LINK AQUACULTURE

FIN 19

A new recirculation system for rearing juvenile halibut using technology from the tropical marine fish industry

A Handbook for BMFA Members Reference

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Chapter 1

WATER QUALITY AND TREATMENT

When water is first introduced to a recirculation system it is essentially an ideal medium for the culture of fish but as time advances, after fish are introduced, certain nutrients will increase. In addition certain essential compounds will be removed. Unless the water is treated to reduce this process the system will eventually break down and become toxic resulting in death of all the fish. Water treatment is therefore essential and the basic principles are set out below:

Biofilter

Central to all water recycling systems is the biofilter. The chemical processes utilised by the respective types of bacteria are described below. The biofilter is a vessel used to contain the various bacteria required to purify the water. They are a living habitat for many micro-organisms presenting them with a substrate of high surface area on which to attach themselves. The overall result of the complex community living on the biofiltrational substrate is to break down complex waste products by mineralisation and nitrification.

Toxins (especially sulphides), effects of disease treatments, oxygen, pH and temperature have a great influence on the efficiency of this delicate community.

Biofiltration in this system is provided by the four trickle towers and four fluidised beds. The trickle towers are termed ‘dry’ biofilters, as they are not submerged. The fluidised beds are ‘wet’ biofilters, the substrate being submerged. Dry biofilters add oxygen to the process whilst wet ones remove oxygen. Different profiles of bacteria are present on both types, increasing their flexibility in the overall water treatment process.

Biofiltration is the process whereby naturally occurring microorganisms are used to convert toxic compounds in the water into less toxic compounds, which are ultimately removed from the system. In order to understand the functioning of the biofiltration process, there are two basic principles, which are outlined below:

Mineralisation

Mineralisation is a general term used to refer to the removal of unwanted organic compounds from the system. Organic compounds increase in the system as a result of waste excretion from fish and other living organisms, uneaten food, lysis the cells of

dead microorganisms. Mineralisation results from the activity of heterotrophic bacteria in recirculation systems. During this process complex organic molecules are broken down into simple inorganic compounds; e.g. proteins into amino acids and ultimately to ammonia, and carbohydrates into carbon dioxide and water.

Nitrification

Nitrification is one of the main processes in the biofilter. Ammonia is excreted by the fish and has to be removed as it is highly toxic. This process is performed mostly by *Nitrosomonas* bacteria by oxidising ammonia to nitrite (equation 1) which is in turn oxidised into nitrate mostly by the bacterium *Nitrobacter* (equation 2).

Equation 1

$$2NH_4^+ + 3O_2 = 2NO_2^- + 2H_2O + 4H^+$$

Equation 2

$$2NO_2^- + O_2 = 2NO_3^-$$

The hydrogen ions produced in equation 1 are responsible for the constant fall in pH within the system. This is more noticeable in fresh water as sea water has a natural buffering effect.

Mineralisation and nitrification occur in the *biofilter* which is described below:

Mechanical Filtration

Most systems utilise a form of removal of solid materials e.g. uneaten food and faeces. Removal of particles > 100um substantially reduces the load on the biofilter. Mechanical filters vary from simple foams and gauzes to more sophisticated belt or drum dynamic filters.

In this system a swirl separator is used. Water enters the separator tangentially and solids fall to the bottom. Solids are removed to waste.

Oxygenation

Oxygen is removed by both the fish and biofilter and must be replaced to support the system. Addition is either by aeration (compressed air) or pure oxygen.

In the first system aeration was applied to the fish tanks and foam fractionation units. Pure oxygen was added to the header tank as required.

In the second system aeration was applied to the foam fractionation units. Oxygen was supplied directly to the fish tanks via a feedback system.

Foam Fractionation (Protein Skimming)

The protein skimmer (foam fractionator) removes dissolved or colloidal material in the water and brings it to the surface as foam. The hydrophobic end of the molecule embeds itself in the rising air bubble and is thus raised to the surface. The foam is removed from the system to waste and decreases the load on the biofilter. Foam fractionation is more effective in salt water.

Ozone (see below for details) is added to the system at this point by a redox potential probe linked to a relay controlling the ozone generator. Thus the redox potential of the effluent water is maintained at a constant 350 mV.

Ultra Violet Treatment

UVc (260 nm wavelength) units are commonly used on recirculating fish farms. Units have been reported to significantly reduce fish diseases. Rainbow trout held in UVc irradiated water infected with *Myosoma cerebralis* (causative agent for whirling disease) for 4.5 months showed very little signs of the disease compared with the control. In addition, the fish in UVc irradiated water had average weights 1.8-2.6 times greater than the control fish. UVc water treatment in a through-flow hatchery reduced deaths from the visceral myosporidian *Ceratomyxa shasta* from 60 % to 2 % in a coho salmon, *Oncorhynchus kisutch* and from 20 % to 1 % in trials on rainbow trout. Atlantic salmon *Salmo salar* were prevented from contracting furunculosis, caused by *Aeromonas salmonicida*, by UVc irradiating spring water introduced to the hatchery. UVc kills the free-swimming stages of the gill parasite *Oodinium* or *Ichthyobodo* (costia) but the adult stages remain on the fish. In this case UVc is used to break the life cycle but for maximal effect it may require to be used in conjunction with ozone.

Uvc is particularly useful in the sterilisation of influent make-up water (as in this system) to avoid the introduction of pathogens such as *M. cerebralis*, *C. shasta* and *A. salmonicida* into the system.

All water is UV treated in the present system before returning to the fish in the tank room.

Ozonation

Ozone, O_3, is produced by passing dry refrigerated air, (O_2), over two arcing electrodes and is a powerful oxidation agent often producing valuable oxygen as a by-product. Redox potential in the recirculating water is a measure of free ions in solution. A negative potential indicates an abundance of reduced (negatively charged) molecules and is considered to be an indication of impure water while a positive redox potential indicates pure water. The addition of organic matter will reduce the redox potential, due to an abundance of reduced molecules, even if oxygen levels are at saturation. The redox potential can be restored by the addition of an oxidising agent

such as potassium permanganate, hydrogen peroxide or ozone. The redox potential of natural sea water at pH 8.0 is 350-400 mV, a level considered satisfactory for aquaculture. At 700 mV the water is considered sterile.

Ozone, when added to recirculation systems, initiates an increase in BOD, as it is responsible for breaking carbon double bonds of large molecules. These molecules which were previously highly resistant to biodegradation and hence unavailable to the heterotrophic bacteria in the biofilter, after ozone treatment can be utilised by these bacteria and hence increase the BOD. Such compounds would normally concentrate themselves in the water, causing discoloration and can only be removed by water changes. The clarity of the water can therefore be significantly improved by use of ozone treatment. Antioxidants, which are common in fish foods, may build up and inhibit the biofilter if not removed by partial water changes or ozonation. Ozone oxidises and breaks down potentially toxic compounds and is also capable of oxidising ammonia and nitrite thus assisting the biofilter in their removal.

The use of ozonation in new recirculation systems seems an attractive option. The major drawbacks are that ozone itself is lethal to fish (and operators) and must be removed from the water before entering the fish holding tanks. In order to avoid stressing the fish, ozone concentrations should be kept below 2 μg/l. There is some evidence to suggest that ozone produces compounds, which may be lethal to oyster larvae. These compounds can only be removed by treatment with activated carbon (this treatment also removes ozone). Ozonation requires expensive equipment to generate it, as does its removal before returning the water to the fish. Ozone addition is via contact chambers and removal by either passing the water through activated carbon, aeration of the water, vacuum degassing or by UVc treatment. Dealing with the removal of ozone from large quantities of water is a problem. There is little opportunity to treat the water in a through flow fish farm as a continuous flow must be maintained. Therefore monitoring ozone is necessary, expensive and not always reliable.

Pathogens such as viruses and bacteria are destroyed by protoplasmic oxidation using ozone, as are protozoans and to some extent rotifers. The growth of juvenile eels, *Anguilla anguilla*, is significantly improved in a partially closed system using ozone compared to identical systems using oxygen and air.

The use of ozone in recirculation systems shows great potential, and many companies are reporting some success in controlling the various problems outlined above. Undoubtedly ozonation of water will play an increasing role in future recirculating aquaculture systems.

The present system utilises ozone introduced by a feed back loop system into the foam fractionator. The use of ozone appears to be very successful and no problems have been encountered in its control and removal.

Chapter 2

INSTALLATION

The installation consists of two rooms, the Plant Room and the Tank Room. Fish are kept in the tanks in the Tank Room, the layout is shown in *figure 1*. Water draining from the fish tanks is passed to the Plant Room where it is treated and returned to the tanks in the Tank Room. A layout of the Plant room can be seen in *figure 2*.

TANK ROOM

Tanks (First Stage)

Tanks are in three rows (*Figure 2:1*) with dimensions are shown in *Table 2:1* as follows:

Table 2:1

Tank Numbers	Length (m)	Breadth (m)	Depth (m)	Basal Area (m^2)	Volume (m^3)
1-8	**1.00**	**1.00**	**0.23**	**1.00**	**0.23**
9-16	**1.00**	**1.00**	**0.23**	**1.00**	**0.23**
R1	**4.00**	**0.44**	**0.12**	**1.76**	**0.21**
R2 & R3	**4.00**	**0.88**	**0.12**	**3.52**	**0.42**

Tank numbers 1-8 and R1-R3 are supplied by recirculated (RC) water. Effluent water from the tanks can be diverted to waste by the operation of a valve. Influent water to the tanks can be supplied by 'fresh' seawater (pumped ashore) by the operation of a valve. By the operation of both of these valves, the recirculation tanks can be operated on throughflow.

As can be seen by the dimensions in *table1*, tanks R1-R3 are raceways.

Tank numbers 9-16 are supplied by 'fresh' (pumped ashore) seawater which flows to waste and termed throughflow (TF).

(TF) water is treated by ozone, activated carbon and UV light before entering any part of the installation.

Oxygenation

Each tank is equipped with a 155 x 30 mm micro bubble diffuser supplied by compressed air at 50 p.s.i. R1, being twice the volume of the other tanks (*table 1*), has two such diffusers.

An oxygen bottle is on standby and can be attached to the airline in the event of power or compressor failure.

Alarm System

Each individual tank is equipped with a low level alarm which activates should the tank water level fall below a pre-set level.

All alarms return to a central control panel. In the event of an alarm being activated, an audible alarm is sounded which is also transferred to a remote pager.

Tanks (Second Stage)

Tanks are in three rows (*Figure 2:1*) with dimensions are shown in *Table 2:2* as follows:

Table 2:2

Tank Numbers	Length (m)	Breadth (m)	Depth (m)	Basal Area (m^2)	Volume (m^3)
1-5	**1.50**	**1.50**	**1.00**	**2.25**	**2.25**
6-10	**1.50**	**1.50**	**1.00**	**2.25**	**2.25**

Tank numbers 1-5 are supplied by recirculated (RC) water. Effluent water from the tanks can be diverted to waste by the operation of a valve. Influent water to the tanks can be supplied by ‘fresh’ seawater (pumped ashore) by the operation of a valve. By the operation of both of these valves, the recirculation tanks can be operated on throughflow.

