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Aquaculture

Aquaculture 270 (2007) 1 – 14

www.elsevier.com/locate/aqua-online

Review article

Nitrogen removal techniques in aquaculture for a sustainable production

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Received 16 February 2007; received in revised form 2 May 2007; accepted 5 May 2007

Abstract

As the aquaculture industry intensively develops, its environmental impact increases. Discharges from aquaculture deteriorate the receiving environment and the need for fishmeal and fish oil for fish feed production increases. Rotating biological contactors, trickling filters, bead filters and fluidized sand biofilters are conventionally used in intensive aquaculture systems to remove nitrogen from culture water. Besides these conventional water treatment systems, there are other possible modi operandi to recycle aquaculture water and simultaneously produce fish feed. These double-purpose techniques are the periphyton treatment technique, which is applicable to extensive systems, and the proteinaceous bio-flocs technology, which can be used in extensive as well as in intensive systems. In addition to maintenance of good water quality, both techniques provide an inexpensive feed source and a higher efficiency of nutrient conversion of feed. The bio-flocs technology has the advantage over the other techniques that it is relatively inexpensive; this makes it an economically viable approach for sustainable aquaculture.

Keywords: Water quality; Sustainability; Biofilters; Periphyton; Bio-flocs technology; C/N ratio

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doi:10.1016/j.aquaculture.2007.05.006

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1. Overview of problem

Aquaculture is a rapidly growing food producing sector. The sector has grown at an average rate of 8.9% per year since 1970, compared to only 1.2% for capture fisheries and 2.8% for terrestrial farmed meat-production systems over the same period (FAO, 2004). In contrast to aquaculture, capture fisheries landings as a whole is stagnant. Although catch rates for some species did not decline during the 1990s, most ocean fisheries stocks are now recognized as fully or over fished. The worldwide decline of ocean fisheries stocks and the further expansion of the human population are an incentive for the further growth of aquaculture. Despite the growth of the sector, aquaculture production still needs to increase 5-fold in the next 2 decades in order to satisfy the minimum protein requirement for human nutrition (FAO, 2004).

The intensive development of the aquaculture industry has been accompanied by an increase in environmental impacts. The production process generates substantial amounts of polluted effluent, containing uneaten feed and feces (Read and Fernandes, 2003). Discharges from aquaculture into the aquatic environment contain nutrients, various organic and inorganic compounds such as ammonium, phosphorus, dissolved organic carbon and organic matter (Piedrahita, 2003; Sugiura et al., 2006). The high levels of nutrients cause environmental deterioration of the receiving water bodies. In addition, the drained water may increase the occurrence of pathogenic microorganisms and introduce invading pathogen species (Thompson et al., 2002).

To produce 1 kg live weight fish one needs 1–3 kg dry weight feed (assuming a food conversion ratio about 1–3) (Naylor et al., 2000). About 36% of the feed is excreted as a form of organic waste (Brune et al., 2003). Around 75% of the feed N and P are unutilized and remain as waste in the water (Piedrahita, 2003; Gutierrez-Wing and Malone, 2006). An intensive aquaculture system, which contains 3 ton tilapia, can be compared on a biomass basis to a human community with 50 inhabitants (Helfman et al., 1997). This intensive aquaculture system can also be compared on grounds of waste generation to a community of around 240 inhabitants (Aziz and Tebbutt, 1980; Flemish government, 2005). It can thus be concluded that live fish biomass generates approximately 5 times more waste than live human biomass. The reason is that the scope of digestion in fish is limited; a relatively large fraction of feed remains undigested and is excreted (Amirkolaie, 2005). The feeding habit of fish is reflected in the digestive anatomy. The gut length of fish is short and the ratio of gut length to body length is small (Hertrampf and Piedad-Pascual, 2000). For instance, the intestine of carp is 2.0–2.5 times longer than the body, while that of cattle and sheep is respectively 20 and 30 times longer. The human intestine is about 3 to 4 times longer than the body. Consequently, in fish, the chyme stays in the gut only for a short time. For this reason, fish feed must have a high digestibility. Typically, fish body contains 65 to 75% protein (Hertrampf and Piedad-Pascual, 2000). In addition, fish use proteins for energy production to a large extent, unlike terrestrial animals that use mostly carbohydrates and lipids (Hepher, 1988). Fish protein requirement, therefore, is about two to three times higher than that of mammals. Ammonium is one of the end products of protein metabolism (Walsh and Wright, 1995). All these factors contribute to the high nitrogen residues in aquaculture water (Fig. 1). In water, NH_3 (ammonia) and NH_4^+ (ammonium) are in equilibrium depending on the pH and the temperature (Timmons et al., 2002). The sum of the two forms is called total ammonium nitrogen (TAN). Although both NH_3 and NH_4^+ may be toxic to fish, unionized ammonia is the more toxic form attributable to the fact that it is uncharged and lipid soluble and consequently traverses biological membranes more readily than the charged and hydrated NH_4^+ ions (Körner et al., 2001). Ammonia-N is toxic to commercially cultured fish at concentrations above 1.5 mg N/l. In most cases, the acceptable level of unionized ammonia in aquaculture systems is only 0.025 mg N/l (Neori et al., 2004; Chen et al., 2006). However, the toxicity threshold depends strongly on the species, size, fine solids, refractory organics, surface-active compounds, metals, and nitrate (Colt, 2006).

