20.80	8.69
28.0	0.045	-95.50	0.1960	20.10	8.76	28	0.0	-100.00	0.151	20.20	8.73
=====						=====					
<u>Chemical 1.00 ppm Addition NH_4Cl By Pass</u>											
BIODRUM I - LOW SPEED						BIODRUM II - HIGH SPEED					
0.5	1.455	-	0.0100	20.00	8.56	0.5	1.435	-	0.020	21.50	8.56
1.5	0.945	-37.00	0.0500	20.00	8.42	1.5	0.560	-60.97	0.075	21.50	8.46
3.0	0.105	-92.30	0.0550	22.70	8.48	3.0	0.025	-98.26	0.035	21.10	8.49
8.5	0.0	-100.00	0.0	20.20	8.59	8.5	0.0	-100.00	0.0	20.60	8.59
=====						=====					

Figure 31. Ammonia and nitrite profile after addition of 5 ppm ammonia (as NH_4Cl)

Δ — Δ = ammonia low speed; $\blacktriangle$ — $\blacktriangle$ ammonia high speed;
 o---o = nitrite low speed; $\bullet$ --- $\bullet$ = nitrite high speed.

Figure 32. Ammonia and nitrite profiles under continuous ammonia input (0.05 ppm/hr)
 Low speed, system I. Δ — Δ = ammonia; $\circ$ - $\circ$ = nitrite.

Figure 33. Ammonia and nitrite profiles under continuous ammonia input (0.05 ppm/hr)

High speed, system II. Δ — Δ = ammonia; $\circ$ — $\circ$ = nitrite.

Figure 34. Ammonia and nitrite profiles under varying inputs - low speed, System I
 Δ — Δ = ammonia; 0---0 = nitrite.

Figure 35. Ammonia and nitrite profiles under varying ammonia inputs - High speed, system II.
 Δ — Δ = ammonia; $\circ$ — $\circ$ = nitrite.

Figure 36. Ammonia and nitrite profiles after transfer of system (II) to brackish water. Arrow signifies addition of submerged gravel filter, and its removal.

Δ—Δ = ammonia; ○—○ = nitrite.

3. After completing the nursery stage, both systems were converted to brackish water and subsequently stocked with larvae at a density of 50/liter. During the period of larviculture (which lasts approximately 30 days) larvae were fed twice daily artemia nauplii at a concentration of 3 nauplii/ml, and once daily 30 grams (wet weight) ground fish (commencing at stage 7) per tank. Figure 36, shows the fluctuations in ammonia and nitrite which occurred after transfer of the systems to brackish water (12 ppt) and stocking with larvae. Ammonia levels increased sharply. The subsequent oxidation of ammonia, resulted in high nitrite levels. Nitrite levels remained constantly high for approximately 2 weeks. Nitrite began to decrease after a shallow gravel-bed submerged biofilter was added in series to the system, the effluent it received being high in nitrite, alone. When nitrite levels decreased sufficiently (below 0.1 ppm) the gravel biofilter was disconnected.

Throughout the production season, (which carried on for a period of 150 days), ammonia and nitrite levels were consistently below 0.1 ppm. During this period 1,700,000 post-larvae were produced at a survival of 67.45% (Survival would have been higher, but on two cycles, artemia shortages resulted in reduction of survival to 58 and 33% respectively. The other three cycles had survival in excess of 80%). The amount of production volume per cycle averaged 8.5 m³ and the amount of post larvae produced per liter was 39. The operation of a U.V. device on water quality was tested during a larviculture cycle. The results are given on Table 24. The system which incorporated the U.V. source revealed a marked improvement in water quality, in both ammonia and nitrite levels.

The system which did not include the U.V. maintained relatively stable values for both ammonia and nitrite. Table 23 shows the effect of U.V. on the respective BOD values for both systems. The results indicate that BOD values decreased by 75% in the system incorporating the U.V. as opposed to a 13% decrease in the system lacking the U.V.

Conclusions

1. The R.B.C. can be applied to conventional closed system aquaculture, and applied to both nursery and hatchery.
2. The rotational velocity of the biofilter affects the rate of removal of the toxic N compounds.
3. The amount of organic matter that can be loaded on to a system is correlated to the rotational velocity.
4. Transfer of system to brackish water requires a period of adaptation. This can be shortened by introducing a temporary stage which includes a submerged gravel filter, which is subsequently disconnected.
5. U.V. treatment improves nitrification, apparently by killing off competitive heterotrophic bacteria resulting in a subsequent decrease in BOD.