Tank numbers 6-10 are supplied by ‘fresh’ (pumped ashore) seawater which flows to waste and termed throughflow (TF). By the operation of valves the TF tanks can be introduced to the RC system.

(TF) water is treated by ozone, activated carbon and UV light before entering any part of the installation.

Oxygenation

Each tank is equipped with a 155 x 30 mm micro bubble diffuser supplied by oxygen via a feed back system. Oxygen can be turned on manually in the event of power failure.

Alarm System

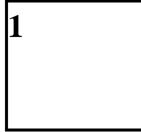
Each individual tank is equipped with a low level alarm which activates should the tank water level fall below a pre-set level this system alerts a remote pager.

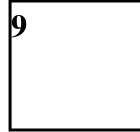
FIGURE 2:1

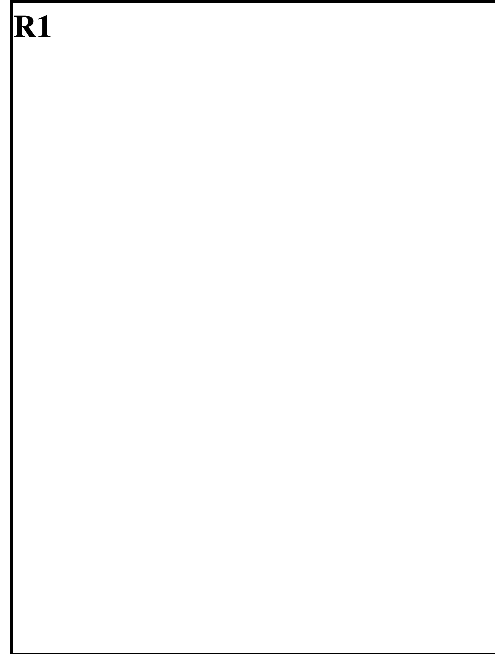
Tank Room Layouts 1&2

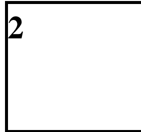
TANK ROOM LAYOUT 1

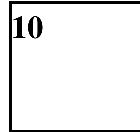
Recirc. Tanks	**T.Flow Tanks**	**Recirc. Raceways**
RC	**TF**	**RW**

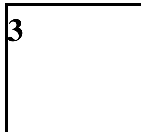


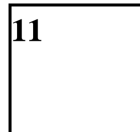


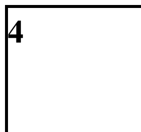


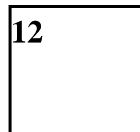


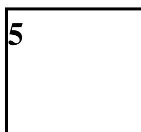


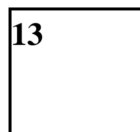


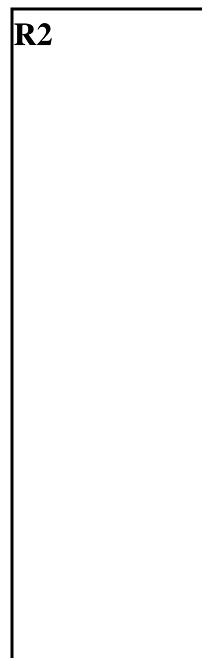


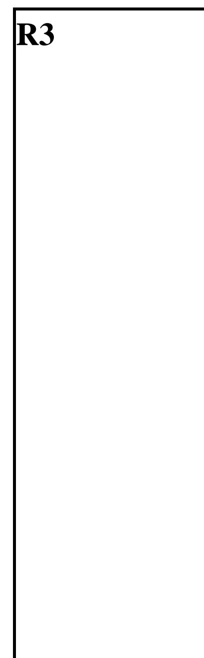


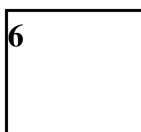


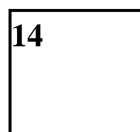


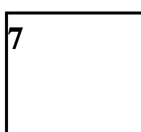


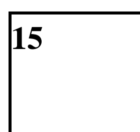


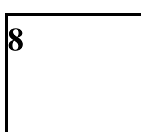


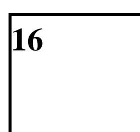












TANK ROOM LAYOUT 2

1	6
2	7
3	8
4	9
5	10

PLANT ROOM

All water draining from the RC tanks is collected in a common pipe and enters the Plant Room. The layout of the Plant Room can be seen in ***Figure 2:2.*** Each item of water treatment equipment is considered separately and approximately in order of use the lettering referring to ***Figure 2:2.***

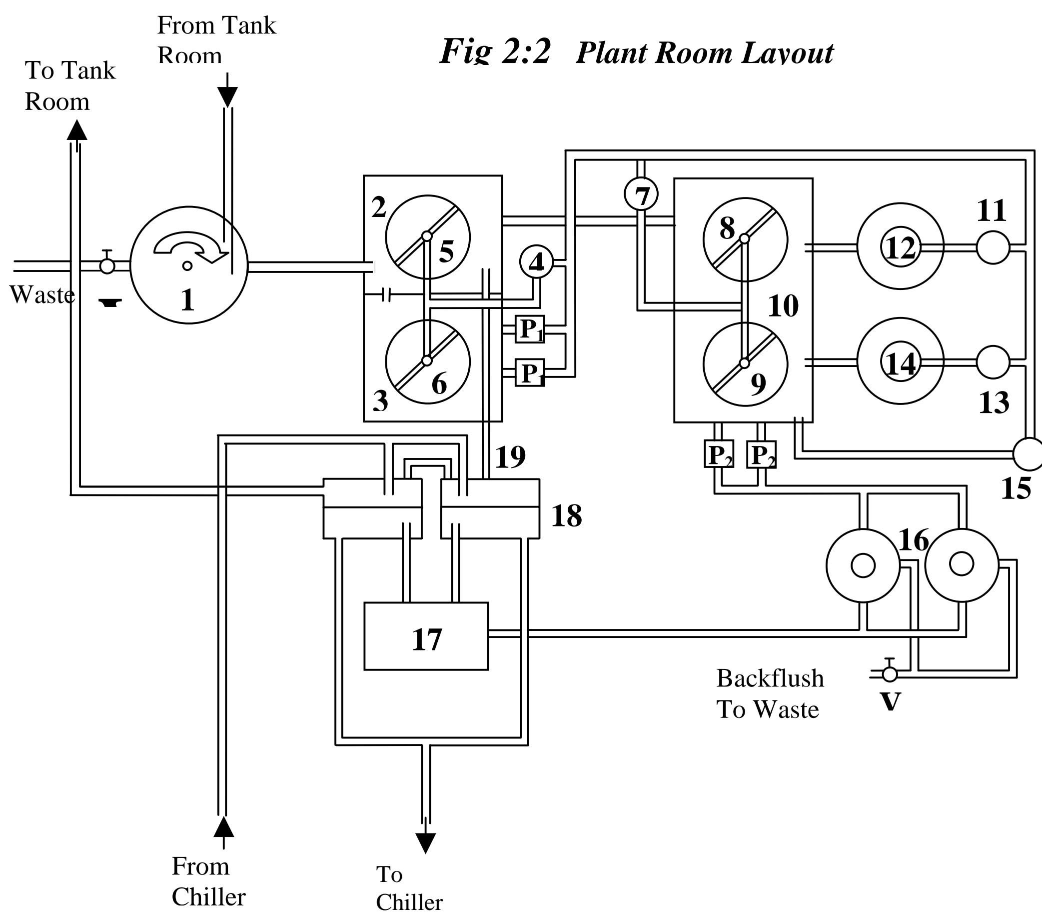


Fig 2:2 Plant Room Layout

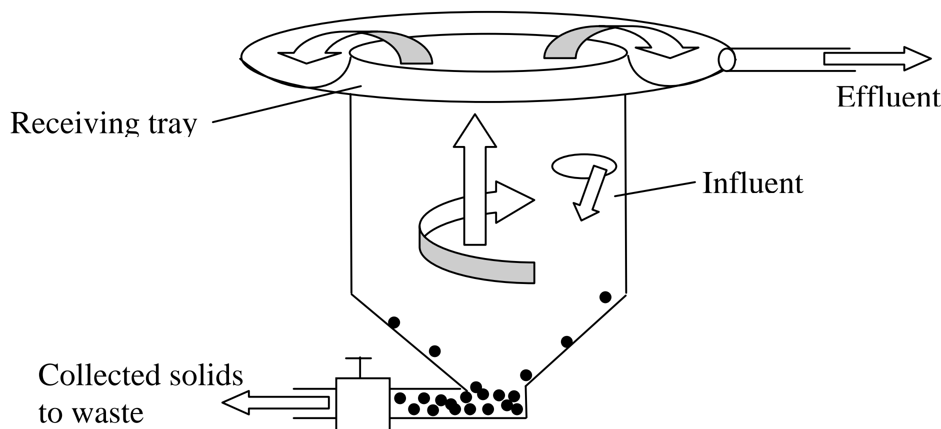
Number	Description
1	Swirl separator
2	Sump (S1)
3	Sump (S2)
4	Fluidised bed (F1)
5	Trickling biofilter (B1)
6	Tricklingbiofilter (B2)
7	Fluidised bed (F2)
8	Trickling biofilter (B3)
9	Trickling biofilter (B4)
10	Sump (S3)

Number	Description
11	Fluidised bed (F3)
12	Foam fractionator (FF1)
13	Fluidised bed (F4)
14	Foam fractionator (FF2)
15	Magnasphere fluidised bed
16	Pressurised sand filters (S1&2)
17	Ultra violet filter
18	Header tanks
19	Overflow
P	Pump
V	Valve

Swirl Separator (*Figure 2:3*)

All the water to be treated enters the swirl separator. Water enters tangentially near the top of the unit, creating a spiral. The rotational flow causes heavier particles to move outwards towards the wall of the unit and downwards. Solids mainly uneaten food and faeces, are removed to waste along with a small proportion of the flow. A low pressure area created at the centre of the spiral carries the clean water up to the receiving tray from where it exits into Sump 1. It is essential to remove solids as soon as possible before they dissolve and increase the organic loading on the rest of the system.

(*Figure 2:3*)



Sumps (S1, S2 & S3)

All water enters S1. From here the water can either overflow the weir into S2 or enter S3 via the balancing pipe. Water exits S3 and is returned to the tanks without undergoing biofiltration.