In addition to the generation of large amounts of waste, the use of fishmeal and fish oil as prime constituents of feed is another non-sustainable practice in aquaculture. Approximately one-third of the global fishmeal production is converted to aquaculture feeds (Delgado et al., 2003). The proportion of fishmeal supplies used for fish production increased from 10% in 1988 to 17% in 1994

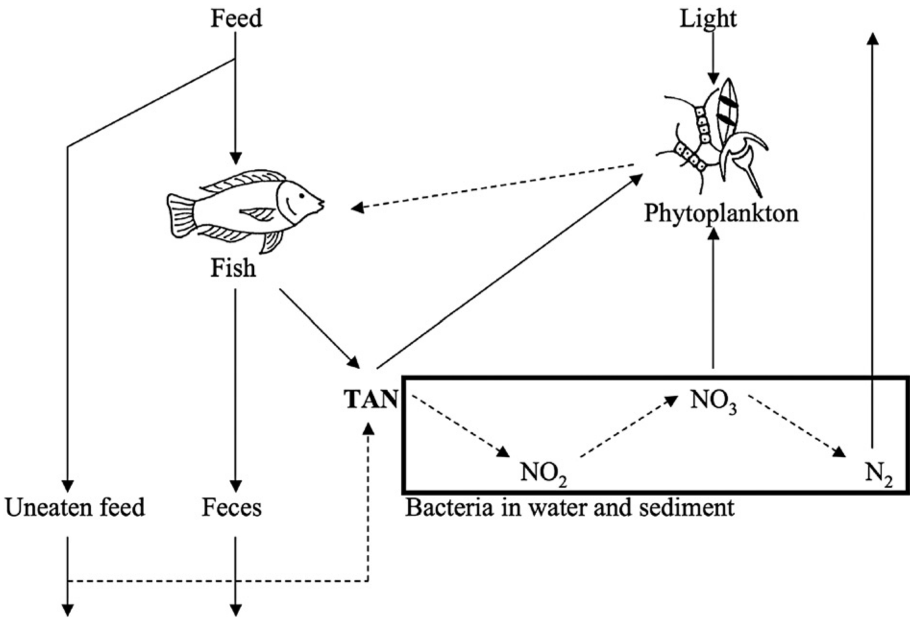

Fig. 1. Nitrogen cycle in aquaculture ponds with a long hydraulic residence time. The N-input considered is formulated feed. A part of the feed remains unconsumed in the system (Franco-Nava et al., 2004b). The consumed feed is partially converted into fish biomass and partially excreted as ammonium or egested as feces (Jiménez-Montealegre et al., 2002). The uneaten feed and feces contribute to the organic mater load of the system. The microbial decomposition of organic matter in the system leads to increased levels of TAN and nitrite, both harmful to fish even at low concentrations (Meade, 1985; Jiménez-Montealegre et al., 2002; Torres-Beristain et al., 2006). The TAN present in the system may be transformed into nitrite, nitrate and gaseous nitrogen. The formation of nitrogen gas is considered negligible in aquaculture ponds (El Samra and Olàh, 1979). The bacteria present in the water and sediment carry out these nitrogen transformations by nitrification and denitrification. Both TAN and nitrate can be assimilated by the phytoplankton, present in the water column. The phytoplankton can be consumed by the cultured organism (Turker et al., 2003). The consumption of phytoplankton by fish is minimal in this network. In stagnant water ponds TAN tends to accumulate within the system due to insufficient nitrification activity (Grommen et al., 2002).

and 33% in 1997 (Naylor et al., 2000). Hence, aquaculture is a possible panacea, but also a promoter of the collapse of fisheries stocks worldwide. The ratio of wild fish:fed farmed fish (both live weight base) is about 1.41:1 for tilapia and 5.16:1 for marine finfish, (Naylor et al., 2000). Purchase of commercially prepared feed for fish culture comprises 50% or more in the production costs; this is primarily due to the cost of the protein component (Bender et al., 2004). On average some 25% of the nutrient input of these feed sources is converted into harvestable products (Avnimelech and Lacher, 1979; Boyd, 1985; Muthuwani and Lin, 1996; Avnimelech and Ritvo, 2003). To make further sustainable increase of aquaculture production possible, the search for inexpensive protein sources and a higher efficiency of nutrient conversion of feed is needed.

2. N removal outside the culture unit

The most common water purification treatments in aquaculture systems can be subdivided in different types of water treatments: 1) earthen treatment ponds or reservoirs, and 2) a combination of solids removal and nitrification tanks as also used in domestic wastewater treatment plants. It should be noted that the real nitrogen removal processes are those that involve the release of fixed nitrogen back to the atmosphere (van Rijn et al., 2006). However, these are not discussed here.

2.1. Earthen treatment ponds or reservoirs

This treatment procedure consists of the direct linkage of, and water recirculation between the intensive production ponds and treatment ponds. The effluent water of the production pond is retained in a basin for several hours to days to allow natural physical, chemical, and biological processes to improve its quality for reuse (Diab et al., 1992; Hargreaves, 2006). Important practical parameters in this system are the 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 use of treatment ponds encounters problems due to algal collapse and anaerobiosis of the sediment (van Rijn, 1996). The main disadvantage is the unstable purification resulting from unpredictable fluctuations of phytoplankton biomass and speciation in the treatment pond (Hargreaves, 2006). An important advantage is that the microalgae grown in the treatment pond can be used to produce a second crop, such as bivalve seed or *Artemia*, which can be sold to generate income (Wang, 2003).

