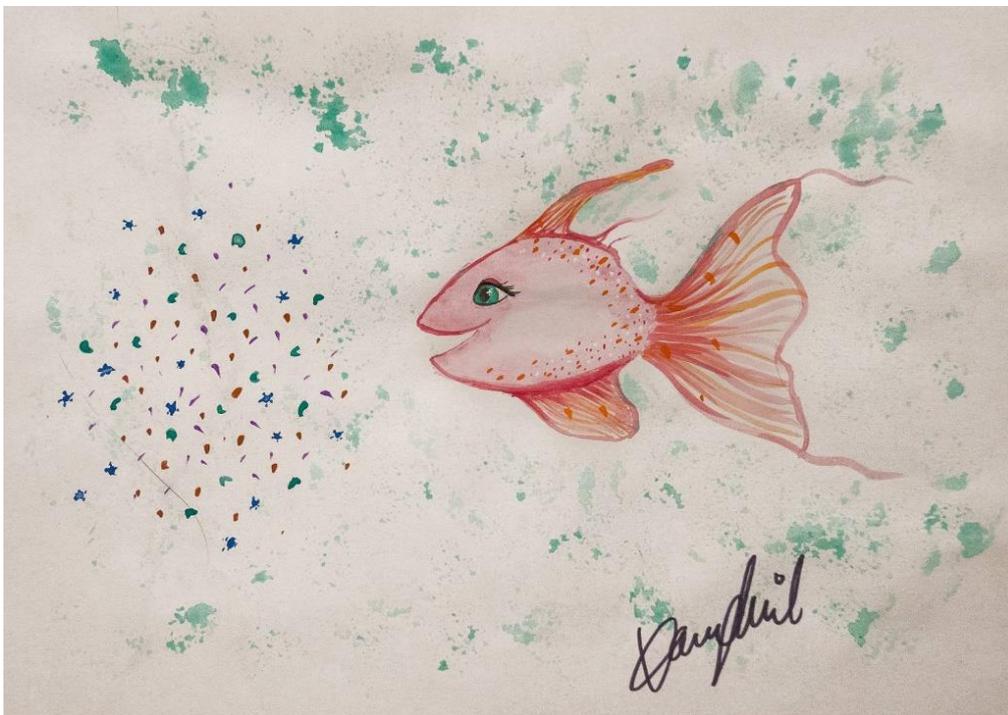




Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Veterinary Medicine and Animal Science
Department of Animal nutrition and management

Biofloc technology - a new concept to produce feed to Swedish aquaculture



Lovisa Granberg

Bachelor project, 15 hp

Agricultural Science Programme – Animal Science

Department of Animal nutrition and management, 579

Uppsala 2016

Biofloc technology - a new concept to produce feed to Swedish aquaculture

Biofloc teknologi - ett nytt koncept för att producera foder till svenskt vattenbruk

Lovisa Granberg

Supervisor: Torbjörn Lundh, SLU, Department of Animal nutrition and management
Examiner: Anders Kiessling, SLU, Department of Animal nutrition and management

Credits: 15 ECTS
Course title: Bachelor project in Animal Science
Course code: EX0553
Programme: Agricultural Science Programme – Animal Science
Level: G2E

Place of publication: Uppsala
Year of publication: 2016
Series name, part no: Examensarbete / Sveriges lantbruksuniversitet, Institutionen för husdjurens utfodring och vård, 579
On-line published: <http://epsilon.slu.se>

Omslagsbild: Hanna Kannerstål

Nyckelord: mikrob protein, tilapia, räkor, foder, miljö
Key words: microbial protein, tilapia, shrimp, feed, environment

Abstract

The aquaculture provides many people with fish and it will increase in scale. To provide the fish with feed that is not a load on the environment the use of microbes as feed either filtrated directly from the water or recovered to become protein meal is very promising. Biofloc technology (BFT) is one method with filtration directly from water. It has traditionally been used in outdoor ponds that contain naturally photoautotrophic algae that provides the water with oxygen and becomes an additional food source to herbivorous and omnivorous species. BFT have shown to reduce feeding costs, maintain water quality and to help to control bacterial infections in the pond. To maintain a better water stability, BFT could be applied indoors, then sunlight becomes restrained and instead of algae, heterotrophic bacteria will dominate. The aim of this literature review was to see if this technology could have potential in Swedish aquaculture that have a colder climate. When higher water temperature can be held during all seasons, it makes it possible to grow tropical fish species. It is possible to have indoor tanks in Sweden, where fish and shrimps can grow in BFT systems. It would decrease the environmental load and an opportunity to take full responsibility for waste material and substances from the water.