Pumps (P1 & P2)

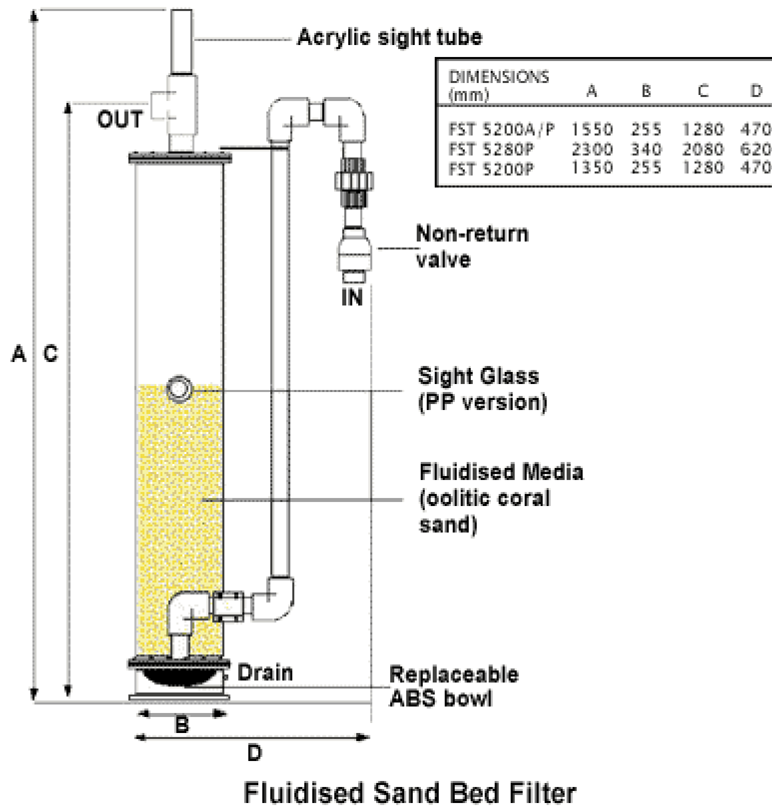
All water entering the treatment plant does so via two pumps (P1).

Biofiltration (B1, B2, B3, B4, F1, F2, F3 & F4) (see Chapters 1&5 for principles of operation)

Water exiting S2 passes through pumps P1 or P2. The water must go through a fluidised bed (either F1, 2, 3 or 4) and then either a trickling filter or foam fractionator. In the biofilters heterotrophic and autotrophic bacteria remove unwanted metabolites and other compounds from the water.

Fluidised beds (*Figure 2:4*). – Coraline sand is contained in these biofilters. Water enters in such a way as to fluidise the sand (keep it in suspension). These are ‘wet’ or submerged biofilters. As a result of no air being added to the biofiltration unit, the heterotrophic and autotrophic bacteria remove oxygen.

Figure 2:4



DIMENSIONS (mm)	A	B	C	D
FST 5200A/P	1550	255	1280	470
FST 5280P	2300	340	2080	620
FST 5200P	1350	255	1280	470

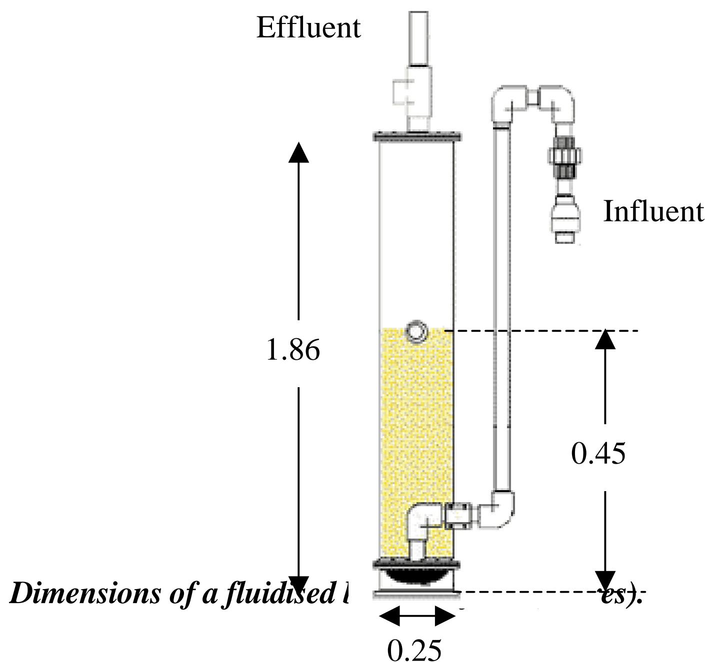
Fluidised bed filter.

The coraline sand is held in suspension by the entry of water at the base through a diffuser bowl.

Expansion of sand is checked through sight glass.

Drawing:

Tropical Marine Centre.



Dimensions of a fluidised l** **es).

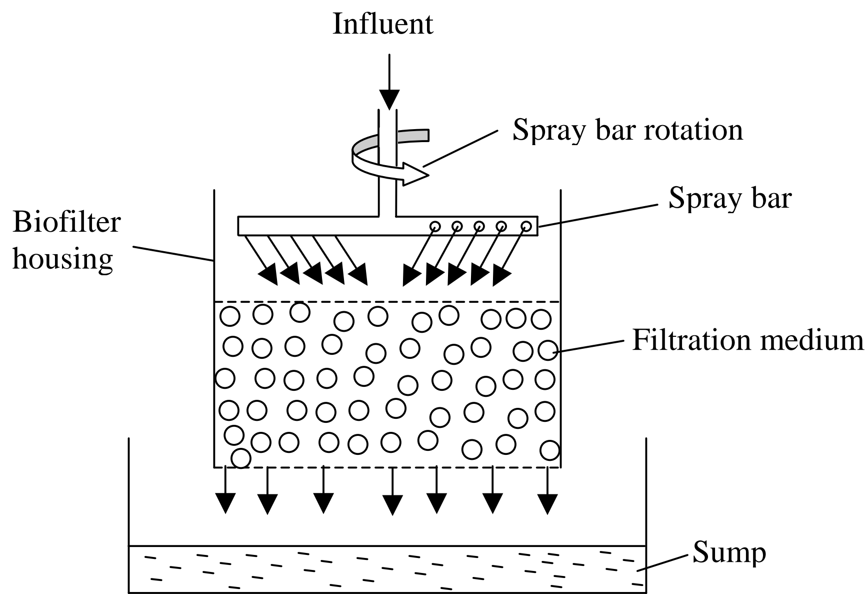
Trickling filters (***Figure 2:5)*** – There are four trickling filters (B1, 2, 3 and 4). Water enters the filters via a spray bar, which rotates under the power of the water running through it. Inside the filters are artificial plastic media providing a high surface area for the colonies of microbes to adhere to. The trickling filters are ‘dry’ or non-submerged

facilitating air to be drawn into the system and thus adding oxygen to the system. This process also removes carbon dioxide.

B1 & B2 drain into S2 and hence allow for the water to be recycled through the system. During a heavy organic load, water can be diverted through this loop by increasing the flow by the operation of a valve. B3 & B4 drain into S3 and allow an earlier escape for the water. During a light organic loading, water is encouraged to take this route by the operation of a valve.

Figure 2:5

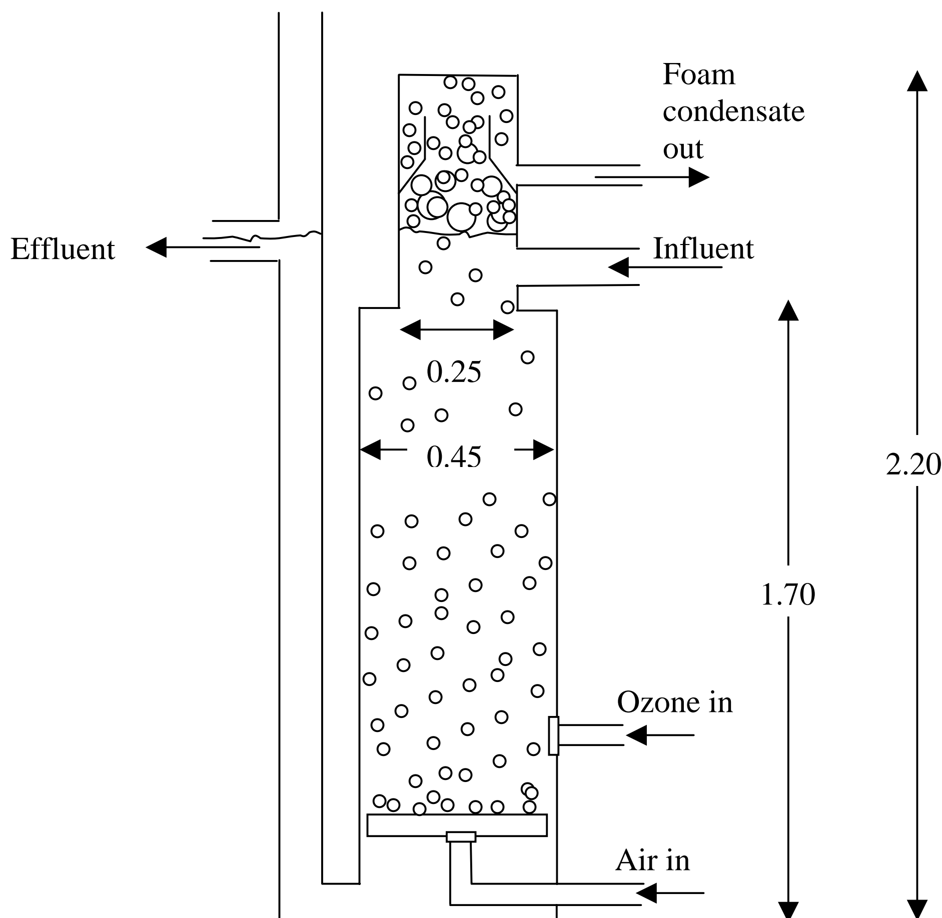
Trickle filters



Foam Fractionation (FF1 & FF2) (***Figure 2:6***) (see Chapters 1&5 for principles of operation)

Approximately one third of the water exiting S2 passes through the foam fractionators. This amount can be adjusted by the operation of valves depending on the characteristics of the water to be treated. Large chained organic materials such as proteins are separated in the foam, which runs to waste. Each fractionators has two pumps which draw water from them and return it together with air introduced at venturis (one per pump). Ozone is introduced to the foam fractionators via these venturis. Redox probes are placed in the effluent of the fractionators and feed back to a control unit. Should the redox potential of the effluent water fall below 350 mV the ozone generator is switched on. The generators are switched off at 400mV. There is one ozone generator, redox probe and control unit per fractionator. The ozone generators are mounted outside the Plant Room to avoid corrosion from the salt atmosphere.

Figure 2:6



Foam fractionator dimensions (metres).

pH Fluidised Bed

Some water from the system is diverted via a valve in series to a fluidised bed filled with magnaspheres (magnesium hydroxide). By controlling the water flow, the pH can be effectively controlled.