A possible system configuration comprises of a fish farm with nutrient assimilation by molluscs and seaweed (Fig. 2). 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 by-product. The nutrient-assimilating photoautotrophic plants can be used to turn nutrient-rich effluents into profitable resources (Neori et al., 2004). Biofiltration by plants generates in the culture system a mini-ecosystem, in which, if properly balanced, plant autotrophy counters fish (or shrimp) and microbial heterotrophy, not only regarding nutrients but also with respect to oxygen, pH and CO_2 (Neori et al., 2004). As a result, plant biofiltration diminishes the net environmental impact of aquaculture production systems. Today's integrated intensive aquaculture approaches, developed from traditional extensive polyculture, integrate the culture of fish or shrimp with vegetables, microalgae, shellfish and/or seaweed (Neori et al., 2004). By dividing the production process into stages, we can increase the constancy of the biomass in the system and improve the utilization efficiency of the physical facility (Wang, 2003).

2.2. Biofiltration

The treatment methods that are applied to treat aquaculture wastewater are broadly classifiable into physical, chemical and biological processes. Physical unit operations apply physical forces to remove contaminants. Solid removal is accomplished by sedimentation (settleable solids) or mechanical filtration (suspended and fine solids) (van Rijn, 1996). Two commonly used types of mechanical filtration in aquaculture include screen filtration and expendable granular media filtration (Twarowska et al., 1997; Franco-Nava et al., 2004a). For fine solids removal, foam fractionation – a process also referred to as air stripping or protein skimming – is often employed (Timmons, 1984; Hussenot, 2003). Chemical unit processes used for aquaculture wastewater treatment are customarily used in conjunction with physical unit operations and biological processes. The inherent disadvantage of most chemical unit processes is that they are additive processes; the chemicals tend to stay for a major part in the water. This is a significant factor if the wastewater is to be reused. The main chemical unit process used in aquaculture is disinfection by means of ozonation (Summerfelt, 2003). Disinfection by UV irradiation is considered as a credible alternative to chemical disinfection, because of the absence of toxic by-products which are usually generated and identified during chemical disinfection (Hassen et al., 2000). These techniques avoid the addition of chemical substances that are hazardous to the cultured organism. Biological processes are the most important ones with respect to aquaculture wastewater treatment and the major biological process is nitrification. Nitrification is carried out in a variety of systems, which can be grouped into 2 general types: emerged (rotating biological contactors, trickling filters) and submerged (e.g. fluidized bed filters, bead filters) fixed film filters (van Rijn, 1996; Ling and Chen, 2005; Malone and Pfeiffer, 2006). Biological filters are used for freshwater

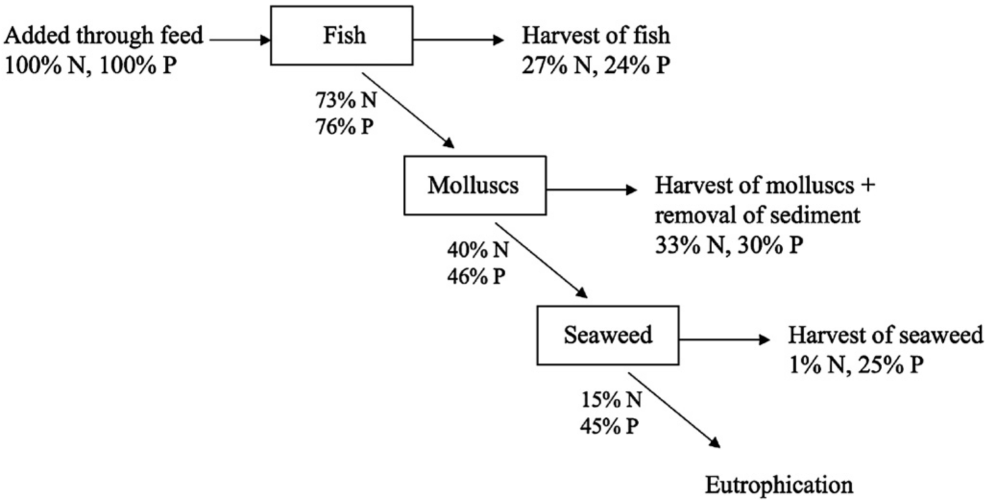

Fig. 2. Integrated farming: nitrogen and phosphorus budget (after Kautsky, 2004).

and marine operations (Hovanec and DeLong, 1996; Gutierrez-Wing and Malone, 2006; Malone and Pfeiffer, 2006). This paper reviews recirculating systems on biofiltration technologies for freshwater systems.

Nitrification in the bacterial film of the biofilter is affected by a variety of parameters such as substrate and dissolved oxygen concentrations, organic matter, temperature, pH, alkalinity, salinity and turbulence level (Satoh et al., 2000; Chen et al., 2006). Nitrifying bacteria are sensitive organisms and are extremely susceptible to a wide variety of inhibitors such as high concentrations of ammonia and nitrous acid, low dissolved oxygen levels (<1 mg/l) and pH outside the optimal range (7.5–8.6) (Masser et al., 1999; Villaverde et al., 2000; Ling and Chen, 2005). Nitrification, and especially the second step ($NO_2 \rightarrow NO_3$), is very sensitive to even traces of sulphides (Joye and Hollibaugh, 1995). Sulphides are present in sediments and in sludges accumulated in intensive aquaculture systems. For higher C/N ratios, the heterotrophic bacteria out-compete nitrifiers for available oxygen and space in the biofilters (Michaud et al., 2006). Hence, nitrification necessitates a low C/N ratio. Fig. 3 illustrates the N cycle in aquaculture systems equipped with an external biofilter.

