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Review

The potential for integrated biological treatment systems in recirculating fish culture—A review

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Abstract

Intensive aquaculture in recirculating systems is rapidly developing, and with it arises the need for reliable treatment systems. To enable reuse of water in these systems, biological treatment is considered the most economically feasible approach. In this review the advantages and disadvantages of some of the most commonly used biological treatment systems are examined. Using as a comparator the main biological processes in extensive static fish ponds, it is explained how most treatment facilities in recirculating systems achieve only partial water purification as sludge and nitrate are produced. Methods for reducing the accumulation of these materials are discussed. It is concluded that incorporation of such methods would result in more stable water quality conditions within the culture units, and also in a considerable reduction of pollution.

Keywords: Biological treatment; Denitrification; Nitrification; Recirculating fish culture systems; Sludge removal; Water quality

1. Introduction

Over the past decade there has been rapid development of intensive fish culture systems. In northern and western Europe (Rosenthal and Black, 1993) and North America (Colt, 1991), in particular, a considerable amount of research and development is being conducted. In aquaculture systems, classification according to water flow provides a key insight with respect to the water quality processes that control fish production (Krom and Van Rijn, 1989). Intensive fish culture systems can be classified as flow-through or recirculating. In the former, clean water is typically directed in a

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single pass through the production unit and discharged or dispersed thereafter. These systems are used to produce both fresh water, brackish water and marine species. Freshwater flow-through or open systems are found in regions with an ample supply of clean water (e.g. trout farming in Idaho, USA). Tidal, earthen ponds for shrimp farming (southeast Asia) are an example of brackish water flow-through systems, while cage culture is the foremost representative of marine flow-through systems and is the main form of aquaculture in many countries (e.g. Norway, Chile, Canada, Japan). A drawback of open systems is that, apart from their site specificity, they may contaminate recipient water bodies and may therefore become limited by environmental constraints. By contrast, recirculating systems incorporate facilities for maintaining adequate water quality. These systems have the potential to be more environmentally sound as water input is minimized, little water is discharged and this can itself be treated. However, adequate water quality control is essential for the successful operation of recirculating systems. In this context, biological control by algae and bacteria has so far been the most economically feasible approach.

A contemporary update on reuse systems with emphasis on engineering and management aspects of these systems, has recently been published (Timmons and Losordo, 1994). The aim of the present review is to focus on the biological processes underlying the treatment systems used in recirculating systems. The main biological process configurations presently applied in recirculating systems are compared with those involved in converting fish metabolites in static-water fish ponds. It is concluded from this that most treatment facilities applied in recirculating systems achieve only partial water purification since, in comparison with static ponds, they do not remove nitrate and organic matter. Little information is available on incorporation of the latter processes in recirculating systems. The review therefore emphasizes these processes, and the means by which they may be incorporated in recirculating systems. It is concluded that such incorporation would result in more stable water quality conditions within culture units and also in a considerable reduction of pollution.

2. Factors leading to intensive fish culture in recirculating systems

While in developing countries traditional fish culture in static earthen ponds is expanding (Hempel, 1993), expansion of conventional fish farms in industrialized countries has almost come to a halt. In Europe (e.g. Denmark, Germany, Holland, Norway), North America and other industrialized countries (e.g. Japan, Taiwan and Israel), fish farming is increasingly characterized by intensification. Factors for this differ from country to country, but the following summary of development in Israel highlights many of these.

In Israel, fish culture began in the late 1940s, based mainly on common carp, and over the following years the production area rapidly expanded. From 1970 onward the industry was characterized by rising area yields with a concomitant reduction in the total area allocated for fish culture (Table 1), due to competition for land between the fish culture industry and other agricultural activities. The bulk of production uses brackish water and/or marginal lands which, in the early years, could not be used for other

Table 1
The development of fish farming in Israel between 1940 and 1990

Year	Area (ha)	Production (t)	Mean yield ($t\ ha^{-1}$)
1940	30	50	1.00
1950	2100	3700	1.76
1960	4180	8432	1.97
1970	4741	11954	2.49
1980	3407	11492	3.37
1990	2818	14586	4.99

From Sarig (1987) and Sarig (1992).

purposes. With the rapid development of agriculture, methods and varieties were developed allowing cultivation of edible (e.g. avocado, citrus) and non-edible (e.g. cotton) crops in such marginal areas using brackish water, causing agriculturalists to reevaluate and, often, reallocate their land use. Limitation of land and water is, therefore, the main reason for intensification of fish farming in Israel, though additional advantages include savings in manpower and easier stock management.

Increased fish yields in conventional, static ponds or reservoirs was accomplished by a combination of management procedures, the most important among them being the use of supplementary feed, polyculture, and auxiliary aeration during the night (Sarig, 1989). Higher yields were obtained in specially designed smaller units, 50–1000 m^3 (Zohar et al., 1985; Van Rijn et al., 1986), which differ from conventional ponds in design. These are made of concrete or are plastic-lined, and their configuration allows periodical removal of organic matter from the bottom. Most of these units are operated in a semi-closed mode, allowing optimal use of water and hence, minimal water discharge. Due to their reduced environmental impact, their development is supported by national and regional authorities. Pollution control is, therefore, another factor underlying the development of intensive systems. Finally, culture of fingerlings (mainly tilapia) during off-season in heated, indoor systems is rapidly expanding, and so, heat conservation can be counted as an additional factor promoting the use of intensive recirculating systems.