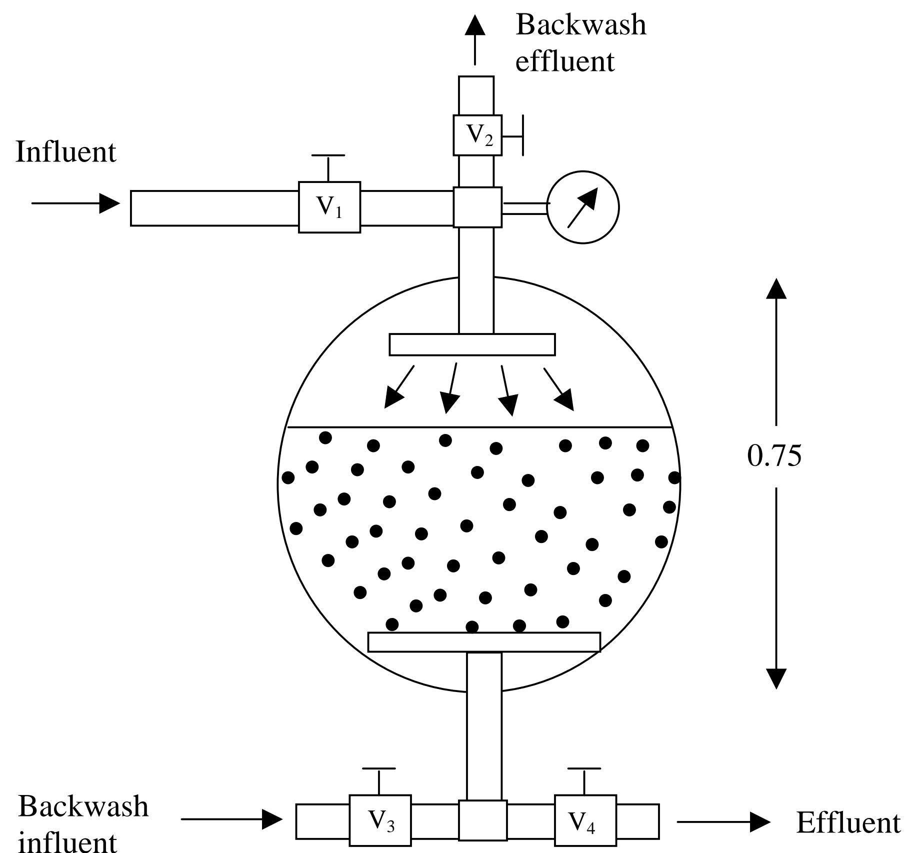
Pumps 3 & 4

All water from the treatment plant is returned to a header tank via two pumps (P2).

Pressurised Sand Filters (S1 & S2) *(Figure 2:7)*

Two pressurised sand filters are run in parallel and valved in such a way as to allow either one to be isolated. The filters are filled with advanced filter material (AFM) which inhibits bacterial growth on the surface of the media and thus alleviates clogging of the filter. Each filter has a multiport valve facilitating backflushing to waste.

Figure 2:7



Dimensions (metres) and valve operation of one of the pressurised sand filters. Valves V_{1-4} are simultaneously operated by a single control; this enables the filter to be backflushed by a single operation procedure.

Ultra Violet Filters

Two UVc filters are run in parallel and valved in such a way as to allow either one to be isolated. Each filter has six UVc, 100 W. bulbs. There are flow meters on the outlets. Should the sand filters become blocked (in need of backflushing) the flow meters will show a decreased reading.

The UV lamp control box is mounted outside the Plant Room to avoid corrosion from the salt atmosphere.

Header Tanks

There are two header tanks raised above the plant on a gantry system. A balancing pipe links the tanks. Both tanks have a weir across the middle and water is discharged from the UV filters into the first stage of the tanks. Water flows over the weir into the next stage, the level of this stage being maintained by a standpipe. An overflow discharges from the header tank into S1. Water exits the stand pipe and returns to the Tank Room.

Chiller

Two 40 kW chillers are outside the plant room. Only one chiller is used at one time the other is a standby. A valve arrangement makes changing chillers simple and rapid in the event of a failure. Water is removed from the header tanks from one side of the weir and returned to the other side. Water is therefore only put through the chiller once.

Oxygenation

The header tank is equipped with a 155 x 30 mm micro bubble diffuser supplied by pure oxygen from a bottle and is utilised as necessary. Air is violently introduced by four venturis as part of the foam fractionation system.

System Monitoring

Water returning to the Tank Room is monitored for pH and redox. Probes for the redox and pH meters are in the header tank.

Alarm System

The header tanks are equipped with a low level alarm which activates should the tank water level fall below a pre-set level.

The control boxes regulating the input of ozone are alarmed. The alarm is activated by high or low redox readings, which are indicative of too much, or too little, ozone.

The temperature of the system is monitored. Should the temperature rise beyond a set point, an alarm is activated.

All alarms return to a central control panel. In the event of an alarm being activated, an audible alarm is sounded which is also transferred to a remote pager.

Chapter 3

DEVELOPMENT AND USE

The recirculation (RC) system was developed artificially without fish until stablity of water parameters was achieved before fish were added. The following describes the development of the system under these conditions and relates to the 'Parameters at System Start-up' ***Table 3:1***.

During the start-up there are two distinct phases:

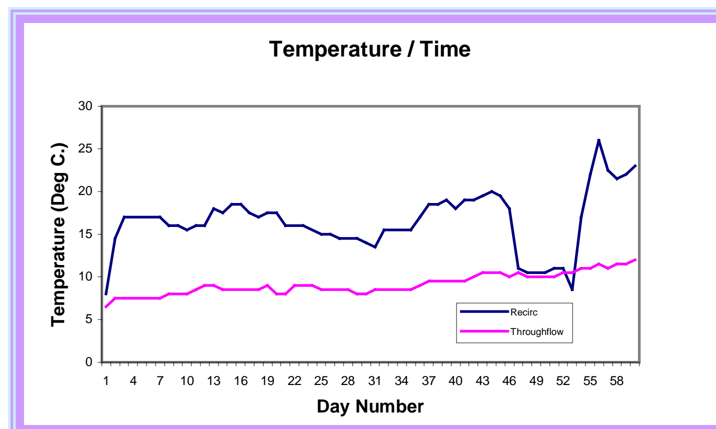
1. Artificial development
2. Initial stocking with fish

Data for the two phases ('Artificial Development' and 'With Fish') are considered separately below as the system behaved differently during each phase. The system was developing from a position of no established biofiltration capacity during the first stage. The system was stabilising in response to a high organic load during the second phase.

Temperature

During Artificial Development
The development of temperature can be seen in ***Figure 3:1:***

Figure 3:1



The temperature of the RC system rose rapidly from 6 °C to 17 °C in three days. ***Figure 3***:1 demonstrates how unstable the RC temperature is in comparison to the TF. The RC temperature was allowed to fluctuate between 13.5 °C and 20 °C to provide a high temperature for the biofilters to develop. On day 46, a 40 kW chiller was hired for a week to establish the size of chiller required to control the temperature of the system. Within 24 hours, the temperature was reduced from approximately 20 °C to

approximately 11 ^{o}C. This temperature of approximately 11 ^{o}C was maintained constantly for six days. On the seventh day, the chiller was turned down and within 12 hours the temperature had dropped to 8.5 ^{o}C. The chiller was then turned off and the temperature rose to 22 ^{o}C within two days.

With Fish

When fish were added to the system a chiller was permanently attached to the system. During experiments, the temperature was set to the same temperature as the throughflow system and remained stable. The chiller was on for approximately 50% of the time, drawing 20 amps when working.

TABLE 3:1

System Start-up Parameters

PARAMETERS AT SYSTEM START-UP

Day No.	Ammonia	Nitrite	Nitrate	Temp RC	Temp TF	
1	0	0	0	8	6.5	
2	0	0	0	14.5	7.5	
3	0	0	0	17	7.5	
4	0	0	0	17	7.5	
5	0	0	0	17	7.5	
6	0	0	0	17	7.5	
7	0	0	0	17	7.5	
8	0	0	0	16	8	
9	1.5	0	0	16	8	"250g Epicin, 60g Ammonia"
10	1.5	0	0	15.5	8	
11	1.5	0	0	16	8.5	
12	1.5	0	0	16	9	
13	1.5	0	0	18	9	
14	1.5	0	0	17.5	8.5	
15	1.5	0	0	18.5	8.5	
16	1.5	0	0	18.5	8.5	
17	1.5	0	0	17.5	8.5	
18	1.5	0	0	17	8.5	T.V.C before U.V. 5.8*103
19	1.5	0	0	17.5	9	
20	1.5	0	0	17.5	8	
21	1.5	0	0	16	8	
22	1.5	0	0	16	9	
23	1.5	0	0	16	9	
24	1.5	0	0	15.5	9	250g Epicin
25	1	0	0	15	8.5	pH 8.2 RC 8.0 TF 40g feed added
26	1	0	0	15	8.5	8.2 8.0 125g feed added
27	1	0.1	0	14.5	8.5	Nitrite developing
28	1	0.1	0	14.5	8.5	
29	1	0.1	0	14.5	8	
30	1	0.2	0	14	8	
31	1	0.2	0	13.5	8.5	
32	1	0.2	0	15.5	8.5	
33	1	0.4	0	15.5	8.5	
34	0.8	0.6	0	15.5	8.5	
35	0.8	1	0	15.5	8.5	
36	0.8	2	6	17	9	
37	0.8	2	60	18.5	9.5	
38	0.8	2	75	18.5	9.5	
39	0.8	2	75	19	9.5	
40	0.6	2	100	18	9.5	
41	0.6	2	100	19	9.5	
42	0.6	2	100	19	10	
43	0.6	2	100	19.5	10.5	
44	0	2	300	20	10.5	1 p.p.m.Ammonia added
45	0.5	2	750	19.5	10.5	
46	0.5	2	500	18	10	40Kw test chiller added
47	0.5	2	500	11	10.5	
48	0.5	2	500	10.5	10	
49	0.5	2	500	10.5	10	
50	0	10	500	10.5	10	1 p.p.m.Ammonia added
51	0.4	20	500	11	10	
52	0.4	20	500	11	10.5	
53	0.2	20	750	8.5	10.5	Chiller decreased to test then turned off
54	0.1	10	750	17	11	1 p.p.m.Ammonia added
55	0.1	10	750	22	11	2 p.p.m. Ammonia added daily point
56	0.1	10	750	26	11.5	
57	0.1	20	1000	22.5	11	
58	0.2	20	1000	21.5	11.5	
59	0.2	20	1000	22	11.5	Water removed from system
60	0.1	15	500	23	12	