Sammanfattning

Vattenbruk bidrar idag med fisk och skaldjur till många människor och efterfrågan kommer att öka. För att kunna ge fisken föda som inte belastar miljön, behövs nya lösningar. Biofloc teknologi (BFT) är en metod som traditionellt sett varit ett system i dammar där det naturligt växer alger i vattnet. Dessa alger tillför syre och blir en näringskälla för herbivora och omnivora fiskarter. BFT har visat sig minska foderkostnader, bibehålla vattenkvalitet och har visat sig hjälpa till mot bakteriella infektioner. Syftet med denna litteraturstudie var att undersöka om denna teknik kan tillämpas i svenskt vattenbruk som har ett kallare klimat. För att bibehålla en bättre vattenstabilitet så skulle BFT kunna användas i inomhus tankar. Då kommer inte solljus ner i vattnet och istället för alger kommer heterotrofiska bakterier att dominera, det innebär att balansen i vattnet förbättras. Dessutom kan man hålla en högre vattentemperatur året runt, vilket passar för tropiska arter. Svenskt vattenbruk kan ha inomhusodling av fisk och räkor där BFT kan vara en av de tekniker som används. BFT skulle ge miljövinster och en möjlighet att kunna ta fullt ansvar för det avfallsmaterial och de ämnen som hamnar i vattnet.

Introduction

How to supply a growing population with high quality protein concerns many people. To produce large quantities of fish or shrimp in a high rate, intensive aquaculture systems is an efficient way (Avnimelech, 2007). One definition of aquaculture is that during controlled conditions, cultivate aquatic animals to reach a goal where a saleable product is made as efficiently and cost worthy as possible (Ebeling *et al.*, 2006). In FAO's report World review of fisheries and aquaculture (2014), the importance of aquaculture is increasing and (Figure 1) shows the rate of its progress. The aquaculture systems can be roughly divided into three general types; extensive- and intensive ponds, intensive recirculating tanks, and raceway systems (Ebeling *et al.*, 2006). However, cages are another important way to housing fish and is used in biggest extent in Europe. The amount of fish is reflected in the different housing systems. In a pond with microalgae about 0.4 kilos/m³/year, with a simple stirring system that aerates the water 1-3 kilos/m³/year. With an intensive heterotrophic bacterial biofloc with an aeration of the water 8-12 kilos/m³/year, and in recirculating aquaculture systems (RAS) and in pure oxygen addition 50 kilos/m³/year (Kiessling, 2016). In systems with higher intensity, there is a demand to feed the fish with better nutrients to maximize growth rate. To minimize capture of marine species as protein feed to aquaculture other protein sources are needed and

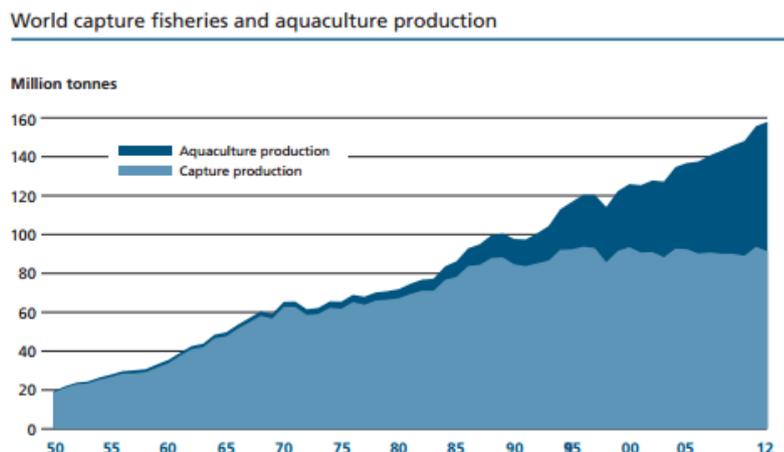


Fig. 1. The world aquaculture production is increasing quickly (FAO, 2014).