3. Biological water quality control in static ponds

Control of water quality in intensive fish culture systems is often the bottleneck for their successful operation. The most common water quality problems in such systems are oxygen depletion and accumulation of organic matter, inorganic nitrogen, particularly ammonia, and CO_2 (Muir, 1982). As with industrial, agricultural and urban waste water treatment (Metcalf and Eddy, 1991), biological treatment of intensive fish culture systems has so far been considered the most economically feasible. The processes that can be utilized to prevent water quality deterioration in intensive systems are also similar to those which occur in static fish ponds. Here, a combination of physical, chemical and biological processes prevents oxygen depletion and accumulation of

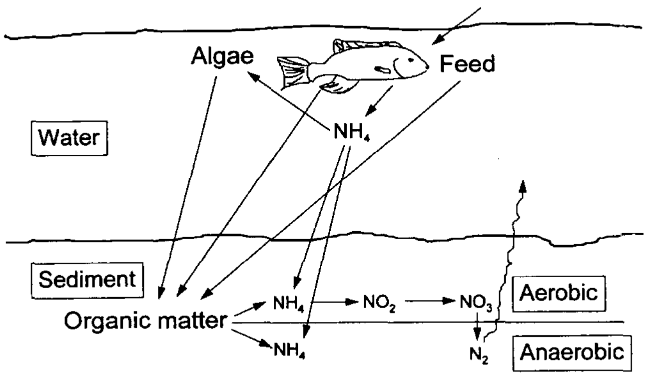

Fig. 1. Nitrogen transformation in a static, earthen-bottom fishpond.

harmful compounds, as long as the carrying capacity of the pond with respect to fish biomass is not exceeded.

Despite the input of protein-rich supplementary feed in most static fish ponds, inorganic nitrogen levels in the water column are usually low. The main processes responsible for nitrogen transformation and removal are depicted in Fig. 1. Transformation of ammonia, excreted by the fish and liberated by decomposition of uneaten feed and fish feces, takes place through the biological processes of algal assimilation, ammonification and nitrification. (Shilo and Rimon, 1982; Van Rijn et al., 1984; Diab and Shilo, 1986). Ammonia in the water column is assimilated and immobilized by algae. Turnover of algae is rapid, as was shown in a study of such ponds, dominated by cyanobacteria, in which the daily turnover was estimated at 10% of the standing cyanobacterial crop (Bejerano, 1978). Decomposition of the algae in the pond sediment and ammonification of nitrogenous cell material results in liberation of ammonia in the pond sediment. The ammonia accumulates in the sediment under anoxic conditions and is oxidized to nitrate by autotrophic nitrifying bacteria under oxic conditions after pond drainage. Removal of the inorganic nitrogen is finally accomplished after refilling the ponds. Renewed anoxic conditions and the presence of high nitrate concentrations in the pond sediment result in the development of a denitrifying population. These heterotrophic bacteria, using organic degradation products as sources of carbon and energy, and nitrate as an electron acceptor, dissimilate nitrate under anoxic conditions, via nitrite, nitric oxide and nitrous oxide to gaseous elemental nitrogen which is subsequently released into the atmosphere (Diab and Shilo, 1986; Hopkins et al., 1994). Accumulation of organic material in the ponds is prevented by rapid bacterial decomposition. Aerobic degradation rates of organic matter in fish pond sediments vary from 0.075 to 0.15 day^{-1} (Boyd, 1973; Y. Avnimelech, personal communication, 1993).

4. Biological water quality control in recirculating systems.

The most common water purification treatments in intensive recirculation systems are depicted in Fig. 2. Three types of treatments can be distinguished: water treatment by (a)

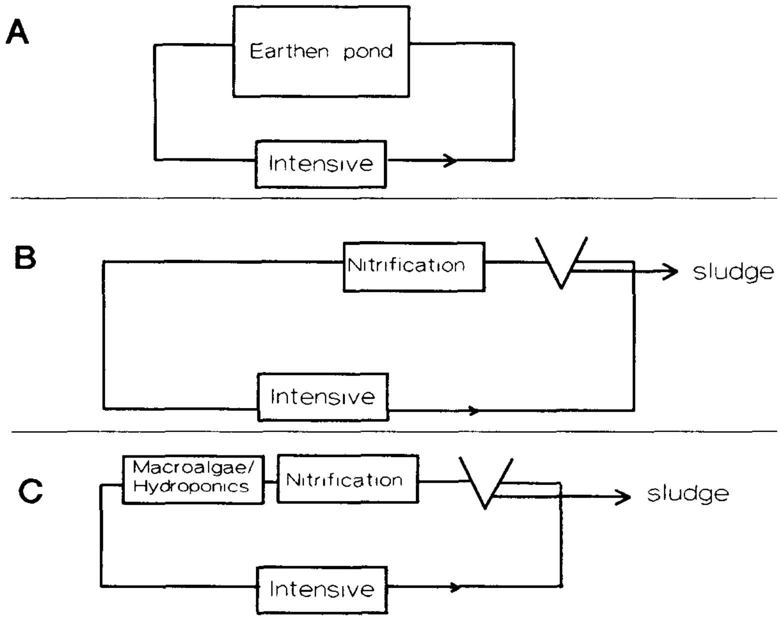

Fig. 2. Treatment configurations for intensive, recirculating fish culture systems.

earthen ponds or reservoirs, (b) a combination of solids removal and nitrification, and (c) a combination of solids removal and macrophytes-based nutrient removal.