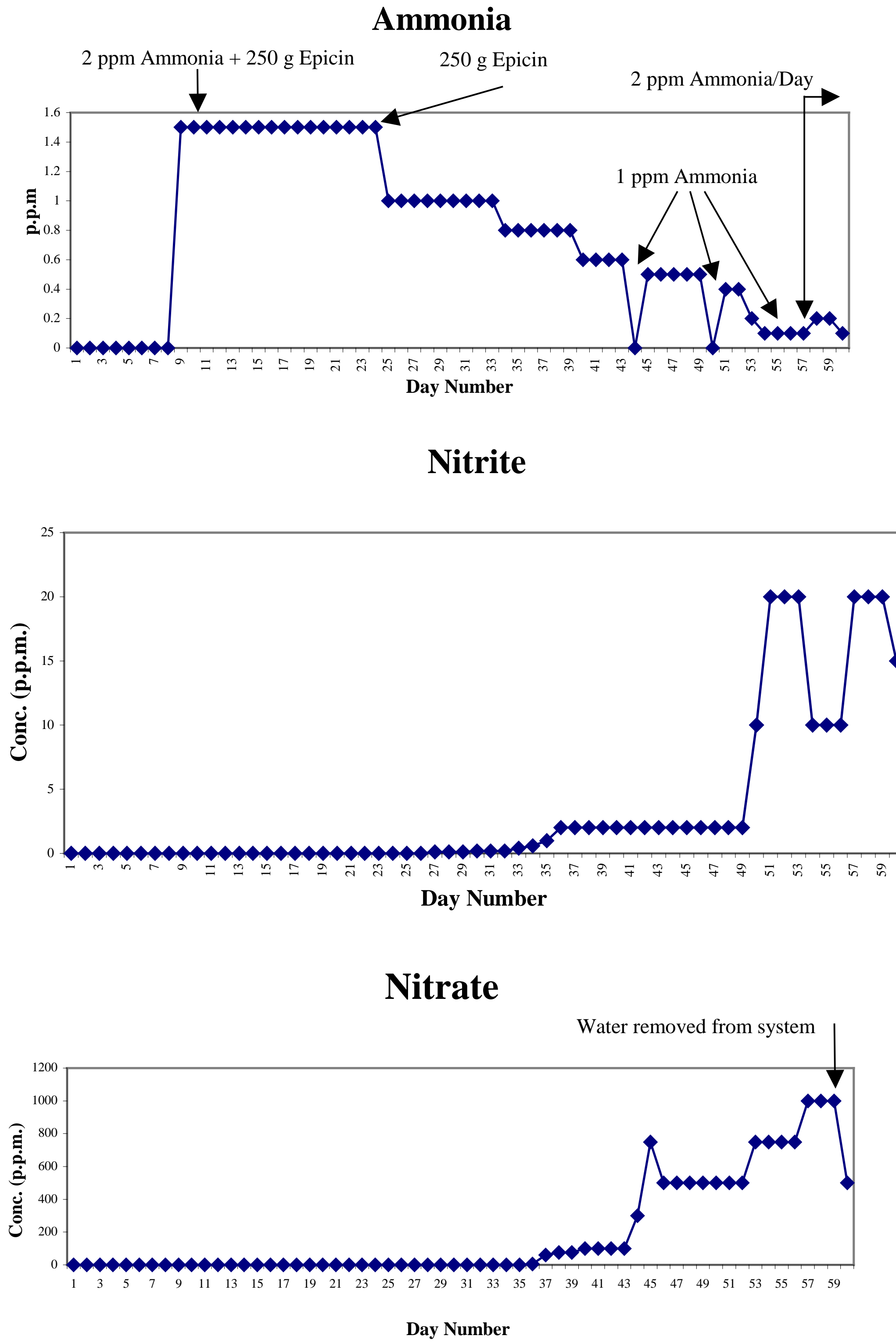
Nitrification

During Artificial Development
The development of the biofilters (trickle towers and fluidised beds) was monitored until stability was reached. The development can be seen graphically in *figures 4*.

Figure 3:2

Development of Nitrification

Figure 3:2



On day 9, after the temperature was stable, 250g of 'Epicin' was added equally to the four trickling filters. Epicin is a commercially available dry preparation of nitrifying bacteria on a substrate of bran. To create ammonia loading, 60g of a mixture of ammonium salts were added. The salts utilised (in equal parts) were $(NH_4)_2 SO_4$, $(NH_4)_2 CO_3$ and $NH_4 PO_4$. The concentration of ammonia immediately rose to 1.5 p.p.m.

On day 18, in order to investigate the degree of microbial activity in the system, the total viable count (TVC) of bacteria was established before the UV filter. The TVC was 5.8 x 10^3 colony forming units (cfu) per ml.

A further 250g of Epicin was added to the biofilters on day 24 as the ammonia dropped to 1 p.p.m.

The drop in ammonia on day 24, together with a TVC of 5.8 x 10^3 cfu/ml was taken as an indication of nitrifying activity (particularly of the *Nitrosomonas*) in the biofilters. At this stage (day 25) 40g of fish food was added to the biofilters to vary the composition of the environment and to encourage heterotrophic bacteria to establish themselves. Heterotrophic bacteria can out compete nitrifying bacteria and so the latter were encouraged first.

Day 26 saw the first sign of nitrite developing. A further 125g of fish feed was added to the biofilters as the *Nitrosomonas* were considered to be established. Nitrite developed rapidly over the next eight days and on day 36 was established at above 2 p.p.m. It was at this stage of the biofilter development (day 36) that nitrate appeared. Nitrate rose steadily until on day 59, upon reaching 1000 p.p.m. the sand filters were backflushed causing a 20% water loss from the system and a fall to 500 p.p.m. Water was removed on a regular daily basis from day 67 causing the drop in nitrate. Volumes removed were up to 10% per day.

Nitrite rose steadily to peak at 20 p.p.m. This level dropped to a 'typical' level of 2 p.p.m. on day 66 and remained at this level, and below, from this point on. When fish were added this level dropped further (see below).

Ammonia was falling and reached 0 p.p.m. by day 44. 1 p.p.m. ammonia was added to the system causing a rise in ammonia to 0.5 p.p.m. When the concentration of ammonia fell to 0 p.p.m. more was added. As the biofilters were firmly established by day 55, 2 p.p.m. ammonia was added daily and utilised fully by the biofilters within 24 hours.

To test the rate of ammonia oxidation, 3 p.p.m. of ammonia was added. The reduction in ammonia per unit time and is given in ***Table 3:2***:

Table 3:2

Ammonia Concentration (p.p.m.)	Time (Hours)
3.0	0
3.0	1
2.5	2
2.0	3
1.0	5
0.6	6
0.6	7
0.2	9
0.0	24

The system was now fully conditioned and ready for fish.

Stability of the biofilters was achieved by approximately day 44. Epicin and ammonia were introduced on day nine. The biofilters therefore took approximately 35 days to develop.

With Fish
When fish were added to the system for experimentation, the ammonia remained constant at 0.1 –0.3 p.p.m. Nitrite rose initially to 2.0 p.p.m. but stabilised to 0.1 – 0.3 p.p.m. within 5 days. The nitrite reducing bacterial colonies were possibly under developed for this sudden increase in organic loading. Nitrate was controlled by water exchanges and was typically between 15 – 35 p.p.m.

pH

During Artificial Development
As can be seen from the Chapter 5, as the oxidation of ammonia proceeds to nitrate, H^+ ions are added to the system. The net result of this process is the reduction of pH. During the development of the biofilters, the pH remained constant at 8.2 – 8.3. This stability of the pH was undoubtedly caused by the strong buffering system in seawater.

With Fish
The pH of the system was relatively constant at 7.5 – 7.7. Make-up and through flow water had a constant pH of 8.2 – 8.3. During the early stages of the trial, there was no facility (such as a fluidised bed of magnaspheres) to increase the pH. Obviously the buffering system of the seawater was unable to cope with the increased addition of H^+ ions into the system. Upon the addition of the pH fluidized bed, the pH was maintained between 7.9 and 8.1.

Redox Potential

During Artificial Development
The redox potential was recorded from the foam fractionator/ozone loop systems and remained relatively stable at approximately 350 mV.

With Fish
Redox was measured at the header tank (return point to the tank room). The redox potential returning to the fish fluctuated between 330 and 390 mV. The readings at the foam fractionator (point of ozone addition) were consistently lower leading to speculation that there is a time lag between the addition of ozone and the increase in redox potential.

Foam Fractionators

During Artificial Development
As there was little organic load on the system, there was very little evidence of the foam fractionators working. Foam was produced especially after water was added, presumably due to the organic content of ambient seawater.

With Fish
The fractionators worked admirably during a high organic load. The levels in the fractionators were set by the valve restricting the outflow, and finely adjusted by the inlet valves. If the level was set too high, too much water overflowed to waste. Production of foam was proliferous when the fractionators were set correctly.

Bromine

During Artificial Development
There was some concern over the intermediate oxidation products of bromine (principally BrO^- at seawater pH, HOBr in more acidic water.) when using ozone, which is a powerful oxidant. These bromites can be toxic to fish and only seem to occur when 'fresh' water (newly introduced saltwater) is added to the system as bromine is oxidised to bromate (BrO^-_3). As it appears to be only the intermediate products that are toxic, the final bromate is considered safe for the introduction to the fish. Should there be a problem with intermediate products, the incoming water must be ozone treated at least 24 hours before use.

Bromine was monitored and found to be less than 2 p.p.m.

With Fish
With fish in the system, the bromine levels never rose above 2 p.p.m. It is not known if this was deleterious to the fish as testing for intermediate oxidation products would be complex and the equipment was not available.

Pressurised Sand Filters

During Artificial Development
The sand filters were not regularly flushed as there was no particulate matter. At one stage the redox potential of the system dropped. It was surmised that there was a bacterial growth in the filters reducing the oxidation capacity (and hence the redox potential) of the water. The filters were backflushed and the redox potential rose. The nitrate concentration also dropped.

With Fish
Sand filters were backflushed daily due to increased particulate matter and the possibility of clogging due to bacterial growth. If the filters became blocked, the flow meters to the header tanks showed a reduced flow. After backflushing the flow rates were restored.

Swirl Separator

During Artificial Development
There was no activity in the swirl separator during this stage.

With Fish
The separator worked well on uneaten fish food with all the pellets falling to the bottom of the unit.

Tanks

During Artificial Development
During development, growth of a 'slime' covering on the surfaces of the tanks was encouraged to help establish biofiltration bacterial colonies.

With Fish
Tanks cleaned themselves well. Raceways did not display such effective self-cleaning properties as the tanks. The sumps below the outlet screens of both tanks and raceways did not clean well. To clean the sumps, the standpipe was removed daily thus flushing any solid build up away. The water was diverted to waste in order to decrease the load on the water treatment plant.