Table 23. The Effect of U.V. on Water Quality Parameters values in ppm.

<u>Date</u>	<u>U.V.</u>	<u>System</u>	<u>NH₃</u>	<u>NO₂</u>
28/3/83	-	1	0.11	0.03
"	-	11	0.08	0.14
31/3/83	-	1	0.085	0.02
"	+	11	0.055	0.08
6/4/83	-	1	0.125	0.035
"	+	11	0.020	0.060

Table 24. The effect of U.V. on BOD (U.V. on system II) values in ppm.

	<u>BOD₅</u> <u>Day 0</u>	<u>BOD₅</u> <u>After 9 Days</u>	<u>ΔBOD₅(%)</u>
<u>System 1</u>			
Effluent	3.50	3.0	-14%
Influent	1.15	1.00	-13
<u>System 11</u>			
Effluent	3.00	1.00	-67
Influent	1.00	0.25	-75

6. The production volume to filter ratio is highly improved in the system using RBC. (The stagnant submerged biofilter uses a production volume to filter ratio of 2:1; the system using RBC used a 4:1 ratio, this calculation includes sedimentation tank, biofilter tank and heating distribution tank as well as production tanks).

Third Year Study

In the third year we directed our efforts to optimizing the biofiltration systems improving design characteristics and developing an alternative to the biodrum which would not fall in its performance. To this end we designed a rotating biological disc. The discs were corrugated (waved) fiberglass sheets. Each sheet was rotated at 90° from the adjacent sheet and revolved around a shaft powered by a pulley/belt driven motor. The active surface area was similar to the biodrum i.e. 20 sqm and the units were rotated at a velocity of 12 r.p.m. A water lift system running the length of the filter was added in order to generate a trickling effect. A total of three biodisc units were constructed, each unit was attached to two production tanks with a volume of 4.5m^3 /tank. The biodisc rotated in a 0.7m^3 tank (stage 1) and an additional 0.7m^3 (stage 2) served as a mechanical filter. Throughout their operation, the systems were closely monitored and their performance was judged in accordance with the following parameters:

1. water quality maintenance
2. length of larval cycle
3. biomass
4. survival
5. mechanical - operational parameters i.e. breakdown and servicing.

Using an air-water lift system, the water was circulated through the biofilters at an exchange rate of six times/24 hours. The larvae were introduced into the tanks at stocking densities of 50 to 70/liter. The larvae were fed a conventional diet of Artemia, to be replaced at a later stage partially by artificial feed. Table 25 shows the production analysis of the experimental units. Tanks 1 and 2 were not connected to a biodisc system, but operated, as a control, with a submerged filter. Tanks 5 and 6 initially operated with a submerged biofilter, but it was later replaced with a biodisc filter. The remaining tanks (3, 4, 7 and 8) operated with biodisc filtration. The results show that the introduction of the biodisc filtration system enabled an increase in larval stocking densities by roughly 27% (from 48 to 67 larvae/liter). The introduction of an artificial feed component to the larvae culture systems resulted in a reduction in survival. However, the reduction was not due to an increase in toxic N levels, but rather to the characteristics of the experimental feed. In order for larvae to feed effectively the particle must be maintained in suspension. The artificial feed has a specific density greater than 1 and in order to maintain this particle in suspension, a great deal of air pressure was necessary to power

Table 25. Production analysis of larvaculture units for Macrobrachium rosenbergii.

Tank #	Larvae stocked	Post-larvae harvested	Survival (%)	Length of cycle (days)	Biodisc +/-	Artificial feed +/-
1	220,000	119,000	54	27	-	-
2	220,000	159,000	72	31	-	-
1	280,000	112,000	40	30	-	+
2	280,000	93,000	33	32	-	+
1	220,000	140,000	63	28	-	-
2	280,000	188,000	65	25	-	-
3	297,000	107,000	36	26	+	+
3	288,000	205,000	71	26	+	-
5	255,000	84,000	33	33	-	-
6	262,000	118,000	45	36	-	-
5	238,000	86,000	36	34	+	+
6	233,000	70,000	30	29	+	+
6	292,000	190,000	65	25	+	-
7	272,000	155,000	57	28	+	+
8	265,000	127,000	48	28	+	+
7	310,000	224,000	72	26	+	-
8	300,000	214,000	71	25	+	-

air water lift inside the production tank. Consequently, the reduction in survival was attributed to a combination of starvation and cannibalism. Upon reintroduction of the Artemia ration an immediate improvement in survival was observed. Figs. 37-40 show the water quality maintained during several cycles. As the graphs reveal, ammonia-N and nitrite-N reflect a reduction in hydraulic load due to a blockage in the effluent lines. The increase in ammonia-N and nitrite-N was controlled and subsequently decreased when the hydraulic load was increased.