4.1. *Earthen ponds or reservoirs.*

The simplest design may be that of an intensive fish culture unit coupled with an earthen pond or reservoir (Fig. 2(a)). This system was applied in Taiwan (Liao and Chen, 1983), in Israel (Hepher, 1985; Mires et al., 1990) and later in Singapore (Chin et al., 1993). Relatively few studies have been conducted on this treatment system. However, information on biological processes taking place in regular conventional fish ponds as well as information on oxidation ponds or stabilization ponds used to purify waste water (Oswald and Golueke, 1968; Uhlmann, 1979; Azov and Shelef, 1982) form a basis for understanding the purification capacity of such ponds. Problems encountered in this system are similar to those encountered in conventional fish ponds, among which are algal collapse and anaerobiosis of the sediment. Important practical parameters in this system are optimization of hydraulic retention times of the intensive fish culture unit and the treatment pond, homogeneous mixing of the treatment pond, and the periodic aeration of the pond sediment by drainage. The system is especially suitable where intensive fish culture units operate in the vicinity of already existing fish ponds or reservoirs. Apart from its purification function, the treatment pond or reservoir can be used for growing fish preferably species that exert a positive influence on the biological stability of the pond (e.g. algal feeders, bottom dwellers). The main disadvantage of this system, as in conventional fish ponds, is the unstable purification resulting from unpredictable fluctuations of phytoplankton biomass and speciation in the treatment pond.

4.2. Nitrification and solids removal.

Treatment based on solids removal and nitrification (Fig. 2(b)) is probably the most widely used amongst today's recirculating systems. Solids removal is accomplished by sedimentation or mechanical filtration (Chen and Malone, 1991). Non-pressurized mechanical filtration systems are preferred. Use of commercially available screen filters (triangle filter, drum filter, Unik wheel filter) has increased in the last 5 years and the performance of such filters has been described in several studies (Liltvedt and Hansen, 1990; Libey, 1993; Bergheim et al., 1993b). The mesh of these filters is as small as 40 μm although, due to the large quantity of wash-water required, filters with a mesh of 70 μm or larger are usually preferred.

Despite these highly improved filtration methods, small suspended solids tend to accumulate in recirculating systems. Studies on three recirculating fish culture systems (Chen et al., 1993b) revealed that more than 95% of the suspended solids had a diameter of less than 20 μm. High concentrations of suspended solids should be avoided as they form an additional source of ammonia, which in its unionized form is highly toxic to fish. Furthermore, they may cause gill damage by fouling, resulting in stress and increased susceptibility to diseases. Removal of small suspended solids can be accomplished by either chemical or biological oxidation. Chemical oxidation by ozonation can be applied to reduce the organic load in conventional waste water treatment (Metcalf and Eddy, 1991). However, in a recirculating system for fish culture, Otte and Rosenthal (1979) found that ozonation did not lead to a significant decrease of organic matter (expressed as BOD). They assumed that in addition to oxidation of degradable organic matter, ozonation also caused release of degradable organic matter by oxidation of recalcitrant organic compounds. Ozonation, however, is effective in degradation of yellow substances (Otte et al., 1977; Takeda and Kiyono, 1990) and is also used for sterilization (Muir, 1982). For these latter reasons it is increasingly being used in mainstream treatment of recirculating systems As an alternative to chemical oxidation, fine suspended material can be removed by biological oxidation. Although this takes place in most recirculating systems (e.g. within nitrifying filters), few such systems induce this process specifically.

Nitrification is typically carried out in a variety of systems, which can be grouped into six general types: submerged filters, trickling filters, reciprocating filters, rotating biological contactors (RBC), rotating drums, and fluidized bed reactors (Wheaton et al., 1991). A number of comparative studies of these systems have been conducted (Kaiser and Wheaton, 1983; Rogers and Klementson, 1985; Miller and Libey, 1985; Knosche, 1994). RBCs were found to give the best performance with respect to specific ammonia removal efficiency (ammonia removal per surface area per time). However, due to operating problems with RBCs, often related to shaft and bearing failures, their commercial use has been limited. The effect of different surface materials (filter media) on nitrification has been examined mainly for trickling filters (Kruner and Rosenthal,

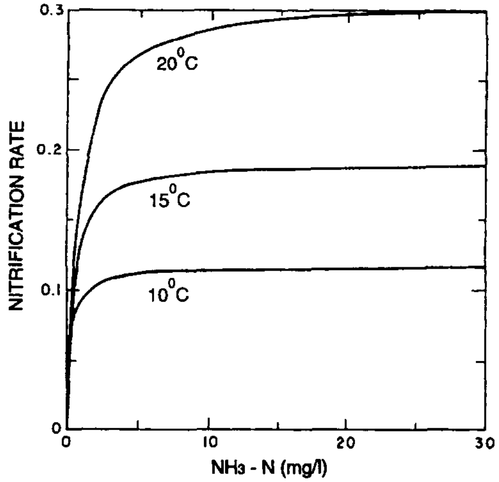

Fig. 3. Nitrification as a function of temperature and ammonia concentration. Nitrification rate is expressed as lb NH_4-N lb^{-1} mixed liquor volatile suspended solids day^{-1}. Adapted from Brune and Gunther (1981).