Rotating biological contactors have been used in the treatment of domestic wastewater for decades and are now widely used as nitrifying filters in aquaculture applications. Rotating biological contactor technology is based on the rotation of a submerged substrate, which is made of high-density polystyrene or polyvinyl chloride, attached to a shaft (Tawfik et al., 2004; Park et al., 2005; Brazil, 2006). Nitrifying bacteria grow on the media and because of the rotation they alternately contacting nitrogen rich water and air. As the rotating biological contactor rotates, it exchanges carbon dioxide, generated by the bacteria, with oxygen from the air. In general, rotating biological contactor systems are divided into a series of independent stages or compartments (Lavens and Sorgeloos, 1984; Brazil, 2006). Compartmentalization creates a plug-flow pattern, increasing overall removal efficiency. It also promotes a variety of conditions where different organisms can flourish to varying degrees. As the water flows through the compartments, each subsequent stage receives influent with a lower organic content than the previous stage; the system thus enhances organic removal (UN, 2003; Watten and Sibrell, 2006). Complimentary, the rotating biological contactor has low head requirements to move water through the vessel. This advantage implies passive aeration and carbon dioxide removal, and low chance of clogging (Brazil, 2006).

Miller and Libey (1985) demonstrated that a rotating biological contactor provided better TAN areal removal rates, in the range of 0.19–0.79 g TAN/m^2 day, than a packed tower or fluidized bed reactor (0.24 g TAN/m^2

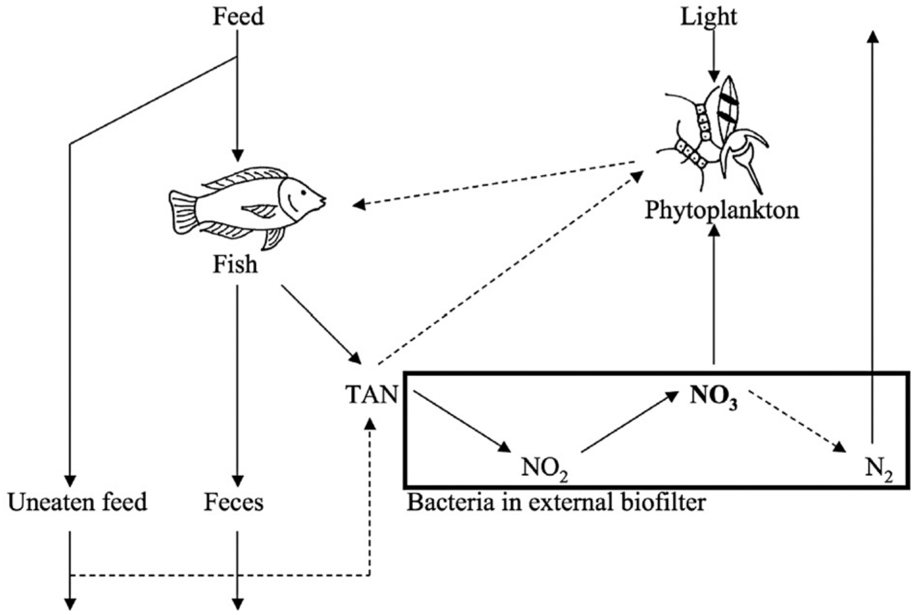

Fig. 3. Nitrogen cycle in aquaculture systems equipped with an external biofilter. The nitrogen cycle is similar to that of in water ponds with a long hydraulic residence time, but now the water rich in TAN is sent to an external biofilter. In this biofilter, nitrification is enhanced and through nitrite, nitrate is formed out of TAN. Nitrate is less toxic to fish than is TAN or nitrite (Meade, 1985; Lyssenko and Wheaton, 2006). Although such a system avoids TAN to accumulate, nitrate build up may take place. The biofilter creates space and optimal conditions for nitrifying bacteria to grow.

day), when treating the same fish culture water at comparable hydraulic loadings. Brazil (2006) described the performance and operation of a rotating biological contactor in a tilapia recirculating aquaculture system. The system obtained an average TAN areal removal rate of about 0.42 g/m^2 day. Increasing influent dissolved organic carbon levels decreased ammonia removal efficiency. However, there was no detectable relationship between the feed loading rate and ammonia oxidation performance. In addition to organic loading, mass and hydraulic loading, rotational speed and staging affected the ammonia oxidation performance.

Trickling filters consist of a fixed medium bed through which aquaculture wastewater flows downwards over a thin aerobic biofilm (Eding et al., 2006). As it trickles down, the water is continuously oxygenated, while the carbon dioxide is degassed and removed by the ventilated air. Trickling medium has a specific surface area ranging from 100 to 1000 m^2/m^3. Finturf artificial grass (284 m^2/m^3), Kaldnes rings (500 m^2/m^3), Norton rings (220 m^2/m^3) and Leca or light weight clay aggregate (500–1000 m^2/m^3) are some of the most frequently used media (Greiner and Timmons, 1998; Lekang and Kleppe, 2000; Timmons et al., 2006a). The organic material present in the wastewater is adsorbed on the biological slime layer and degraded by aerobic microorganisms.

Kamstra et al. (1998) reported TAN areal removal rates between 0.24 and 0.55 g TAN/m^2 day for a commercial-scale trickling filter. For three different applied filter medium types in commercial farms and a range of hydraulic surface loading conditions, the highest observed TAN areal removal rate for a trickling filter was 1.1 g TAN/m^2 day, with an average TAN areal removal rate of about 0.16 g TAN/m^2 day (Schnel et al., 2002; Eding et al., 2006). Lyssenko and Wheaton (2006) reported TAN areal removal rates of 0.64 g TAN/m^2 day. In the same study they found similar TAN areal removal rates for a submerged expandable upflow sand filter.