The significance of the hydraulic load is demonstrated in Table 26. While maintaining high stocking densities and commensurate feeding regimes we found that the level of ammonia-N would increase inside the production tank irrespective of the high removal efficiency of the biofilter 87% (Table 26). The ammonia-N level would attain levels in excess of 0.3 ppm (which is well beyond the LC_{50} reported by Armstrong et al. 1978), in the production tank, irrespective of the fact that the influent had low ammonia-N concentration 0.04 ppm. The build up of toxic ammonia-N levels in the production tanks resulted in mortalities, consequently we had to increase the hydraulic load considerably even though it would mean a reduction in the detention (retention) time in the biofilter. Fortunately, by increasing the hydraulic load to $0.27 \text{ m}^3/\text{m}^2/\text{h}$, the water in the production tanks improved markedly (Table 26). The detention time was reduced from 16 minutes to only 8 minutes per stage. In spite of this reduction the level of nitrification was sufficiently high to balance the degree of ammonification in the production tanks. This balance is reflected in the decline in the ratio of ammonia-N in the effluent and influent.

In conclusion, the rotating biological contractor (RBC) has proven to be an adequate instrument for maintaining a stable environment for prawn production. Furthermore, the biodisc developed and designed in our laboratory fulfills our water quality requirements and it proved to be an excellent and superior alternative to other devices tried in our laboratory and production facilities.

The measure of success of a given research project in an applied discipline, (such as this project), is the actual implementation of the developed technology on an industrial level. As a direct result of this project, four kibbutz aquafarms in Israel have adapted our biofiltration technology and others have expressed an interest in acquiring the technology. This has opened the door to many new exciting developments and applications.

Figure 37. Ammonia-N and Nitrite-N profiles during larval cycle.

Figure 38. Ammonia-N and Nitrite-N profiles during larval cycle.

Figure 39. Ammonia-N and Nitrite-N profiles during larval cycle.

Figure 40. Ammonia-N and Nitrite-N profiles during larval cycle.

Table 26. Removal Efficiency of Biofilter as a function of Hydraulic Load

Hydraulic load	Effluent	Influent	Removal Efficiency
$\text{m}^3/\text{m}^2/\text{hr.}$	Ammonia-N ppm	Nitrite-N ppm	%
0.125	0.230	0.025	89
0.125	0.310	0.040	87
0.270	0.060	0.050	19
0.270	0.100	0.040	55
0.270	0.060	0.045	25

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D. EVALUATION OF RESEARCH ACHIEVEMENTS

Description of Problem

Water quality is a limiting factor wherever high aquatic animal yields are expected or when the organisms are intensively raised in foreign environments, necessitating closed, recirculating culture systems. The natural, and yet toxic wastes, produced by the animals in an intensive aquaculture system have high concentrations of ammonia. These wastes must be removed from the waters before they can be recycled back to the aquaculture basins for reuse by the animals. The usual and most effective tool for controlling water quality in such systems is the biological filter (biofilter). The biofilter functions by allowing natural bacterial populations of the nitrogen cycle to oxidize toxic nitrogenous substances and to remove them from the culture medium. It is a complex biological entity, composed of a polyculture of bacteria, protozoa, and algae with different environmental and nutritional requirements.

The problem as discussed involves a system analysis of the water quality needs of the entire aquaculture system for Macrobrachium production and the design and evaluation of biofilter systems to produce the recycled water quality and optimum animal production. Both the engineering and economic efficiencies of the water treatment system and the factors of animal production were evaluated during the course of this study.

Scope of Work

The tasks of this research project were divided between the United States and Israeli research teams. The work in the United States was originally based at Colorado State University but this location was changed during the second and third years to The Brigham Young University. The work in Israel was coordinated from The Hebrew University of Jerusalem. In general, the work in the United States involved the engineering and economic analysis of the biofilter wastewater treatment systems, and the work in Israel concentrated on the effects of the water quality on the factors of animal production. The results of each organization were exchanged regularly and used to modify the subsequent direction of the work. The specific objectives for the United States work are presented below.