1983; Kikuchi et al., 1994). The ideal material (a) has a high surface area per volume, (b) is low in cost, (c) is durable, (d) does not clog easily and (e) promotes a uniform spread of water to be treated. Plastic materials fulfill most of these criteria and are increasingly being used. Various configurations are commercially available; the specific surface area usually varies from 150 to 350 m^2 m^{-3}, with maximum ammonia removal rates (where NH_4-N stands for total ammonia-N) between 0.28 and 0.55 g NH_4-N m^{-2} day^{-1} (Kruner and Rosenthal, 1983; Nijhof and Bovendeur, 1990; Van Rijn and Rivera, 1990; Kikuchi et al., 1994). Reported maximum ammonia removal rates are sometimes misleading as they are measured at unrealistically high ammonia levels. Nitrification follows classic Michaelis–Menten kinetics where

$$v_o = (V_{max}[S])/K_s + [S] \quad (1)$$

where (in case of ammonia oxidation) v_o is ammonia oxidation rate at ambient ammonia concentration ($[S]$), V_{max} is maximum ammonia oxidation rate and K_s is half saturation constant.

Hence, ammonia oxidation follows a hyperbolic trend with increasing ammonia concentrations. An example for a nitrifying trickling filter in a recirculating system is given by Brune and Gunther (1981) and shown in Fig. 3. Maximum ammonia removal rates are found at concentrations too high to be tolerated by fish. A more realistic approach is therefore to assess rates at ammonia levels typical of fish culture units (e.g. Malone et al., 1993).

Based on fixed-film nitrification kinetics described by Harremoes (1978), Harremoes (1982), Williamson and McCarty (1976a) and Williamson and McCarty (1976b), Bovendeur (1989) provided a design and performance prediction for a fixed-film reactor for aquaculture. This was applied in a study on recirculating systems for eel culture (Heinsbroek and Kamstra, 1990) and in a seawater recirculation system (Nijhof and

Bovendeur, 1990). The design concept is based on homogeneous distribution of nitrifying bacteria in the filters. However, in reality, distribution of nitrifying bacteria is heterogeneous in space and in time. This was illustrated by scanning electron microscopy studies on RBCs used in wastewater treatment (Alleman et al., 1982; Kinner et al., 1983) and on a trickling filter used in a recirculating aquaculture unit (Sich and Van Rijn, 1992). In the latter study the filter was characterized by a heterogeneous distribution of nitrifiers, a high diversity of non-nitrifying bacteria and protozoa and a high spatial variability in nitrifying activity. A design approach which takes account of heterogeneity would be a useful development in understanding and predicting the performance of fixed film reactors. Bioanalytical tools, recently employed in modern environmental biotechnology research (Sayler and Fox, 1991), may be useful in accomplishing this goal.

A new type of biofilter combining sludge removal and nitrification has recently been described by Malone (1992). In this 'expandable granular biofilter', solids settle following their capture by floating beads, which concomitantly serve as substrata for nitrifying and other bacteria. The system is periodically agitated in order to maintain aeration and allow trapped solids to be flushed. Loads as high as 32 kg feed m^{-3} day^{-1} have been achieved, with total ammonia concentrations lower than 1.0 mg NH_4-N l^{-1} and organic matter concentrations lower than 20 mg BOD_5 l^{-1} in the effluent. Ammonia removal rates (normalized to a total ambient ammonia concentration of 1 mg NH_4-N l^{-1}) varied between 0.25 and 0.50 g NH_4-N m^{-2} day^{-1} (Malone et al., 1993).

Accumulation of nitrite due to incomplete oxidation of ammonia is often observed in biofilters (Otte and Rosenthal, 1979; Manthe et al., 1984; Miller and Libey, 1985; Nijhof and Bovendeur, 1990; Van Rijn and Rivera, 1990). As nitrite is highly toxic to fish and other cultured aquatic species (Armstrong et al., 1976; Colt et al., 1981), its accumulation should be avoided. Thermodynamically, under optimal conditions, nitrite oxidation by *Nitrobacter* is approximately ten times as fast as ammonia oxidation by *Nitrosomonas* (Grady and Lim, 1980). In filters, however, conditions are often far from optimal and, in particular, nitrite oxidation is less than might be expected from laboratory studies. Nitrite accumulation as a result of differential inhibition of *Nitrosomonas* and *Nitrobacter* at low oxygen concentrations have been reported in various studies (Helder and De Vries, 1983; Alleman, 1985). Due to accumulation of organic matter, semi-anaerobic zones, resulting from microbial respiration, are present in most biofilters. Oxygen limitation is thus likely to be the main cause for nitrite accumulation (Manthe et al., 1984; Van Rijn and Rivera, 1990). Other factors causing nitrite accumulation are: suboptimal pH (Fenton and Mills, 1980; Alleman, 1985), substrate and product inhibition (Focht and Verstraete, 1977), and high light intensities (Olsen, 1981; Diab and Shilo, 1988). Denitrifying bacteria may also cause nitrite accumulation (see later).