the use of microbes as feed either filtrated directly from the water or recovered to become protein meal is very promising. According to Avnimelech (2007) Biofloc technology (BFT) can contribute as an important environmental advantage when feeding aquaculture. The main principle of BFT is that microbes accumulates of excess feed and, if added, carbohydrates. The held water animal can filtrate particles consisting of microbes as a complementary feed. The aim of this literature review is learn about BFT as a complementary feed to fish and further on discuss the potential of the technique in colder climates, such as Sweden.

Swedish aquaculture has three different specializations, the first is food production. Second, the production of smolt (young salmons) that is being released in rivers that have gotten natural salmon habitats destroyed. Thirdly, the production of game fish that is being released in waters that they naturally do not live (KSLA, 2004). Year 2001 the Swedish aquaculture produced 6300 tonnes of fish (KSLA, 2004) but have increased to be 11 700 tonnes 2013 (Svenskt vattenbruk, 2015). Traditional aquaculture is outdoors, in lakes and dams with cold-water fish species like rainbow trout, salmon, arctic charr and eel (Jordbruksverket, 2016).

Literature review

Nutrition for aquatic animals

Aquatic animals that lives on small particles must separate them from water. When microbial flocs is a part of the diet, an important factor is that bacterial cells form clusters due to flocculation, with or without particles from feed or mud (Harris & Mitchell, 1973). Both bacteria and algae produces extracellular organic matter that adhere to other particles and tend to link together to an assorted mix of algae, bacteria, detritus and mineral particles (Seki, 1972; Bowen, 1978; Paerl, 1978, in Bowen, 1987). Detritus is an expression for all non-living organic material in the aquaculture. It can be dissolved materials as well as particles (Moriarty, 1997). The detritus derives mainly from compounds from dead plants, soluble fractions as carbohydrates and some proteins dissolve rapidly while lignin and cellulose contribute to the sedimentation (Bowen, 1987). Detritus is important to the cultures as feed, but also as an attachment surface for bacteria which may also use some of the detritus as substrate (Bowen, 1987).

Fish gut anatomy and nutrient digestion

The fish have solved the water separation problem with modified gill rakers disposed on the gill arches. Depending on the particles size that the fish prefer it is more or less densely organised (Hepher, 1988). However, while species such as carp or tilapia thrives in nutritious waters, salmonids prefer clearer water and cannot cope with too much particles through the gills (Kiessling, 2016). The collected particles move along a channel to the pharynx. The intestine is quite simple, long and coiled but is not separated in clear transitions between oesophagus, foregut or hindgut (Hepher, 1988). The anatomy derives from their feeding habits. The length of the fish guts is small when compared to the body length. For example, the intestine of a carp is 2.0-2.5 times longer than the body, compared to cattle and sheep that has 20 and 30 times longer respectively. In humans, it is 3-4 times longer (Hertrampf & Piedad-Pascal, 2000). Consequently, the feed rapidly moves through the gut and has to have a high digestibility. The fish has requirements of two to three times more protein than mammals and typically, the fish contains 65-75 % protein (Hertrampf & Piedad-Pascal, 2000). However, the fish have lots of protein available and therefore they can afford to have a shorter intestine (Kiessling, 2016).

Much of the energy derives in aquatic animals from catabolism and oxidation of proteins, in difference from terrestrial animals that use mostly carbohydrates and lipids. Therefore, they need proteins in a high concentration in the feed. (Hepher, 1988). In a trial with Tilapia it was seen that even though the fish is not capable of digesting cellulose, an addition of cellulose to the diet did support growth. This is due to the intake and digestion of bacteria that used cellulose as substrate or for attachment surface (Avnimelech, 1999).