Electrical Consumption

During Artificial Development
No assessment of electrical consumption was made at this stage as pumps and chillers were not constantly in use.

With Fish
Electrical consumption averaged out at 282 units (kW-hr) of electricity per day.

Water Consumption

During Artificial Development
A minimal amount of water was used during the development of the system as no water was exchanged until the system was fully developed (Nitrate 1000 p.p.m.). Water was removed on a regular basis after day 67 as a nitrate management policy.

With Fish
Water usage was mainly due to the flushing of the tanks and backflushing of the pressurised sand filters in almost equal proportions. Daily use was approximately 1.4 m^3/day. This figure equates to 16% of the system volume per day. To reduce this figure would require either redesigning the tanks to negate the requirement for tank flushing or treat the water used for flushing and backflushing.

Water exchange reduced the residual end product of nitrification (nitrate) and controlled, to some degree, the fall in pH.

Chapter 4

The daily routines outlined below are the actual working schedules carried out during the FIN 19 project and are included to illustrate the type of work and time required to run a RC system for producing juvenile halibut. They can be used as a useful guide for farm managers in planning the work and work-loads required to operate a recirculation system.

DAILY ROUTINES

1. On entering the building, water flow and aeration to all tanks should be visually checked.
2. On entering the plant room, check the U.V. unit before turning on the light. A violet glow should be seen from all of the 12 U.V. tubes.
3. With the light now turned on, check for solids in the swirl separator. Checking at this stage gives an indication as to the state of the system.
4. Check that the spray bars in all four trickling filters are turning.
5. Check that sand in the four fluidised beds is fluid and visibly upwelling in the lower window.
6. Check the pH tower for magnaspheres and also ensure they are upwelling.
7. Check both pumps evacuating sump No. 1 are running. These are pumps 3 & 4.
8. Check both pumps evacuating sump No. 2 are running. These are pumps 1 & 2.
9. Check all four pumps supplying the protein skimmer venturis are operational.
10. Take water meter, redox and pH readings and enter them in the log.
11. Flush protein skimmers:
    a) Fully open the freshwater tap (in-between the sand filters and U.V. unit). This activates the spray bars in the skimmers. The pressure is pre-set.
    b) Open the blue flush valves on both the skimmers for 5 - 10 seconds. This will normally cause the skimmers to create more foam and is of no concern.
    c) When all foam has subsided, turn off fresh water tap.
12. Record water temperatures.
13. Flush fish tanks (see procedure). Remove and record any mortalities at this stage.
14. Feed fish. Weights and size of food per tank are entered on the chart on the notice board by the scales. Numbered containers are provided. Feeders to be wiped daily with paper towelling.
15. The water meter by the swirl separator should now be stationary. Backflush the pressurised sand filters (see procedure). This is best performed in the afternoon to allow the temperature drop due to the tank flush to recover and hence overall be minimal.
16. Daily test water for ammonia and nitrite. Weekly test for nitrate. Record.
17. Mondays and Thursdays - clean tanks with green panscrub.
18. Lights in the plant room should be turned out at night. (Lights in the tank room are on a time switch.

DAILY ROUTINE FOR BACKFLUSHING PRESSURISED SAND FILTER

Backflushing of the pressurised sand filters is carried out daily to remove any build up of debris filtered from the system water. This debris can add to the oxygen demand of the filter by colonising heterotrophic bacteria. These bacteria remove oxygen in the water returning to the fish tanks. Any organic content in the filter will be oxidised thus lowering the redox potential of the effluent water.

Signs of the filters needing backflushing are:

- Reduced water flow to the header tanks possibly resulting in a header tank alarm (normal flow rates to the header tanks are marked on the flow meters above the U.V. unit by the red slides).
- Redox meter reading in the header tank lower than usual. The header tank redox meter is located above pumps 3 & 4.
- Reduced clarity of the water viewed through the clear top of the filter or in the fish tanks.

The backflushing procedure is as follows:

1. Disable the tank level and header tank alarms on the alarm panel above the bench. Pressing the respective yellow button does this.
2. Turn off the chiller. The chiller control is found in the electrical cabinet on the end wall of the plant room behind the protein skimmers. The control is to the left of the electricity meter and marked ‘chiller pump’. Press the red button.
3. Turn off pumps 1 & 2 (located to the right of the sand filters). These pumps drain sump No. 2 and return water to the fish tanks via the header tanks.
4. Turn the multi way valves (attached to the filters) to backflush, this is marked ‘F’ in red and is 180° from the normal running setting (marked ‘R’ in red).
5. Turn on pumps 1 & 2 for one minute. ***NB*** if these pumps are left on too long, sumps 1 & 2 will empty causing all pumps to stop until the sumps are refilled.
6. Turn both pumps off.
7. Turn the multiway valves to rinse.
8. Turn on pumps 1 & 2 for 10 seconds or until the water in the filters is clear.
9. Turn both pumps off.
10. Turn the multiway valves to filter (marked ‘R’ in red).
11. Turn on pumps 1 & 2.
12. Check that water is flowing to the header tank (flow valves located above the U.V.)
13. Turn on the chiller when water returns to the fish tanks (green button).
14. Enable the alarm system by first pressing the red button if lit (this will cancel any alarm). Then press the yellow button to extinguish the light.

DAILY ROUTINE FOR FLUSHING TANKS

Flushing of the fish holding tanks is carried out daily to remove unwanted waste collected in the sump below the screen. This waste will unnecessarily load the treatment system and must be flushed to waste.

The procedure is as follows:

1. Disable the tank level alarm on the alarm panel above the bench.

2. Ensure that the water meter reading in the plant room is recorded in the log. This meter is found on the floor to the left of the steps when walking from the tank to the plant room.

3. Close the valve returning water to the swirl separator. This valve is located between tanks 1 & 2 running through the wall.

4. Open the valve to waste. This valve is located in the tank room, next to the wall, to the right of the door from the tank to the plant room.

5. Removing the external standpipe for 5 - 10 seconds flushes tanks. Flush the recirculating tanks (1 - 8) and raceways (R1 –R3). Only tanks with fish in them need be flushed.

6. After the last tank has been flushed, wait approximately 30 seconds for the flushed water to flow to waste.

7. Close the valve to waste.

8. Open the valve to the plant room.

9. The swirl separator is not overflowing as its supply has been cut off. Clean the swirl separator with the brush provided. This is best done at this stage, as there is not a strong current to obscure visibility.

10. As the water returns from the tank, it will contain debris from the tank flush procedure. As this water enters the separator, open the valve at the bottom of the separator. This will draw the solids to waste. When the majority of solids are removed, close the valve. Some floating solids will inevitably overflow and pass through the treatment plant.

11. Flush the throughflow tanks (9 – 16) as per section 5.

12. Enable the alarm system by first pressing the red button if lit (this will cancel any alarm). Then press the yellow button to extinguish the light.

Chapter 5

CHEMICAL BACKGROUND

Nitrogen

Perhaps the compounds causing most concern in recirculating systems are the nitrogen compounds. Atmospheric or molecular nitrogen although omnipresent is of little importance as it is an inert gas. It can be of some concern if introduced to aquaculture systems under pressure such as from a leak in a pump inlet drawing in air. Such supersaturated nitrogen gas can cause death by gas bubble disease as it comes out of solution in the blood, a situation similar to decompression sickness sometimes referred to as the 'bends' in subaqua divers.

Of much more concern to the commercial aquaculturist are the major detrimental effects of the nitrogen containing compounds such as ammonia, nitrite and nitrate as a result of protein metabolism and nitrification by nitrifying bacteria.

Examples of other nitrogen containing compounds entering into the system are proteins, amino acids, urea (CH_4ON_2) and uric acid ($C_5H_4O_3N_4$). These compounds are invariably broken down into ammonia by the mineralisation process of heterotrophic bacteria and then enter the nitrification cycle to be removed from the system.

Ammonia

Ammonia is the primary nitrogen compound excreted as a result of protein metabolism especially in teleost fish fed on high protein diets. Ammonia occurs in the natural aquatic environment in small amounts as a result of excretions of fish. Increased concentrations may occur mainly from pollution especially from sewerage discharge or agricultural silage, manure or fertilizer leaking into the water course.

In fish, ammonia originates in the liver and is excreted through the gills via the blood system (Smith, 1929; Wood 1958; Goldstein *et al*., 1964; Janicki & Lingus, 1970). Deamination of plasma amino acids in the gill tissue is another source (Smith, 1930; Goldstein & Forster, 1961; Goldstein *et al*., 1964). Ammonia is transported across the gills by two mechanisms; by diffusion down the concentration gradient in the case of the unionised form (NH_3) and also by active transport by exchanging the ionised form (NH_4^+) for a similarly charged ion in the exterior environment such as sodium ions (Maetz & Garcia-Romeu, 1964; Whitelaw, 1973). Similar exchanges have been observed in euryhaline teleost fish adapted to sea water (Motais, 1970; Evans, 1973).

Ammonia exists in two forms: ionised NH_4^+ and unionised NH_3. The sum of these two forms is termed the total ammonia.. Concentrations referred to in terms of nitrogen

(NH_3-N) can be converted to NH_3 by multiplying by 0.8235 (Meade, 1985) and NH_4^+-N is converted to NH_4^+ by multiplying by 1.3 (Spotte, 1979). Total ammonia exists in an equilibrium which is dependent on pH, temperature and dissolved oxygen concentration (Downing and Merkens, 1955). This is described in equation 1.

Equation 1:

$$NH_3 + H_2O = NH_4OH = NH_4^+ + OH^-$$

Lowering the pH will drive the equilibrium in equation 1 to the right, one pH unit increase causes the percentage of un-ionised ammonia to rise by approximately tenfold (Spotte, 1979). The 24h-LC_{50} of total ammonia nitrogen for the eel *Anguilla japonica* at 25°C. was estimated by Yamagata and Niwa (1982) to be 2,844 mg/l at pH 5, 820 mg/l at pH 7 and 16.8 mg/l at pH 9. By keeping the pH below 7 (see table 1 for fresh water, similar results are displayed by Whitefield (1974) in sea water) the ammonia can be, in effect, stored in a non-toxic form until removed from the system by the nitrifying bacteria in the bio-filter. Increasing the temperature will increase the relative concentration of NH_3 at constant pH (*Table 5:1*) as will decreasing salinity although this is a less dramatic effect than increasing pH. It is suggested by Sousa *et al*. (1974) using chinook salmon smolts *(Oncorhynchus tshawytscha)* that not only is it practical to shift the ammonia into the ionised form by decreasing the pH but the toxicity can also be reduced by using intermediate salinities. Their reasoning is that this salinity dependent reduction in toxicity may be related to an ionic exchange relationship between sodium and ammonium across the cellular membranes of the gill branchial cells.