4.3. Macrophytes, nitrification and solids removal

The third recirculating system configuration comprises mechanical solids removal, nitrification, and nutrient assimilation by plants, macroalgae or molluscs. Here, nutrients released in the culture system can be converted into plant or other biomass which can easily be removed and may often be a valuable byproduct. This more 'ecological' approach has considerable appeal and has led to increasing research efforts (e.g. Naegel, 1977; Sutton and Lewis, 1982; Zweig, 1986; Rennert and Drews, 1989; Rakocy et al., 1989; Quillere et al., 1993). Most such research has been conducted on hydroponic cultivation of vegetables for which technical feasibility has been demonstrated and commercial interest is widespread (Rakocy and Hargreaves, 1993). Commercially, however, fish culture and plant growth are not fully integrated and emphasis may be placed on one at expense of the other. With respect to the biology of these systems, a number of topics are only partially understood and deserve further attention, including: (a) stabilization of the nutritional status of water in the fish culture system, to meet the plant nutritional and physiological requirements, (b) prevention of salt accumulation, (c) disease and pest control of plants and fish, and (d) treatment of the waste generated in the integrated system (Rakocy and Hargreaves, 1993). Apart from vegetables, aquatic plants and macroalgae have been cultured in combination with recirculating fish culture systems, although mainly on a experimental scale (e.g. Rakocy and Allison, 1981; Ng et al., 1992; Porath and Pollock, 1992). Effluent treatment by macroalgae in land-based mariculture facilities has recently been revived. This procedure, first examined at Woods Hole Oceanographic Institution (Goldman et al., 1974a; Goldman et al., 1974b; Ryther et al., 1975) for removing inorganic nitrogen and phosphorous from tertiary waste, is now being used to treat mariculture effluents (Vandermeulen and Gordin, 1990; Cohen and Neori, 1991; Neori et al., 1991). The system is based on sludge removal followed by nutrient stripping by macroalgae (usually indigenous species). The process lends itself to the development of a multi-component food chain system. Research at the National Institute for Mariculture in Eilat, Israel, has examined the feasibility of a treatment facility comprising an earthen settling basin, an oyster culture facility for removal of organic matter and, a macroalgae culture facility (*Ulva lactuca*) for removal of inorganic nutrients (Shpigel et al., 1993).

4.4. Constraints of biological treatment systems presently used in recirculating aquaculture systems

Each of the above treatment systems has specific advantages (Table 2). Treatment by earthen ponds is simple with low operating costs, treatment by nitrification and solids removal is relatively stable, while treatment by plants or macroalgae and solids removal results in an extra crop in addition to the fish. If heat conservation is the main reason for using recirculation, earthen ponds are impractical. However, compared with the earthen pond treatment system, the other systems only achieve partial purification; solids and nitrate accumulate.

5. Solids and nitrate accumulation and their control in recirculating systems

5.1. Solids

In terms of BOD, production of 1 t of fish was estimated to produce organic matter equivalent to the untreated sewage load from 20 people (Solbe, 1982). Given an annual

Table 2
Advantages and disadvantages of main treatment configurations currently used in recirculating aquaculture facilities

Treatment system	Advantage	Disadvantage
Earthen pond or reservoir	Low operating costs	Large area required
	Additional fish crop	Unstable performance
	Little sludge production	Only for outdoor use
Sludge removal/nitrification	Stable performance	Sludge production
	Indoor and outdoor use	Nitrate accumulation
		pH control required
Sludge removal/hydroponics (macrophytes)	Low operating costs	Unstable performance
	Additional crop	Sludge production
	Indoor and outdoor use	Energy demanding when used indoors
		Limited commercial use of macroalgae

production of 15 million tons (Hempel, 1993), solids output by aquaculture is significant. In Norway, for example, it was estimated that the waste output by salmonid farming in 1989 was comparable to a pollution load by a human population of 2.7 million (Seymour and Bergheim, 1991).

Solids production in recirculating systems depends largely on the type of culture system and its management, the quality and type of feed used, and the size and type of aquatic species cultured. Uneaten feed and excreta are the main sources of solids in aquaculture. Production from non-utilized feed can be reduced by implementing more sophisticated feeding methods through which losses as low as 1% of the feed input can be obtained (Beveridge et al., 1991). In data compiled by these authors it was estimated that fecal production of salmonids, catfish, carps and shrimps ranged from 26 to 46% of the ingested feed (on a dry weight basis). Reported solids production values (uneaten feed and excreta) in fish culture systems range from 11 to 38% of the applied feed (McLaughlin, 1981; Mudrak, 1981; Chen et al., 1993a). The large variation can be attributed to differences in feed, species, management, and differences in decay of organic matter within the culture unit.

Methods for solids removal from recirculating systems have been discussed earlier. Filtration methods lead to a sludge concentrate which is commonly discharged with the effluent water. Growing legislative pressure in many parts of the world has increased the incentive to treat sludge produced in aquaculture systems. Application of liquid aquaculture sludge to fertilize agricultural crops is being conducted (Mudrak, 1981; Olsen et al., 1993). However, thickening of aquaculture sludge prior to its use as fertilizer is preferred (Willet and Jacobson, 1986; Bergheim et al., 1993a) since it reduces the loss of sludge to ground water. Composting as with manure from animal husbandry, and its subsequent use as fertilizer, has been only partially explored (Vette, 1988 as cited by Bergheim et al., 1993a).