The Brigham Young University

- (a) To identify and define the usable ranges and design limits of the approximately 35 most important water quality parameters.
- (b) To assess the relative influence of the design factors (area, bed materials, grain size and distribution, hydraulic loading rate, aeration supplements, and salinity) on each of the recognized biofilter types: Biodisk, biodrum, trickling filter, and submerged filter.

- (c) To evaluate the effects on filter design of the use of microbial organisms specifically cultured or recommended by the reserachers at the Hebrew University of Jerusalem.
- (d) To select several possible United States sites for Macrobrachium production potential and evaluate water quality conditions. Evaluate water quality treatment needs on the basis of prameters established in task (a) above.
- (e) To develop a mathematical site evaluation model of biofilter water treatment systems to assist in the design of an optimum system for given animal production and water quality conditions.

The Hebrew University of Jerusalem

- (a) To identify and define quality and quantity of wastes produced in each production stage, monitoring by APHA (1977) standard methods, pH, dissolved oxygen (DO), biochemical oxygen demand (BOD), ammonia (NH₃), nitrite (NO₂), nitrate (NO₃), and bacterial analysis.
- (b) To analyze biofilter operation as it relates to the three components of Macrobrachium production using live animals and selected biofilters.
- (c) To culture, identify, and select suitable biofilter organisms for use in the studies in the United States.
- (d) To perform bioassays on growths of Macrobrachium larvae, post-larvae, and adult; evaluationg larvae ovegenesis; larvae period; metamorphosis; size of metamorphosis; size distribution of post-larvae population; , class; molt frequency; , and reproduction as a function of density and ability of the biofilter water treatment system to allow proper animal development.

Evaluation of Research Achievements

The Brigham Young University

- (a) The important water quality parameters related to the growth of Macrobrachium were determine from published literature and personal contacts. In addition, laboratory work conducted in both the United States and Israel were utilized to fill the gaps in the existing knowledge.
- (b) Four types of biofilters (rotating biological contactor, biodrum, trickling filter, and anaerobic submerged filter) were evaluated through extensive laboratory testing. The rotating biological contactor was selected for mathematical analysis and modeling as well. Design methods were developed for the first three units.

- (c) It was originally planned to use microorganisms from biofilters treating the water of the live systems in Israel. However, the difficulties of getting them to the U.S. research site make it more desirable to produce the microorganisms directly in the United States from systems treating live Macrobrachium and other species. In addition, a detailed microscopic examination of the microorganisms on the biofilters at The Brigham Young University and several places throughout the United States could be included in the study. Considerable data were collected.
- (d) Numerous sites throughout the world were evaluated in this portion of the study utilizing the MAPT (Maintenance of Aquaculture Pond Temperature) Model. A site development checklist was developed and utilized for the site evaluation in Colorado. Economic data were collected and presented in a form useful to potential aquaculture developers.
- (e) Biofilter model development and design methods, site evaluation checklists, and the pond temperature prediction model were all parts of the mathematical site evaluation model. The data from each phase is necessary to understand the site development needs. Each of these areas were summarized in the previous section.

The Hebrew University of Jerusalem

- (a) Early in the study and through out the study data were collected on the water quality of recycled water in the Macrobrachium production system. This data was used for operation and evaluation of the biofilter water treatment system and summaries of this data were forwarded to the U.S. research team to guide their selection of synthetic wastewaters for detailed biofilter studies.
- (b) The study of the biofilter systems included improvements during each year of the study. In the first year baseline data were collected using existing systems. During the second year a commercial biodrum system was analysed, and in the final year improvements were made on that system. This approach allowed for both an analysis of biofilter requirements but also improvements in the operational system as troubles arose during the operation of a live system.
- (c) The organisms on the operating biofilters were going to be cultured and sent to the U.S. investigators but it was decided early in the study that this could be achieved more effectively by having the U.S. investigators obtain biofilter samples from throughout the United States. The approach worked very well.
- (d) Records were kept on the growth behavior of the animals during each phase of growth to determine the effects of biofilter operations and water quality on animal production. This data enabled the several stages of redesign of the biofilter system during the study period.

E. DESCRIPTION AND RESULTS OF COOPERATION

The economic production of an aquatic animal in an intensive growth environment requires that the grower understands all of the interactions between the animal and its environment. It is unlikely that one person will have the background to be able to successfully obtain optimum animal growth and also the background to create and evaluate all of possible environmental conditions that the animal could live in.