Water temperature

Aquatic animals are poikilothermic which means that they have the same temperature as their surroundings. So called warm water fish grows best between 25-30 °C and cold water fish in < 20 ° C. (Hertrampf & Piedad-Pascual, 2000). Aquaculture in Sweden is adjusted to colder climate. Reaction rates is connected to temperature, is it often governed by the van't Hoff

relationship, which says that reaction rates doubles with every 10° C increase until maximum rate (Hargreaves, 2006). Water temperature have effects on fish metabolism, which pre-eminently is connected with feed intake and growth. Extreme temperatures can be very stressful to the animal, and led to diseases when the immune system is affected. Water temperature also effects chemical reactions and microbial growth (Hargreaves & Tucker, 2003).

Tilapia and Pangasius

The fish can be classified after feeding choice in their natural conditions, the three prime groups are; carnivores (e.g. salmon and trout), herbivores (several different carps) and omnivores (e.g. tilapia and pangasius) (Hertrampf & Piedad-Pascual, 2000). Tilapia (*Oreochromis niloticus*) and pangasius (*Pangasius hypophthalmus*) have a diet consisting of mainly proteins and lipids that is derived from vegetable sources (Van Leeuwen *et al.*, 2009). Pangasius is mostly cultured in Vietnam in outdoor ponds. Tilapia is traditionally cultured in Southeast Asia and South America in rice fields, ponds and net-cages (Van Leeuwen *et al.*, 2009). However, Nile tilapia is used in many studies with BFT with positive results. A big constraint that is of importance is the fact that tilapia is cold sensitive (Charo-Karisa *et al.*, 2005). Nile tilapia reduces feeding and activity at 20° C and at 16° C growth stops. If it becomes 10° C the fish won't survive for many days (Chervinski, 1982 in Dan & Little, 2000). In China and Egypt, the over-wintering of tilapia is a big economical problem, because of the mass mortality if a cold winter (< 15° C in water) (Charo-Karisa *et al.*, 2005). Different strategies of holding tilapia in tempered water exists. To maintain water temperature in ponds, water exchange rate can be minimized and ponds or tanks can be covered with plastic sheets. This was done by (Crab *et al.*, 2009) after positive results from Chinese insulated greenhouses covered with plastic sheets (Jiazhao, 1991 in Cruz & Ridha, 1994). Other strategies that have been tried successfully is heated facilities (Behrends *et al.*, 1990), heated effluents from power plants (Behrends *et al.*, 1981 in Cruz & Ridha, 1994) and warm underground seawater (Cruz & Ridha, 1994).

Arctic charr and Eurasian perch

The fish that is most adapted to coldwater is the arctic charr (*Salvelinus Alpinus L.*) that have an optimum temperature of 5-12° C (Johnson., 1980 in Dalsgaard *et al.*, 2013). Their growth may continue until just above 0° C (Brännäs & Wiklund, 1992 in Brännäs & Linnér, 2000). In a study about feeding frequencies and stocking density by Brännäs & Linnér (2000) results indicates that feeding frequently and a high densities increased feeding opportunities and reduced aggressive behaviour between individuals in cold water. Another fish adapted to Swedish conditions is the eurasian perch (*Perca fluviatilis*). They demand a higher temperature for growth, 10-22° C (Fishbase, 2016) and is favoured during summer temperatures. Arctic charr and eurasian perch are carnivorous fish that is being fed with high concentrations of fish and plant-based protein (Langeland *et al.*, 2014). Even plants as a source of protein have limitations, e. g soybean meal have many antinutritional factors among others; protease inhibitors, phytic acid and saponins (Francis *et al.*, 2001).

Microbial protein

Bacteria grows very fast and contains DNA and big amounts of messenger RNA (mRNA) to be able to have a quick protein synthesis. Bacteria that grows more slowly have a smaller content of nucleic acid (Kiessling, 2016). Crude bacterial protein contains 30% nucleic acid (Dostalek & Molin, 1975; Gow *et al.*, 1975 in Avnimelech & Mokady, 1988). This could lead to a reduction in nutritive value and possible toxicity (Avnimelech *et al.*, 1988). It was shown that when feeding fish with a nucleic acid extract up to 5 % of the diet the fish did not show any harmful effects. When increasing the amount up to 10 % it had detrimental outcome (Tacon & Cooke, 1980 in Avnimelech *et al.*, 1988), similar results were shown in trial by Kiessling & Askbrandt (1993) where two different bacterial strains (*Brevibacterium lactofermentum* and *Bacterium glutamicum*) were added in separate trials in 4-,8-,16 % of diet to rainbow trout. Results showed that *B. glutamicum* led to depressed growth rate and feed efficiency with significance when compared to the control diet consisting of fishmeal. This occurred in both 8 % and 16 % level of added single cell protein (SCP). *B. lactofermentum* should be able to be added in feed even in 16 %. The diets and strains had the same fatty acid- and amino acid composition, which could imply that SCP from some bacterial strains have antinutritional/toxic effect.