Biological degradation of aquaculture solids has received little attention to date. The chemical composition of solids from aquaculture was found to be similar to that from sewage plants (Seymour and Bergheim, 1991). As such, classical methods such as aerobic degradation in oxidation ponds, anaerobic degradation in septic tanks or a combination of both, by means of activated sludge treatment, may be applied. Oxidation ponds have also been used to degrade aquaculture solids (Mudrak, 1981; Muir, 1982). Activated sludge treatment in an experimental fish culture system, combining nitrification, denitrification and organic matter degradation, has also been described by Meske (1971) and Meske (1976). Commercial application of this system was however limited, probably stemming from the fact that stable performance of such a system is difficult to maintain (Muir, 1978). Sand infiltration basins, in which partial degradation of solids takes place prior to reaching the ground water, are presently under examination in Sweden (Alanara et al., 1992).

Based on experience in treatment of urban and industrial waste water, it can be observed that aerobic degradation of organic solids is often coupled with excess sludge production, i.e. much of the energy released by oxidation of organic compounds is converted into algal or bacterial biomass. This is in contrast to anaerobic treatment which results in a more positive energy balance, i.e. less energy is converted into biomass growth, resulting in lower sludge production (Ten Brummeler et al., 1985; McClintock et al., 1988; Mergaert et al., 1992). As a result of further research on anaerobic treatment, various systems are now successfully used in treating a variety of waste waters from municipal, industrial and agricultural origin. Different types of 'high rate' anaerobic systems have been developed, including the anaerobic filter, the upflow anaerobic sludge blanket (UASB) and expanded/fluidized bed reactors (Hickey et al., 1991). In all of these systems, solids and hydraulic retention times are effectively separated, allowing accumulation of high biomass concentrations in the reactor and use of relatively high hydraulic loadings. In anaerobic filters and expanded/fluidized bed reactors this is accomplished by the development of biofilms on support surfaces within the reactor. In UASB systems, this is accomplished by the development of granules or flocs that have extremely good settling properties. There is little reported information on the treatment of aquaculture solids by high rate anaerobic reactors, though the construction of such a reactor in a freshwater facility in Allentown, Pennsylvania, was reported by Kugelman and Van Gorder (1991).

The anaerobic digestion of organic matter in these reactors has been described as a four-stage model (Zeikus, 1982; Zoetemeyer, 1982; Samsoon et al., 1987) in which four metabolic groups of microorganisms are recognized (Fig. 4): (1) hydrolytic bacteria that ferment a wide variety of complex organic molecules, such as polysaccharides, lipids and proteins, to acetate, H_2 and CO_2, other one-carbon organic compounds, organic acids larger than acetate and neutral compounds larger than methanol, (2) hydrogen-producing acetogenic bacteria that ferment organic acids larger than acetate (e.g. propionate and butyrate) as well as neutral compounds larger than methanol (e.g. ethanol and propanol) to H_2 and acetate, (3) homoacetogenic bacteria which ferment a wide spectrum of one- or multi-carbon compounds and catalyze the reduction of CO_2 to acetate, (4) methanogens which ferment acetate, H_2, CO_2 and other one-carbon compounds such as methanol and methylamine to methane. The main products of complete anaerobic digestion are therefore biomass (sludge), gaseous products (CO_2, H_2 and CH_4) and inorganic nutrients, primarily nitrogen, sulfur and phosphate. Breakdown of protein-rich organic matter results in the release of relatively large amounts of ammonia by hydrolysis of protein and ammonification of amino acids. During anaerobic

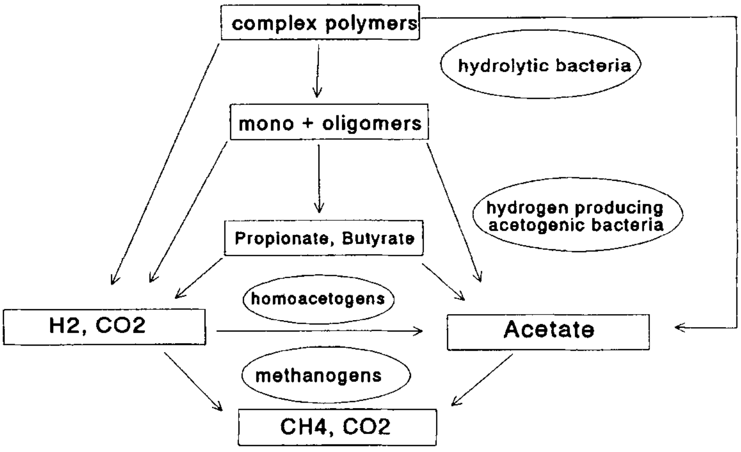

Fig. 4. Microbial interactions in anaerobic digestion.

digestion most of the ammonia is released and only a small part of the ammonia is reassimilated into bacterial biomass (sludge).