Downflow microbead filters are combinations of trickling filters and granular type biological filters (Timmons et al., 2006a). The use of floating media in downflow configurations has the advantage of being capable of using smaller media and the associated higher specific surface areas. As the recirculating water passes through the packed bed, suspended solids are captured and biofiltration processes are active (Malone and Beecher, 2000). The configuration offers the added advantage of using high hydraulic loadings without the need for sophisticated mechanical structures in the reactor to retain the media within the reactor vessel (Greiner and Timmons, 1998). The medium consists of polystyrene beads that are 1–3 mm in diameter and have a porosity of 36–40% (Timmons et al., 2006a). Depending on these features the specific surface area ranges from 1150 to 3936 m^2/m^3 (Greiner and Timmons, 1998; Malone and Beecher, 2000; Timmons et al., 2006a).

Greiner and Timmons (1998) observed TAN areal removal rates of about 0.45–0.60 g/m^2 day. A study using a commercial microbead filter system reported an average TAN areal removal rate of 0.30 g/m^2 day (Timmons et al., 2006a).

Fluidized sand biofilters have been widely adopted in recirculating systems that must reliably maintain excellent water quality (Summerfelt, 2006). Filter sand has a high specific surface area, i.e. 4000–20000 m^2/m^3 and has a moderate cost (Summerfelt, 2006). A disadvantage of the FBS is that they do not aerate, as do trickling filters (Summerfelt, 2006). Therefore, additional aeration is needed. These filters also must operate within a narrow water flow range in order to maintain proper bed expansion (Summerfelt, 2006).

Miller and Libey (1985) demonstrated that the TAN removal rate of a fluidized bed reactor was around 0.24 g N/m^2 day. Timmons and Summerfelt (1998) found similar rates in their research.

Table 1 gives an overview of the average TAN areal removal rate and the cost per kg of fish produced per

Table 1
General overview of the average TAN areal removal rate for frequently used biofilters in aquaculture systems

Biofilter type	Average TAN areal removal rate (g TAN/m^2 day)	Cost[a] (Euro/kg yr)	References
Rotating biological contactor	0.19–0.79	1.143	Miller and Libey, 1985; Brazil, 2006
Trickling filter	0.24–0.64	1.036	Kamstra et al., 1998; Schnel et al., 2002; Eding et al., 2006; Lyssenko and Wheaton, 2006
Bead filter	0.30–0.60	0.503	Greiner and Timmons, 1998; Timmons et al., 2006a
Fluidized sand biofilter	0.24	0.198	Miller and Libey, 1985; Timmons and Summerfelt, 1998

Also the costs for various biofilter choices based upon their capitalization cost to support a 454 ton per year tilapia farm are summarized.

[a] Data from Timmons et al. (2006b).

year for each biofilter type. Rotating biological contactors have the highest TAN areal removal rate, followed by bead biofilters and trickling filters, and fluidized sand biofilters. Although rotating biological contactors show good performance concerning TAN removal rate, they are together with trickling filters more expensive then the other biofilter types discussed. Fluidized sand biofilters and bead biofilters are the least expensive options for water treatment when the cost per kg of fish produced per year is considered.

3. N removal within the culture unit

The three nitrogen conversion pathways naturally present for the removal of ammonia–nitrogen in aquaculture systems are photoautotrophic removal by algae, autotrophic bacterial conversion of ammonia–nitrogen to nitrate–nitrogen, and heterotrophic bacterial conversion of ammonia–nitrogen directly to microbial biomass (Ebeling et al., 2006).

Developing and controlling dense heterotrophic microbial flocs in the water column or attached microorganisms called periphyton can accelerate the biological removal of organic and inorganic wastes in ponds (Avnimelech, 2005; Azim et al., 2003a,c). These processes are integral parts of the culture unit (Hargreaves, 2006). An important advantage is that microbial bio-flocs and periphyton can be consumed and used as a source of feed by the cultivated organisms (Burford et al., 2003, 2004; Hari et al., 2004; Azim and Wahab, 2005; Keshavanath and Gangadhar, 2005). As explained in the following paragraphs, both approaches are possible solutions for water quality problems, and can decrease the use of fish oil and fishmeal utilization in aquaculture.

3.1. The periphyton treatment technique

The periphyton community consists of attached aquatic biota on submerged matrices. It harbours algae, bacteria, fungi, protozoa, zooplankton and other invertebrates (Azim et al., 2005). As with phytoplankton, periphyton can be found in almost every type of water body from small ponds to large oceans and in trophic conditions that range from the most oligotrophic to the most eutrophic (Azim and Asaeda, 2005). Given adequate light, up to about 0.5 m depth in the water, high rates of photosynthesis and autotrophic production can be achieved (Craggs et al., 1996; Vermaat, 2005). Values for periphyton productivity are typically in the range of 1–3 g C/m^2 substrate day or 2–6 g dry matter/m^2 day (Azim et al., 2005). Periphyton entraps organic detritus, removes nutrients from the water column and helps