It has been concluded through many studies that SCP can replace other protein sources in some extent (Avnimelech *et al.*, 1989; Kiessling & Askbrandt, 1993). The biggest issue concerning SCP cultures as feed is the problems with harvesting, dehydration and packaging. When having bacterial protein directly in the pond as a complementary feed to the aquatic culture these expensive stages of processing disappears because it is managed directly by the fish (Avnimelech, 1999). Humans cannot utilize microbial protein because of the high amount of uric acid that is formed during degradation. This is a big advantage to the fish, which have other metabolic enzymes that are able to degrade nucleic acids to less toxic products (Rumsey *et al.*, 1992).

In a Swedish trial by Langeland *et al.* (2014) four protein-rich alternative feeds were given to arctic charr and eurasian perch. The different diets consisted of Baker's yeast (*Saccharomyces cerevisiae*) in an intact form or in an extracted, zygomycete (*Rhizopus oryzae*) or blue mussel (*Mytilus edulis*). The diets contained 30 % of the test-ingredient and were fed twice a day until rejection. Results showed that digestibility was higher for extracted *S. cerevisiae* and *M. edulis* than intact *S. cerevisiae* and *R. oryzae* fed to Arctic Charr but to Eurasian perch, no distinct differences were seen (Langeland *et al.*, 2014). When the test period became longer (99 days) when feeding the mentioned diets to Arctic charr, the results were different. Growth was restricted in the extracted *S. cerevisiae* and it was concluded that *M. edulis* and intact *S. cerevisiae* is promising as a protein-feed to Arctic charr (Vidakovic *et al.*, 2015).

Nitrogen removal strategies

To remove detrimental nitrogen from the pond, there are three ways (Ebeling *et al.*, 2006);

1. Photoautotrophic removal by algae (photoautotrophic organisms is self-nourishing through photosynthesis).

2. Autotrophic bacterial conversion of inorganic ammonia-nitrogen to nitrate-nitrogen (autotrophic organisms is self-nourishing through oxidation of inorganic substances).
3. Heterotrophic bacterial conversion of ammonia-nitrogen directly to the Biofloc (heterotrophic organisms requires organic substances to survive, e.g. animals).

When stimulating the growth of heterotrophic bacteria it gives an advantage – the microbial flocs can be a source of feed to the aquatic culture (Burford *et al.*, 2004, 2003; Ekasari *et al.*, 2015; Emerenciano *et al.*, 2012).

Protein metabolism

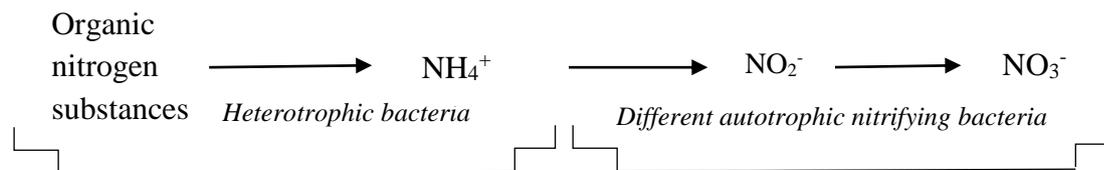


Fig. 2. (Anthe Ammonification in sufficient amounts of dissolved Nitrate. Nitrification after, bacteria oxidize ammonia to nitrite and other bacteria oxidize the nitrite to nitrate. Ammonia, nitrite and nitrate are all soluble in water.