5.2. Nitrate

As the final product of ammonia oxidation, nitrate reaches remarkably high concentrations in nitrifying recirculating systems. Reported maximum concentrations are as high as 400–500 mg NO_3-N l^{-1} (Otte and Rosenthal, 1979; Honda et al., 1993). Maximum levels vary and are dictated mainly by the rate of water exchange and the extent of denitrifying activity taking place in the system. Denitrification is carried out by facultative anaerobic bacteria which, in the absence of oxygen and presence of metabolizable organic matter, are capable of using nitrate, nitrite, nitric oxide or nitrous oxide as terminal electron acceptors with N_2 as the end product (Payne, 1973). Although meant to be aerobic, most recirculating systems harbor anoxic zones which develop mainly in areas where organic matter accumulates. This is common in nitrifying filters used for wastewater treatment (Dalsgaard and Revsbech, 1992; Watanabe et al., 1992) and has also been demonstrated aquaculture facilities (Kawai et al., 1964; Hirayama, 1974; Bullock et al., 1994).

Unlike ammonia and nitrite, nitrate is relatively non-toxic to aquatic species. However, high concentrations in culture water of low alkalinity and low pH were shown to adversely affect octopus respiration (Hirayama, 1966) and were found to inhibit spawning in some fresh-water ornamental fish species (Y. Yirshkovich, personal communication, 1994). Despite the low toxicity, nitrate concentrations should be controlled. Environmental and public health considerations have lead to stringent regulations on nitrate discharge in many countries and permissible nitrate levels in effluent water are now as low as 11.6 mg NO_3-N l^{-1} (European Community Directive). As many recirculating systems are operated with partial water exchange, local effluent taxes might endanger their profitability. There is also the possibility of nitrite accumulation in systems containing high levels of nitrate. This results from incomplete reduction of nitrate by nitrate-reducing microorganisms, including denitrifiers (Betlach and Tiedje, 1981; Wilderer et al., 1987). In intensive fish culture systems nitrite was found to accumulate as a result of incomplete denitrification at low oxygen concentrations, or where denitrification was inhibited by limitation of organic matter (Van Rijn and Rivera, 1990; Van Rijn and Sich, 1992).

An additional factor favoring the use of denitrification is its ability to stabilize the buffering capacity of culture water. Acidification is often observed in nitrifying recirculating systems (e.g. Kaiser and Wheaton, 1983), where alkalinity decreases by 6.0–8.6 mg HCO_3^- for each milligram of ammonium oxidized to nitrate (Sharma and Ahlert, 1977; Grady and Lim, 1980). However, release of hydroxyl ions by denitrification raises alkalinity; it was estimated that each mg of nitrate reduced to N_2 causes alkalinity to increase by 3.57 mg (Jeris and Owens, 1975). The overall effect of denitrification in stabilizing the pH in recirculating systems has been described (Otte and Rosenthal, 1979; Kaiser et al., 1989).

5.3. Combined nitrate and solids removal in recirculating systems

Relatively few studies have been conducted on nitrate removal from recirculating systems. Nitrate removal by means of activated sludge treatment was studied by Meske (1971) and Meske (1976), while Otte and Rosenthal (1979) used an activated sludge tank, fed from the bottom and stirred with a propeller, to induce denitrification, using glucose and methanol as carbon and energy sources. A similar system was described by Kaiser et al. (1989) in a recirculating trout culture unit, using hydrolyzed corn starch as a carbon substrate. A commercial installation (Stahlermatic®, Muhlenhof, Germany) based on an integrated fixed film-activated sludge treatment (Gabel, 1984) was recently described for a recirculating eel culture system (Knosche, 1994). Another commercial system for ammonia and nitrate removal from intensive aquaculture systems based on fluidized bed technology is marketed by the German AquaPlan Company (Uwe Sonnenrein, personal communication, 1993). A marine system using denitrification in a fixed bed column was described by Balderston and Sieburth (1976). A similar recirculation marine system has successfully been used for cephalopods (Whitson and Lawrence, 1991; Whitson et al., 1993). Denitrification by a submerged filter in a recirculating system for culture of Japanese flounder was also recently described by Honda et al. (1993).

Reduction of sulfate to sulfide (extremely toxic to aquatic organisms) during anaerobic treatment is more likely to occur in marine systems, as sea water contains much higher sulfate concentrations. However, from the above studies on marine anaerobic reactors as well as studies on anaerobic treatment of sulfate-rich waste water such as pulp, paper and food oil industry effluents (Yoda et al., 1987), it can be concluded that the relatively high oxidation/reduction potential resulting from denitrification prevents the reduction of sulfate.

Studies such as these have demonstrated that such treatment is feasible and performance-stable. Except for the activated sludge treatment, most of the sludge is periodically removed from all the systems described above. In all these systems, denitrification