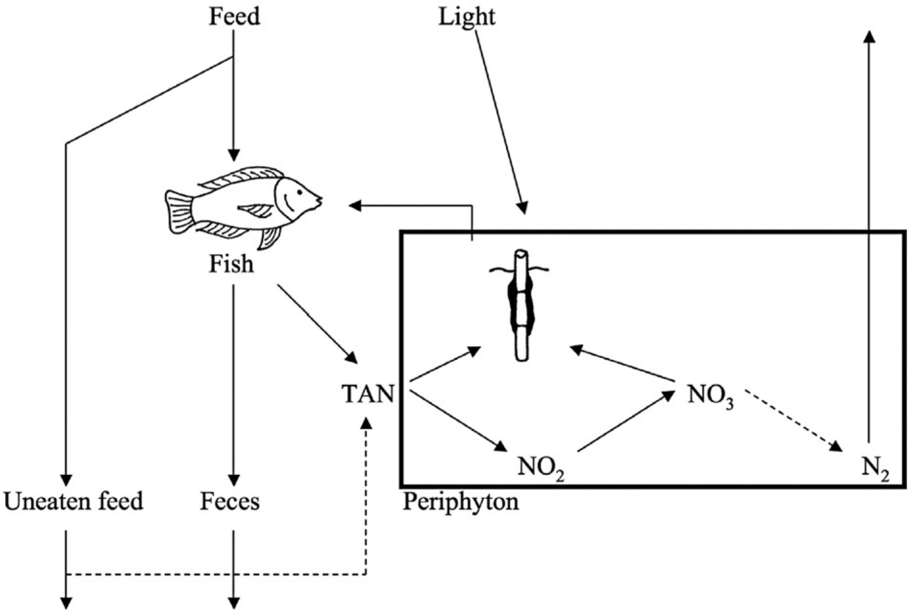

Fig. 4. Nitrogen cycle in extensive aquaculture ponds with substrates for periphyton growth. The nitrogen cycle is similar to that of in water ponds with a long hydraulic residence time, but now TAN concentration does not build up in the water column, neither is the nitrate concentration. The periphyton community takes up both TAN and nitrate and edible biomass is formed. The cultured fish can graze on the periphyton community and hence nitrogen, originating from wasted feed and excretion by fish, is redirected towards the cultured organism and therefore, this technique enhances the overall efficiency of nutrient conversion of feed.

control the dissolved oxygen concentration and the pH of the surrounding water (Azim et al., 2002; Dodds, 2003; Bender et al., 2004).

Supplying substrates improves the nitrogen-related processes developing in the water column and the nitrogen flow is mainly linked to autotrophic and heterotrophic activity that takes place in the periphyton (Fig. 4) (Milstein, 2005). The beneficial influence of periphyton on the water quality in different aquaculture systems has been investigated, as well as the impact of grazing by fish on periphyton communities (Huchette et al., 2000; Azim et al., 2001, 2002, 2003a,b,c, 2004). Not all fish are able to graze on periphyton; morphological and physiological adaptations to periphyton grazing are required (Azim et al., 2005). Although direct experimental evidence is scarce, the aquaculture fish species that can effectively utilize the periphyton assemblage are probably more numerous than those that are exclusively phytoplanktivorous (van Dam and Verdegem, 2005). Besides specialist (macro)herbivores, more general detritus and benthos feeders can also thrive on periphyton (van Dam et al., 2002).

Periphyton has an average C/N ratio of 10 (Azim and Asaeda, 2005). Its assimilation capacity is around 0.2 g N/m^2 day. From this it is clear that one needs a large surface, which allows periphyton growth, to treat intensive aquaculture wastewater without compromising the water quality. Besides N removal, biomass is formed. The yield is around 4 g dry matter/m^2 day and the protein content of periphyton is around 25% of the dry matter (Azim et al., 2002, 2005). This corresponds to a particular feed quantity that can diminish the overall feed cost.

Besides the large area needed, the problem within this system is that the process is completely dependent on the availability of sunlight (Azim and Asaeda, 2005). On cloudy days or on days with insufficient sunlight, the maximum nitrogen uptake rate will not be reached. Another problem is the laborious task to harvest the periphyton. One can conclude that application of the periphyton treatment technique in the intensive aquaculture sector is not feasible. Nevertheless, the technique of using this natural feed may be significant, particularly in smaller, extensive-level aquaculture systems in developing countries. The addition of the ‘periphyton loop’ in aquaculture ponds can be accomplished by adding static substrates to the pond (Azim et al., 2005), such as poles horizontally planted in the ponds. Substrates used are bamboo, hizol and kanchi (Azim et al., 2002, 2003c). Since periphyton can be easily cultured in modified fishponds and demands little management, the benefits may be substantial.

3.2. Bio-flocs technology

Suspended growth in ponds consists of phytoplankton, bacteria, aggregates of living and dead particulate organic

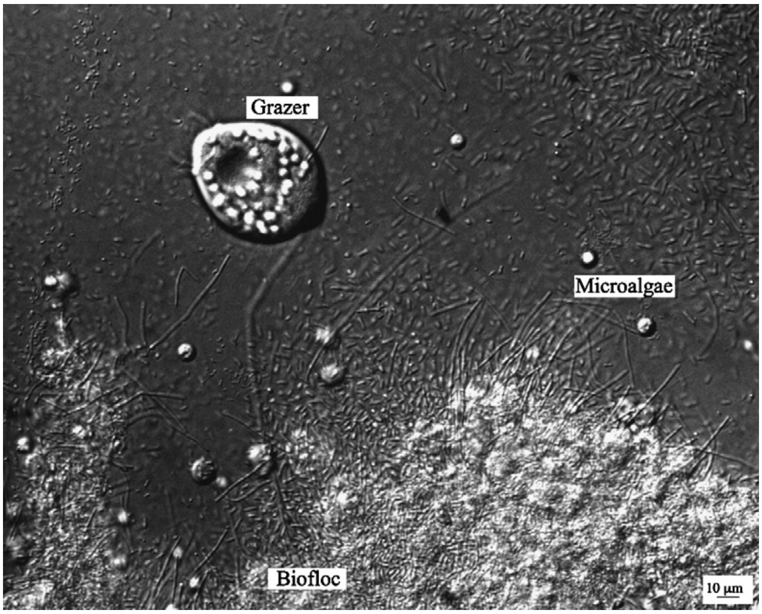

Fig. 5. Amorphous aggregate, which consists of phytoplankton, bacteria, aggregates of living and dead particulate organic matter, and grazers (The flocs were examined with light microscopy, and digital images were captured with a 1-CCD camera).