One of the end products in protein metabolism is ammonia (Walsh & Wright, 1995; Azim *et al.*, 2008), the degradation (Figure 2) to other substrates is important for aquatic animals. The sum of NH₃ (ammonia) and NH₄⁺ (ammonium) is the total ammonium nitrogen (TAN) (Anthonisen *et al.*, 1976). In water the balance between the ionized and un-ionized ammonia depends on pH, temperature and salinity (Emerson *et al.*, 1975; Anthonisen *et al.*, 1976). In a study by Körner *et al.* (2001), both NH₃ and NH₄⁺ proved to be toxic to fish. The un-ionized ammonia proved to be more detrimental because it is lipid soluble and is uncharged which makes it easy to move across the biological membranes than the charged NH₄⁺ (Körner *et al.*, 2001). The ammonia is toxic in above 1.5 mg N/l to most cultured fish (Neori *et al.*, 2004). To the species Gilthead seabream (*Sparus aurata*) the value should not exceed higher than 1.2 mg/l TAN (0.064 mg/l NH₃-N). Theoretically the fish will not suffer any deleterious effects from the presence of ammonia up to this level (Wajsbrodt *et al.*, 1991).

Biofloc technology

The degradation process see (Figure 3) is completed under both aerobic and anaerobic conditions but it has been shown that the aerobic pathway is much quicker (Reddy & Patrick, 1975). Therefore, it is very important that the water is aerated to provide a good environment to the culture. It has been shown that lack of oxygen is the first limiting factor. Bacteria and other microorganisms generate energy from cleaving carbohydrates (cellulose, sugars and starch) to produce proteins and new cells. Organic C → CO₂ + energy + C assimilation in microbial cells (Avnimelech, 1999). During aerobic condition, degradation of organic carbon by microbes to make new bacterial cells stands for 40-60% of the total amount organic carbon (Paul & van Veen, 1978).

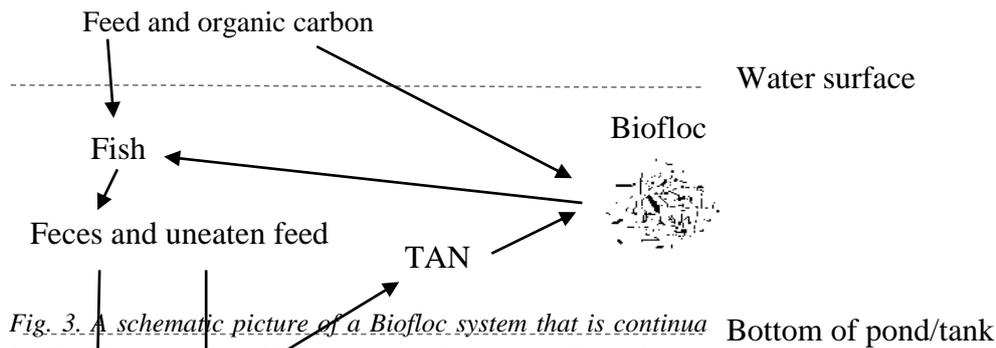


Fig. 3. A schematic picture of a Biofloc system that is continuous. Bioflocs consume the added organic carbon material together with microbial cells are formed and becomes clusters that the fish can eat. (Azim *et al.*, 2012). The nitrogen (TAN). New

In the early eighties, the BFT in aquaculture was developed and were referred to as Activated suspension technique (AST) (Serfling, 2006 in Azim *et al.*, 2007). It was based on constant aeration to allow degradation under aerobic condition to maintain high levels of bacterial floc in suspension (Avnimelech *et al.*, 1986). When suspending solid particles and have a continuous aeration to the system it supports efficient metabolic processes (Avnimelech & Mokady, 1988). The technology of using microbes instead of the traditional phyto-plankton based system has a big advantage, the sunlight is not the limitation, instead the lack of substrate or organic matter. This could make the technology to function in indoor condition (Azim *et al.*, 2008). A negative aspect of an indoor production is the higher demand for oxygen and more aeration. However, without organisms that is dependent on photosynthesis, the growth of bacteria continues during day and night. That results in less fluctuation in water quality and has a better stability over time (Hargreaves, 2006).