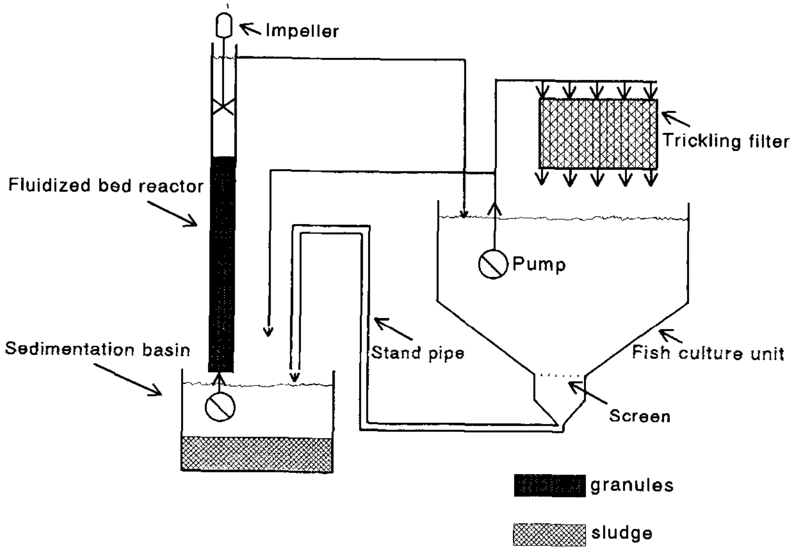

Fig. 5. Aerobic and anaerobic biofiltration in a recirculating fish culture system

is supported using an external source of carbon, often with methanol as the chosen source. However, sludge from aquaculture facilities forms an excellent source of carbon for denitrifying bacteria and so anaerobic decomposition of aquaculture solids and denitrification can be combined. By adding nitrate during decomposition of solids, anaerobic digestion, as described earlier, can proceed until the denitrifying stage. In the presence of nitrate, the methanogenic stage is absent due to the fact that, thermodynamically, the energy gain from denitrification is far larger than that from methanogenesis. Furthermore, the high redox potential resulting from denitrification inhibits methanogenic activity. Consequently, degradation of organic matter in the presence of nitrate proceeds at a faster rate than degradation in its absence (Haltrich, 1972; McClintock et al., 1988). In fact, by combining denitrification with degradation of organic matter in systems such as these, the biological processes responsible for reduction of nitrate and organic matter in static, earthen fish ponds are simulated.

This combination, with aerobic nitrification has successfully been applied in a pilot recirculating carp culture system (Van Rijn and Rivera, 1990; Arbiv and Van Rijn, 1995). A trickling filter was used to oxidize ammonia to nitrate while a fluidized bed reactor was used to oxidize organic matter and reduce nitrate. Predigestion of solids was carried out in a settling basin, and from here the supernatant water, rich in dissolved organic matter, was led into the fluidized bed column. A schematic presentation is given in Fig. 5. The rate of anaerobic digestion of organic matter was examined and the main products identified (Van Rijn et al., 1995). Carbon compounds triggering denitrification in the fluidized bed reactor were identified and kinetic studies on these compounds with bacterial isolates and natural populations derived from the fluidized bed reactor were conducted (Aboutboul et al., 1995). The treatment system has successfully been employed in a closed, intensive aquaculture unit, where it was found that levels of both inorganic nitrogen and organic matter could be kept sufficiently low to ensure adequate water quality for fish growth without water exchange (Arbiv and Van Rijn, 1995).

6. Bioaugmentation

Although published literature data are scarce, bioaugmentation, i.e. seeding with selected bacterial populations, is increasingly being promoted for recirculating systems. Suggested benefits range from removal of inorganic nitrogen, phosphate and organic matter to algal control. Arguments against bioaugmentation, largely in the 'gray' literature, are plentiful, and relate to the uncertainty of bacterial competition, the excessive claims made by commercial promoters and the difficulty of manipulating microbiological ecosystems with added seed material. Bioaugmentation may be beneficial for more rapid start-up of biofilters, although other, environmental, factors are also required to enhance start-up (Malone and Manthe, 1985). It might be beneficial for short-term reduction of wastes in effluents from open flow-through systems, as retention times are too low for establishment of an endemic bacterial population. Their use in more closed systems, including recirculating systems and static ponds, appears to be much less justifiable. Endemic bacteria develop under prevailing environmental conditions in such systems and there is no need to add more bacteria. If endemic bacteria do not develop they are usually inhibited by poor or fluctuating environmental conditions, which would also prevent the development of bioaugmented bacterial populations. Evaluations of various commercial bacterial suspensions in catfish ponds revealed no significant differences from controls (Boyd et al., 1984; Tucker and Lloyd, 1985; Boyd and Pippopinyo, 1994).

7. Concluding remarks

Research on recirculating aquaculture systems is developing at a rapid pace. Water quality control in such systems requires more than biological management of water quality. The improvement of (1) diets and delivery methods, (2) culture systems, (3) aeration and circulation devices, (4) filtration methods and (5) automatic monitoring devices are all significant and deserve future research. The grow-out of more sensitive species is increasingly coming within reach and a wider range of systems and locations can be expected. The economic feasibility of recirculating systems remains to be confirmed, however, and depends on the balance between their high capital and operating costs, and better performance and environmental management which might be attained. Environmental charges can be expected to have an increasing impact, and may shift the balance towards systems such as these.

Biological water quality control in recirculating fish culture systems has so far focused mainly on the prevention of accumulation of ammonia through the induction and management of nitrification. Accumulation of other inorganic nutrients such as nitrate and phosphate has received little attention but will deserve increasing consideration. Solids removal and discharge, but not treatment, is a common practice in most recirculating systems. However, environmental regulations are likely to encourage treatment not only of culture water but also of effluent water in recirculating systems, and so more complete approaches to nutrient management will increasingly be required. Here, the rational application of natural processes such as occurring in static fish ponds, is likely to hold an important position in future system development.

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