TABLE 5:1

Variation in % NH_3 in an Aqueous

Ammonia Solution With Temperature and pH

Ammonia Solution With Temperature and pH

Temperature Deg. C	pH Range							
	6.0	**6.5**	**7.0**	**7.5**	**8.0**	**8.5**	**9.0**	**9.5**
0	0.008	0.026	0.083	0.261	0.820	2.550	20.700	45.300
5	0.013	0.040	0.125	0.400	1.230	3.800	28.300	55.600
10	0.019	0.059	0.186	0.590	1.830	5.560	37.100	65.100
15	0.027	0.087	0.273	0.860	2.670	9.790	46.400	73.300
20	0.040	0.125	0.400	1.240	3.820	11.200	55.700	79.900
25	0.057	0.180	0.570	1.770	5.380	15.300	64.300	85.100
30	0.081	0.254	0.800	2.480	7.460	20.300	71.800	89.000

After Emerson *et al*. (1975)

The toxicity of ammonia is of much debate; it is, however, generally agreed that the unionised form is extremely toxic while the ionised form is considerably less toxic (Smart, 1981; Yamagata & Niwa, 1982). Sadler (1981) concludes that, in the European eel *Anguilla anguilla*, elvers are more susceptible to NH_3 than mature eels with growth being halted at a concentration of 0.5 mg/l NH_3-N. Some toxicity has been attributed to the ionised form NH_4^+ in the Japanese eel *Anguilla japonica* although this is in effect negligible (Yamagata & Niwa, 1982). Thurston *et al*. (1981) speculated that NH_3 is 300 to 400 times as toxic as NH_4^+.

Nitrite

Recently, the effects of nitrite in fish has been of increasing interest, in the aquaculture industry possibly due to the trend towards recirculation of water. Its effects are well studied in man and higher vertebrates leading to the World Health Organisation recommending maximum dietary intakes of less than 1 mg/l as N-NO_2 (WHO, 1978) (N-NO_2 * 3.3 = NO_2 (Spotte,1979)).

Nitrite is present in recirculation systems as an oxidatory product of the breakdown of ammonia by the bacterium *Nitrosomonas sp.* In the natural aquatic ecosystem nitrite is present mainly as a product of the reduction of nitrate by the activity of phytoplankton and some atmospheric nitrogen is fixed by lightning and oxidized to both nitrite and nitrate. Normal levels in the environment vary but typically range from 1-5 mg/l. Levels of 5-20 mg/l have been recorded in a survey of 65 Italian lakes previously considered to be unpolluted (IRSLA 1980). 10 mg/l is considered to be a warning sign of pollution (EIFAC 1984). Generally, poor quality salmonid fisheries are associated with high nitrite levels of 60-200 mg/l although this is not evident in coarse fisheries (Solbe, 1981).

Nitrite exists in an equilibrium according to equation 2:

Equation 2

$$HNO_2^- = H^+ + NO_2^-$$

The reaction is pH dependant with the relative concentration of HNO_2^-, the conjugate acid, being decreased as the pH is decreased. As the temperature of the water rises, the disassociation constant of the HNO_2^- increases and hence the relative concentration of NO_2^- rises. Both temperature and pH effects are minimal at pH levels common to aquaculture as, for example, at pH 7 12.5°C., the relative concentration of NO_2^- is 99.978% and at the same temperature at pH 6, the NO_2^- relative concentration is 99.781 (EIFAC, 1984). Therefore at the temperature and pH values used in typical recirculating aquaculture systems (pH 6-8 and temperature 10-25 °C) the relative concentration of NO_2 is practically 100%

One of the reasons that nitrite is well studied in man is due to a condition called methaemoglobinaemia which can be caused by the iron in haemoglobin, normally being in the divalent (Fe^{2+}) form, being oxidised by nitrite to the trivalent (Fe^{3+}) form when it forms methaemoglobin. Methaemoglobin is unable to combine reversibly with oxygen in the blood as occurs with haemoglobin and the result, in severe cases, is anaemic hypoxia. In fish, normally 5% of the haemoglobin is in the form of methaemoglobin,

(Eddy *et al*., 1983) and this is maintained constant by a methaemoglobin reductase in the red blood cells.

High levels of haemoglobin in fish appear to be of less importance than in higher vertebrates. In experimentally induced high concentrations of plasma methaemoglobin (by the increase of nitrite concentrations), over 70% methaemoglobin in rainbow trout *Oncorhynchus mykiss* (Smith and Williams, 1974) and chinook salmon *Oncorhynchus tschawytscha* (Brown and McLeay, 1975), did not induce mortalities. The fish were however stressed. Some Antarctic fish, having no haemoglobin at all, rely on the plasma to carry the oxygen as the reduced temperature allows a high plasma concentration of dissolved oxygen, (Holeton, 1970).

Early studies on the toxicity of nitrite in fish gave widely varying results until Perrone and Meade (1977) discovered that the chloride ion concentration in the environment strongly counteracted the toxicity of nitrite in coho salmon *Oncorhynchus kisutch.* This was confirmed later in rainbow trout *Oncorhynchus mykiss* (Bath and Eddy, 1980). The ratio of chloride to nitrite is critical and protection can be given against nitrite toxicity to the fish by increasing the chloride concentration in the environment. A $Cl^-/N.NO_2^-$ ratio of 15:1 in rainbow trout *O. mykiss* is sufficient to render nitrite non toxic (Bath and Eddy, 1980). A figure of 18:1 has the same effect in coho salmon *Oncorhynchus kisutch* (Perrone and Meade, 1977) and 41 for the channel catfish *Ictalurus punctatus* (Tomasso *et al*., 1979).

Nitrite in the environment enters the fish through the gills via the branchial epithelial cells in rainbow trout *Oncorhynchus mykiss* (Gaino *et al*., 1984). The concentration of methaemoglobin in the blood of fish has been shown to closely follow the trend of nitrite in the aquatic environment with the gills concentrating the nitrite up to ten times the external level in the blood and tissues (Eddy *et al*., 1983).

Rainbow trout *Oncorhynchus mykiss* exposed to 1.5 mg/l nitrite (0.45 mg/l $N\text{-}NO_2$) for 72 hours showed inhibited lysosomal activity in the liver and fragile lysosomal membranes. Liver damage was observed which was typical of that caused by anaemic hypoxia induced by methaemoglobin (Mensi *et al*., 1982). Plasma concentrations of corticosteroids in channel catfish *Ictalurus punctatus* were raised after exposure to ammonia and nitrite (Tommasso *et al*., 1981). Fish recovered more rapidly when ammonia rather than nitrite was the cause of the plasma corticosteroid increase. A concentration of 5 mg/l NO_2^- (1.5 mg/l $N\text{-}NO_2^-$) in the environmental water was sufficient to raise the corticosteroid level by ten times the normal amount.

The toxicity of nitrite can be enhanced by its reaction with certain amines causing the formation of compounds such as N-nitrosodimethylamine which when fed to rats at a level of 5 ppm caused tumours in 70% of the experimental group (Wolff and Wasserman, 1972). This effect is uninvestigated in fish.

Environmental pH is another important factor affecting NO_{2-} toxicity with an increase in pH reducing its toxicity (Wedermeger and Yasutake, 1978). Over a pH range of 6.4-9.1 the toxicity of nitrite has been found to decrease as the pH is increased (Russo *et al*., 1981).

Nitrate

Comparatively little is known about the effect of nitrate in intensive aquaculture although it has been speculated that pale gills in captive fish are a result of elevated nitrate in the environment (Spotte, 1979). Levels of 400 mg/l have been found to have no effect on the mortality and growth in the fresh water large mouth bass *Micropterus salmoides* and channel catfish *Ictalurus punctatus* (Knepp and Arkin, 1973). It is claimed that nitrate is 2,000 times less toxic than nitrite in coho salmon *Oncorhynchus kisutch* and rainbow trout *Oncorhynchus mykiss* kept in fresh water, with no sign of stress after exposure to 1,000 mg/l for over 5 days. Toxicity increased by up to 1.41 times in brackish water at 15 ppt. (Westin, 1974). Nitrate is considered harmless at relatively large concentrations in eels with 24 hour LC50 3,130 mg/l for the Japanese eel *Anguilla japonica* and no acute toxicity reported at concentrations below 1,150 mg/l (Mie *et al*., 1984).

Nitrate is the end product of the oxidation of ammonia, either directly by some bacteria or via the toxic intermediary nitrite by the various species of *Nitrobacter sp*.

NO_3^- can be removed by nitrate respiration performed by certain microorganisms under anaerobic conditions. This is achieved by NO_3^- accepting hydrogen rather than oxygen. Dissimilation is a term synonymous to nitrate respiration and applied to the conversion of nitrate to ammonia, nitrite, nitrous oxide or molecular nitrogen depending on the species of microorganism employed (Painter, 1970). Nitrogen is only removed completely from the system when the partial pressure of the gas exceeds that of the atmosphere and nitrogen gas escapes from the water. This latter form of dissimilation is termed denitrification. Many species of bacteria capable of dissimilation, under the right conditions, have been identified (Painter 1970). Interestingly Ozretich (1997) cites the bacterium *Vibrio anguilarum* (a scepticaemic pathogen to fresh water eels) as capable of reducing nitrate.

Carbon

Dissolved organic carbon (D.O.C.) is present in aquaculture systems as a result of uneaten food, faecal and urine production and as carbon dioxide as a result of respiration. Heterotrophic microorganisms convert faecal and urinary carbon to produce CO_2. Excess CO_2 is removed in recycling systems by mechanical evasion (removal by mechanical means) either using a cascading system or by additional aeration

In a natural environment, the CO_2 is reduced back into organic carbon by plants. Bicarbonate can also be photosynthetically reduced into organic carbon by algae (Blinks, 1963).

D.O.C. has been attributed to the yellow pigment in sea water and is termed "Gelbstoff" by Kalle (1966). Although Gelbstoff is largely of plant origin it would appear to have an animal content which together with nitrogenous wastes could account for the persistent yellow/brown colour characteristic in many recycling fish farm waters.

Phosphorous

There are three forms of phosphorous: dissolved inorganic phosphorous (DIP and also termed phosphate, orthophosphate or reactive phosphate), dissolved organic phosphate (DOP) and particulate organic phosphate (POP).

In the natural environment DOP is released from dead or ruptured animal cells or excreted by macroalgae. Heterotrophic bacteria mineralize DOP into DIP. DIP is precipitated out into calcium salts (Goldizen, 1970) or calcium and magnesium salts (Saeki, 1962). In sea water, in the presence of magnesium and ionic ammonia, the mineral struvite ($NH_4MgPO_4.6H_2O$) is formed by certain bacteria (Malone and Towe, 1970). The DIP is removed by precipitation in an albeit minor way. In fresh water DIP combines with the ferrous ion to precipitate out (Adey and Loveland, 1991).