matter, and grazers of the bacteria (Fig. 5) (Hargreaves, 2006). If carbon and nitrogen are well balanced in the solution, ammonium in addition to organic nitrogenous waste will be converted into bacterial biomass (Schneider et al., 2005). By adding carbohydrates to the pond, bacterial growth is stimulated and nitrogen uptake through the production of microbial proteins takes place (Avnimelech, 1999). This promoted nitrogen uptake by bacterial growth decreases the ammonium concentration more rapidly than nitrification (Hargreaves, 2006). Immobilization of ammonium by heterotrophic bacteria occurs much more rapidly because the growth rate and microbial biomass yield per unit substrate of heterotrophs are a factor 10 higher than that of nitrifying bacteria (Hargreaves, 2006). The microbial biomass yield per unit substrate of heterotrophic bacteria is about 0.5 g biomass C/g substrate C used (Eding et al., 2006).

In natural environments, microorganisms tend to form amorphous aggregates. The settling velocity of these flocs appears not to relate to the square of the size, as expected from Stokes' law (Logan and Hunt, 1987, 1988). If an aggregate is highly porous, fluid streamlines will penetrate the aggregate resulting in advective flow through it. This will improve the supply of nutrients to the cells present in the aggregate and will decrease the settling velocity of the flocs in the pond.

Using the relative uptake factor γ, defined as growth rate of aggregated cells/growth rate of free cells, one can make a comparison of the substrate uptake by aggregated versus dispersed cells. Fig. 6 depicts the relative uptake predictions for microbial cells in permeable flocs (Logan and Hunt, 1988). The power input to the fluid originates from the aeration of the ponds. Different aeration techniques are available, such as diffuser aeration, mechanical aeration and packed column aeration. For turbulent fluids, the mean shear rate G is determined from the power input to the fluid per unit volume of the fluid. In intensive aquaculture systems the average power input to the fluid is around 1–10 W/m^3 = 10^1–10^2 cm^2/s^3 or $10 < G < 100$ s^{-1} (Boyd, 1998; McGraw et al., 2001; Schuur, 2003). At these moderate mixing rates, cells growing in permeable aggregates can profit from advective flow and grow better than single dispersed cells ($\gamma > 1$). One can calculate that the relative growth rate of aggregated cells in this energy regime is greater than the growth rate of free cells (Logan and Hunt, 1987, 1988). When more intense aeration is applied, the advantage of growing in flocs disappears and cells growing solely show higher growth.

We can conclude that the biological flocs can be considered as a kind of fast growing microbial mixed culture in which the 'waste'-nitrogen is recycled to young cells, which subsequently are grazed by the fish (Fig. 7). Uptake of the bio-flocs by fish depends most probably on the fish species and feeding traits, fish size, floc size and floc density (Avnimelech, 2007). With respect to feeding, this technique operates at "neutral cost", because it upgrades starch to protein. Moreover, one does not need to invest in an external water treatment system. This method is applicable to extensive as well as intensive aquaculture systems. In addition, the heterotrophic microbial biomass is suspected to have a controlling effect on pathogenic bacteria (Michaud et al., 2006). Preliminary results at our laboratories have shown the presence of poly-β-hydroxybutyrate in bio-flocs. PHB-accumulating bacteria may abate pathogenic bacteria in aquaculture (Defoirdt et al., 2007; Halet et al., 2007).

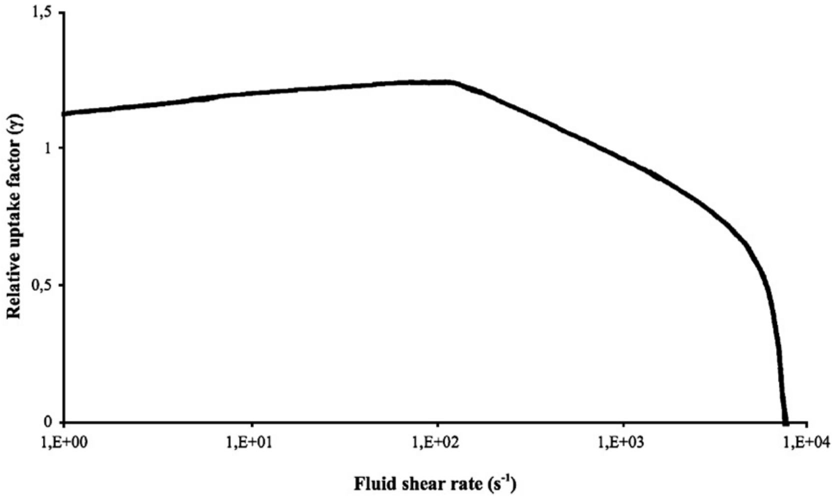

Fig. 6. Relative growth prediction for microbial cells in permeable flocs (after Logan and Hunt, 1988).