This project has taken advantage of the animal culturing and production skills of Dr. Dan Cohen of The Hebrew University of Jerusalem and the engineering skills of Dr. Stanley L. Klemetson of The Brigham Young University in the United States. Dr. Cohen collected significant data on the environmental requirements for animal production and forwarded them to Dr. Klemetson to assist in his evaluation of several types of biofilter systems. This information was then made available to Dr. Cohen for his redesign of the biofilters for the live systems. In Israel the biofilters had to be operated in a manner that would insure the survival of the animal population. In the United States the work with synthetic wastewaters permitted the operation of the biofilter systems over a wide range of operating conditions that were not possible with the live systems in Israel. This data permitted the development of biofilter design methods that will enable others to utilize the results of this research effort.

The work in the United States on site selection criteria for aquaculture development will enable others to economically evaluate their possible sites. This work was correlated with the information available in Israel as well as several other places in the world.

This project would not have been possible without the efforts of the researchers from the two countries. The results of this work will aid aquaculture development in both the United States and Israel.

F. THESIS

Three theses and one dissertation were completed in the United States during the course of this study. They are summarized below. Many papers were presented at professional conferences and several papers have been submitted for publication.

Lang, Mark E., "Treatment of Saline Wastewaters Using a Rotating Biological Contactor." Thesis for Master of Science in Civil Engineering, Colorado State University, Fort Collins, Colorado, Fall 1980 (90 pages)

Alarcon, Anibal, "Nitrification in Fresh-Water and Semi-Saline Water Using Rotating Biological Contactors." Report prepared for Master of Science in Civil Engineering, Colorado State University, Fort Collins, Colorado, Summer 1981 (70 pages)

Baird, Drew C., "Biofilter Treatment of Waters in Aquatic Animal Production Facilities." Thesis for Master of Science in Civil Engineering, Brigham Young University, Provo, Utah, Aug 1983 (170 pages)

Rogers, Gary L., "Nitrifier Populations and Kinetics in Selected Aquaculture Water Reuse Biofilters." Dissertation for Doctor of Philosophy in Civil Engineering, Colorado State University, Fort Collins, Colorado, Summer 1983 (135 pages)

G. BENEFITS TO AQUACULTURE

There were significant benefits to aquaculture derived from the work of this research project. Prior to this study little useful information was available on the treatment requirements for fresh water aquaculture utilizing the *Macrobrachium rosenbergii*, or the design requirements of the biofilter treatment systems needed to maintain intensive aquaculture development.

In Israel work was conducted to determine the wastewater characteristics produced by living production facilities of all three stages of *Macrobrachium* production. Also at these sites work was done to determine the treatment efficiency of a standardized biofilter unit supporting the living system.

In the United States the work was concerned with the engineering of biofilter systems operated over a wide range of water quality conditions to determine the sensitivity of the treatment units to changes in water quality. The study stressed a comparison of the four types of biofilters: Rotating Biological Contactor (RBC), Biodrum, Trickling Filter, and Submerged Anaerobic Filter. The work resulted in information necessary for optimizing the sizing of the treatment units for varying animal loads.

The work also included information on site selection criteria, site evaluation methods, and the economics of aquaculture development. While some of this data was site specific, it did provide valuable information on the additional data to be collected for any site. The use of MAPT (Maintenance of Aquaculture Pond Temperature) model was also shown to be a valuable assistance in site evaluation.

The overall benefit to aquaculture was an improved understanding of site evaluation and design requirements for water quality control in intensive aquaculture facilities.

H. INVENTIONS

No development was started or completed by this project that is being considered for registration as an invention.

I. PUBLISHED REPRINTS AND MANUSCRIPTS

Numerous presentations were given and papers were submitted. Abstracts have also been submitted to future conferences but these have not been included in this list. These are summarized below:

Presentations Given:

- ✓ Lang, M.E., and Klemetson, S.L., "The Treatment of Saline Wastewaters Using a Rotating Biological Contactor." Proceedings: First National Symposium/Workshop on Rotating Biological Contactor Technology, Champion, Pennsylvania, Feb 5, 1980, pages 691-710.
- ✓ Rogers, G.L., and Klemetson, S.L., "Evaluation of Biofilter Water Reuse Systems for Macrobrachium rosenbergii Prawn Aquaculture Ponds." Presented at the Second Water Reuse Symposium in Washington, D.C., August 1981.
- ✓ Klemetson, S.L., and Rogers, G.L., "The Future of Biological Fixed-Film Processes and Their Application to Environmental Problems." Presented at First International Conference on Fixed-Film Processes, April 20-23, 1982, Kings Island Resort, Kings Island, Ohio 45034
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