In an aquaculture system, fish faeces and uneaten food contain DIP and POP. POP is converted into DIP by heterotrophic bacteria. DIP can be physically removed by aeration; the phosphate bound to organic molecules is adsorbed into air bubbles and dissipated as aerosol droplets at the surface when the bubbles burst (Sutcliffe *et al.*, 1963).

The phosphate is removed at a rate proportional to its concentration (Bayler *et al.* 1962). Thus:

$$Ct = Coe^{-kt}$$

where Ct is the concentration of reactive phosphate at time t; Co is the concentration of reactive phosphate at the beginning time 0; e is the base of natural logarithms and k is a rate constant dependant on the surface area created by the bubbles.

DIP levels in recycling systems stabilise very quickly, rarely exceeding 3 mg/l, and not too much concern should be given to removing it in recirculating systems as it is considered harmless.

Oxygen

Oxygen makes up 21% of the atmosphere but is relatively insoluble in water the solubility decreasing with temperature rise, increased salinity, a reduction in barometric pressure (increased altitude) and impurities (see *Table 5:2*).
The oxygen consumption in fish depends on many variables which include;

Temperature - fish consume more oxygen at higher temperatures due to increased metabolic activity

Dissolved oxygen (DO) in the water - increased DO increases the diffusional gradient across the gills.

Activity - the more active the fish the more oxygen consumed.

Feeding - more oxygen is consumed both during the activity of feeding and post feeding, in digestion and assimilation. For example oxygen demand doubles one to six hours after feeding in channel catfish (Tucker and Robinson, 1990).

Ammonia - high levels of ammonia cause large and prolonged increases in post feeding oxygen consumption. For example as reported in 1-2 g. eels *Anguilla anguilla* (Knights, 1989).

TABLE 5:2

Solubility of Oxygen in Fresh and Sea Water at 100% Saturation

After Shepherd and Bromage (1989)

SOLUBILITY OF OXYGEN IN FRESH AND SEA WATER AT 100% SATURATION

	Solubility mg/l	
Temperature Deg. C	**Fresh Water**	**Sea Water (35 ppt)**
0	14.6	11.3
5	12.8	10.0
10	11.3	9.0
15	10.2	8.1
20	9.2	7.4
25	8.4	6.7
30	7.6	6.1
35	7.1	5.7
40	6.6	5.3

Size of fish - the smaller the fish the more oxygen per unit body weight the fish utilises. The oxygen consumption of eels with reference to size in a farm situation is given by Stahler GmBH (Hadamar, Germany) as follows:

Weight (g)	O_2 Consumption (mg/kg/h)
1	1,100
10	600
50	420
300	250

Species - minimum required levels of oxygen are higher for salmonids (5.0-5.5 mg/l for fish and 7.0 mg/l for eggs) than for species such as carp, catfish and tilapia which can withstand DO levels below 2.0 mg/l for periods of about 12 hours (Shepherd and Bromage, 1992) and adult channel catfish can survive short periods at less than 0.5 mg/l (Tucker and Robinson, 1990).

Minimum oxygen levels vary for a particular species according to the variables listed above. Oxygen concentration in water may be expressed either as % saturation or as mg/l and is dependant on environmental water temperatures. Colt and Orwicz (1991) suggest 90% saturated water to be the minimum. Fish are reported to exhibit poor growth at DO concentrations of less than 25% (Romaire 1985). When 2-5g eels (*Anguilla japonica*) were exposed to DO levels of less than 40% saturation, growth feeding and food conversion rates decreased but were not affected when maintained at 60% saturated water (Rowchai *et al*., 1986). An oxygen concentration of about 1 mg/l at 25°C. produced high mortalities in 2g. eels *Anguilla japonica* (Yamagata *et al*., 1983). As oxygen levels decrease, fish will respond by decreasing their activity, cessation of feeding and eventually 'gasping' at the surface. Oxygen starved fish eventually die due to a decreased pH in the blood as a result of increased glycolysis to meet energy demands due to stress. Decreased pH disables the efficiency of haemoglobin to extract oxygen from the blood.

The bacteria in the biofilter are essentially aerobic. How much oxygen they require is the source of some debate. The sea water bacterium *Nitrocystis oceanus* is capable of nitrification at oxygen levels as low as 0.05 mg/l (Gundersen, 1966). Kawai *et al.* (1971) demonstrated that total ammonia concentration in fresh and sea water was maintained at an acceptable level (0.465 mg/l) at an oxygen concentration of 34% saturation whereas at DO levels of 6% saturation, the total ammonia was 173.6 mg/l.

pH

pH is the negative logarithm of the hydrogen ion concentration. In mathematical terms the prefix p denotes a negative logarithm of the following term, pH is therefore $-\log_{10}[H^+]$. Pure water ionises only very slightly producing 10^{-7} moles of hydrogen and hydroxyl ions per litre. The pH of pure water at 25°C. is therefore 7. At pH values above 7 the hydroxyl ion predominates and the water is alkaline (the hydrogen ion concentration being less than 10^{-7}). At pH values below 7 the hydrogen ion predominates and the water is acidic (hydrogen ion concentration greater than 10^{-7}). As the pH scale is logarithmic, each decrease of one unit represents a ten fold increase in hydrogen ion concentration.

Extremes of pH in natural fresh water range from 5-10 (Boyd, 1990) and sea water remains constant at a pH of approximately 8.2 due to the bicarbonate buffering system. Acid rain producing mineral acids, salts and weak bases plus other man made pollutants are recent phenomena in industrial environments which are responsible for decreasing pH values of natural water courses. Other factors affecting pH in natural waters are CO_2 from respiration of fish and other aquatic organisms, carbonic acids from soils, swamps forests and bogs and mineralisation and nitrification in organic decay in bottom sediments. The pH value of natural ecosystems increases during the day due to phytoplankton utilizing CO_2 during daylight to perform photosynthesis and decreases during darkness due to the phytoplankton excreting CO_2 during respiration. The effects of changes in pH on the environment are important in all biological systems including in recirculating aquaculture systems. Perhaps the most important effect in this respect is that of pH on the ionised/unionised condition of ammonia as outlined above. However the concentration of hydrogen ions has an effect on all chemical dissociation equilibria in the chemistry of water, the most important of which are covered in this section.

Effects of pH on fish have been observed in salmonids below pH 5 where the fish start to lose the ability to regulate plasma sodium concentration leading to the loss of integrated body movements due to drastically reduced sodium chloride concentrations in the plasma (Leivestad and Muniz, 1976).

In recirculation systems pH is constantly decreasing due to the heterotrophic microorganisms and the release of hydrogen ions by nitrifying bacteria as ammonia is reduced to nitrate. This process is more apparent in soft water as the buffering capacity is weak (Bisogni and Timmons, 1991.)

The effect of pH on the biofilter is equally important in a recirculation system. The preferred pH is slightly higher than neutral with minimum requirements of pH 6.5-7 (Petit, 1990) below this the bacteria can slowly adapt to a range of pH 5-10 but cannot respond to rapid changes of more than 0.5-1 pH unit (Wheaton *et al.*, 1991).

Alkalinity

Alkalinity is a measure of total concentration of titratable bases expressed as millequivalents per litre or meq/l. Alkalinity has been simplified by expressing it as mg/l of equivalent calcium carbonate ($CaCO_3$) thereby standardising the many bases (mostly CO_3^- and HCO_3^-), one meq/l equals approximately 50 mg/l $CaCO_3$. The total alkalinity reflects the buffering capacity of the water by neutralising any addition of carbon dioxide and repressing any fluctuations in hydrogen ions thus maintaining a stable pH. Acceptable limits for aquaculture are reported to be 20-400 mg/l $CaCO_3$ (Tucker and Robinson, 1990) any less leading to a depletion in CO_2 and a resultant rise in pH (Sawyer and McCarty, 1978). A minimum of 40 mg/l is recommended for the efficient functioning of biofilters (Paz, 1984).

Hardness

Hardness is the total concentration of metal ions expressed in meq/l or in terms of mg/l of equivalent calcium carbonate ($CaCO_3$). The main metal ions are magnesium (Mg^{2+}) and calcium (Ca^{2+}) with lesser amounts of iron and manganese. Equal amounts of hardness and alkalinity are preferred to keep the pH stable (Romaire, 1985). The recommended levels of hardness are 20-300 mg/l (Boyd and Walley, 1975).

Invertebrates are particularly affected by soft water where a certain amount of calcium availability is required for shell formation although this is not a problem in sea water with an average hardness of 6,600 mg/l (Boyd, 1990).

Calcium and sodium have been shown to have a sedative effect on fish in high concentrations and also in certain concentrations lead to a reduction in oxygen absorption and mucous secretion. Calcium is known for its reduction of the toxic effects of nitrite (Perrone and Meade, 1977; Bath and Eddy, 1980) similar to that described for chloride ions above. Heavy metals are usually toxic and care should be exercised to avoid copper and brass fittings in recirculation systems.

Temperature

Teleost fish inhabit waters from below 0^oC. to 45^oC. although individual species have a preferred temperature range where the enzymatic activity in the fish is at an optimum for maximum growth and performance. A temperature above the upper limit of thermal tolerance will result in death as the life supporting enzymes cannot function; similarly a temperature below the limit of thermal tolerance leads to reduction in enzyme activity. In particular sudden changes in temperature will kill fish due to "thermal shock". Certain species may have limited physiological control over body temperature through processes such as countercurrent heat exchange mechanisms between arteries and veins and the ability to reduce circulation to the surface of the body. In the wild, fish are capable of limited body temperature control by certain behavioural responses, such as migration. Under cultured conditions fish are totally reliant on the temperature controlled by the aquaculturist.

Although the optimum temperature from the farmer's point of view is that at which the fish grows most rapidly, and usually at the upper end of the range to which the fish is tolerant, it is important to note that diseases are more active at high temperatures. For example vibriosis, caused by the bacterium *Vibrio anguilarum,* is associated with increased environmental temperatures and does not appear to infect salmonids and turbot below 10-11^oC. and Anguillidae and Pleuronectids below 15-16^oC (Roberts, 1989). Conversely, low temperatures have certain diseases associated with them such as the disease caused by the bacterial group known as myxobacteria producing thickening of fins, due to epithelial hyperplasia, and eventual necrosis; this condition is termed ‘cold water disease’. Some other common temperature related problems are:

1) Wounded fish, although capable of healing faster at high temperatures, they are in greater danger of bacterial and fungal infections.
2) Increased temperatures can heighten the fish's susceptibility to toxins.
3) Oxygen is less soluble at higher temperatures.
4) Fish, having a higher metabolic rate at high temperatures, have a higher oxygen demand and care must be exercised to ensure that it is met.

Temperature affects all the chemical reactions mentioned in this section which are essential in maintaining a balance in managing a recycling unit. The rate of a chemical reaction approximately doubles with a 10^oC rise and so the recirculating system must be carefully observed as the temperature is increased.

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