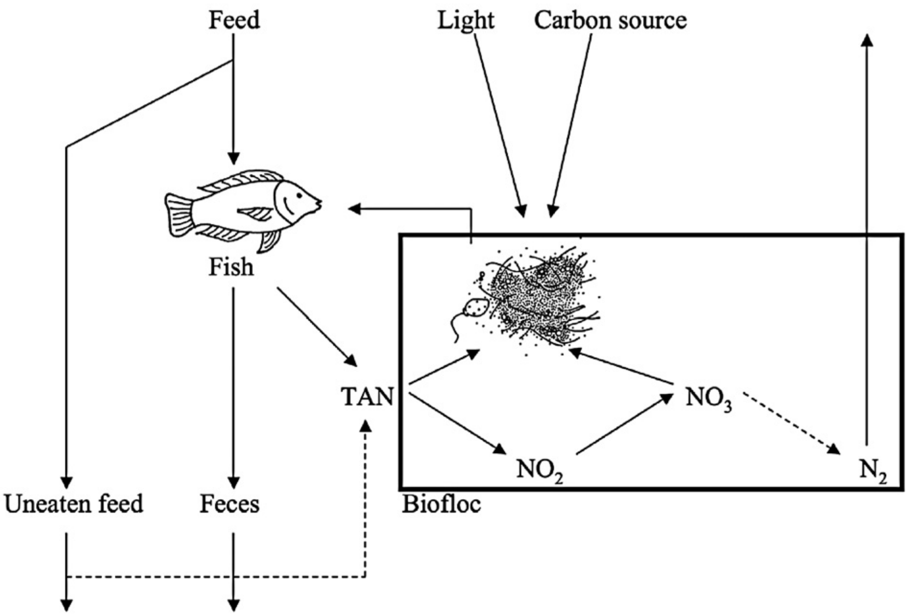

Fig. 7. Nitrogen cycle in bio-floc ponds. The nitrogen cycle is similar to that of in water ponds with periphyton. In contrast to the periphyton system, this system is also applicable to intensive systems. The added carbon source, together with the waste nitrogen, is converted into microbial bio-flocs, which in turn can be eaten by the cultured organism. This technique provides an inexpensive protein source with a higher efficiency of nutrient conversion of feed.

In what follows, some examples are discussed concerning performance of the bio-flocs technology in practice in freshwater systems.

Avnimelech (1999) pointed out the use of the C/N ratio as a control element in aquaculture systems. Nitrogen control was induced by adding carbohydrates to the water, and through the subsequent uptake of nitrogen by heterotrophic bacteria. This resulted in the synthesis of microbial proteins that can be eaten by the cultured fish species. Experiments with sediment suspension amended with about 10 mg N/L ammonium and glucose at a concentration 20 times higher than that of the TAN showed that almost all the added ammonium disappeared over a period of about 2 h. Avnimelech et al. (1994) found that protein utilization by fish in intensive bio-floc systems is almost twice as high as the protein utilization in conventionally fed intensive aquaculture ponds, due to a recycling of the excreted nitrogen into utilizable microbial protein. Protein recovery by tilapia rose from 23% in the control to 43% in the floc treatment. It was concluded from this study that the price of feed for fish production using sorghum supplemented granules (pellets containing only 20% protein and sorghum as a carbonaceous substrate) is just about 50% of the conventional cost when 30% protein pellets were used.

The bio-flocs technology is also applicable to saline systems, as discussed below.

Hari et al. (2004) facilitated the development of heterotrophic bacteria and the related *in situ* protein synthesis by increasing the C/N ratio of the feed and by further increasing the C/N ratio through carbohydrate addition to the ponds. The added carbohydrate facilitated increased heterotrophic growth thereby augmenting shrimp production. The levels of inorganic nitrogen species in the water column were lower due to uptake by heterotrophic bacteria, making farming more sustainable. The TAN levels in the water column in the study were 0.01 mg/L, which is low compared to levels reported in other studies (0.5–3.0 mg/L) (Hopkins et al., 1993). Consumption of microbial flocs increased nitrogen retention from added feed by 13% (Hari et al., 2004).

Burford et al. (2004) promoted the growth of the natural microbiota in ponds by routine addition of grain feed (18–22% protein) and molasses as carbon sources. Fishmeal-based feeds and ammonia from shrimp excretion were used as nitrogen sources. The study supports the theory that natural biota can provide a nitrogen source for shrimp, and that flocculated particles are likely to be a significant proportion of this.

Hari et al. (2006) reported that carbohydrate addition in combination with a decreased dietary protein level improved the sustainability of shrimp farming in extensive shrimp culture systems through 1) increased nitrogen retention in harvested shrimp biomass, 2) reduced demand for feed protein, 3) reduced concentrations of potentially toxic TAN and NO_2–N in the system, and 4) reduced water based nitrogen discharge to the environment. If carbohydrate was added to the water column to enhance heterotrophic bacterial protein production, the protein level in the diet could be reduced from 40% to 25%, without compromising shrimp production.

4. Conclusions and future perspectives

Possible effluent treatment technologies in aquaculture are diverse. The challenge to the designers of aquaculture systems is to develop systems that maximize production capacity per cost unit of capital invested. To do so, components used in recirculating systems need to be designed and developed to reduce the cost of the unit while maintaining reliability. The bio-flocs technology, the periphyton treatment technique, integrated treatment ponds, fluidized sand biofilters, bead filters, trickling filters and the rotating biological contactors can be considered as good effluent treatment technologies. The bio-flocs technology provides a sustainable method to maintain water quality in aquaculture systems and moreover concurrently fish feed is produced. Since the purchase of commercially prepared feed in fish culture has a share of 50% or more in the production costs, an effluent treatment technique that maintains water quality and simultaneously produces *in situ* fish feed has a large asset over other techniques. Additional research in this field concerning management of the floc production, the floc dynamics in intensive aquaculture systems, the nutritional value of flocs and the health effects of flocs is needed, more specifically the effect on growth and survival of the cultured organisms. Also microbiological aspects need further investigation, particularly the microbiological characterization of the flocs, possible manipulation of the microbial community and presence of pre/probiotic organisms in the microbial community of the flocs are challenging fields of interest.

Acknowledgements

This work was supported by the “Instituut voor de aanmoediging van Innovatie door Wetenschap en Technologie in Vlaanderen” (IWT grant no. 51256). A special thank to the reviewers (ir. Han Vervaeren and ir. Peter Deschryver) for critically reading the manuscript.

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