In a study by Azim *et al.* (2008), the composition of the Biofloc did not depend of the composition of the feed that was applied. Analysing the Biofloc revealed that it had over 50 % crude protein (CP), 2.5 % crude lipid, 4 % fibre, 7 % ash and gave 22 kJ/g energy in dry matter (DM). It was suggested by Jauncey (2000) that Tilapia diets would contain 25-30 % CP and 6-8 % crude lipid for larger fish. This combination of nutritional values, together with a satisfying amount of Biofloc should be advantageous as fishfeed to herbivorous and omnivorous fish species (Azim *et al.*, 2008). Emerenciano *et al.* (2012) found that Biofloc increased growth performance and improved the water quality in tanks with Pink shrimp (*Farfantepenaeus brasiliensis*). According to Michaud *et al.* (2006) the heterotrophic bacteria in the Biofloc may protect the fish from harmful pathogens. A trial with Brine shrimp (*Artemia franciscana*) and the pathogenic bacteria *Vibrio harveyi* showed that a live Biofloc indeed could protect the young shrimps from vibriosis and decease (Crab *et al.*, 2010). It is believed that the biofloc use the available carbohydrates which makes it hard for the opportunistic pathogens to survive. The biofloc might even engulf pathogenic microbes. Further research might show if the biofloc also can protect or improve the situation to already infected animals or animals that is exposed for an infection (Kiessling, 2016).

Carbon:Nitrogen ratio

To maintain a controlled environment in the Biofloc it is desirable to be able to remove the excessive ammonium and nitrite that risks being toxic to the fish (Colt *et al.*, 1981; Lewis & Morris, 1986) Ammonium is assimilated into microbial protein in the water when adding

some source of organic carbon which is increasing the ratio between carbon and nitrogen (C:N). It could be an answer to the inorganic nitrogen build-up (Avnimelech, 1999). In a study by Avnimelech *et al.* (1992) different ways of feeding were compared; a control with conventional pellet containing 30 % CP, a smaller proportion of conventional pellet (30 % CP) and an addition of wheat flour, and a third alternative that consisted of pellet with 20 % CP and crushed sorghum. After a few days and continuing up to 30 days, the sum of ammonium, nitrite and nitrate showed highest amounts when fed with the control than in the alternatives. The lowest value was found in the diet with the addition of wheat flour that is a source of organic carbon. Body composition of the fish were the same in the different treatments and growth rate were best in the treatment with 20 % CP and sorghum followed by treatment with 30 % CP and added flour. The fish that were fed with pellets consisting of a smaller amount of CP, could utilize microbial protein. Because of the high growth rate that were seen and the body composition that were the same compared to the other treatments. It was concluded that it is possible to grow a dense fish biomass in a closed system if a method to control the inorganic nitrogen accumulation is practiced (Avnimelech *et al.*, 1992).

When adding carbonaceous material to the pond, several things changed: (Avnimelech *et al.*, 1992)

1. The amount of inorganic nitrogen was reduced and organic nitrogen was increased.
2. Protein utilization was improved.
3. It gave significantly lower feed costs.

To maintain a high C:N ratio there are two ways. Have a low protein content in the fishfeed or have some kind of organic carbon input in the water (Crab *et al.*, 2007; Hargreaves, 2006). When having available carbon and the C:N ratio is 15 or higher, an effective removal of nitrogen is reached and protein production occurs (Diab & Avnimelech, unpublished results, in Avnimelech *et al.*, 1992). Different sources of locally produced carbohydrates has been used as input (Hargreaves, 2006). Studies have been done among others: cellulose and sorghum meal (Avnimelech & Mokady, 1988), wheat flour and crushed sorghum (Avnimelech *et al.*, 1992), glycerol (Crab *et al.*, 2010), wheat and corn meal (Burford *et al.*, 2004), sugar (glucose) and cassava meal (Avnimelech, 1999). A disadvantage when adding carbohydrates could be that it may increase the sedimentation to the bottom of the pond where the microbial biomass will not be utilised by the fish. That could result in negative organic load (Avnimelech, 1999).

The accumulation of nitrogen has traditionally been removed in intensive aquaculture but it is limited by three main factors (Avnimelech, 1999):

1. Regulations concerning environment, prevents effluents with nutrient rich water.
2. The different pathogens that could spread to external water.
3. The high costs associated with moving large amounts of water through pumping systems.

In an experiment made by Avnimelech *et al.* (1986) only 25 % of the organic carbon added was found again. The remaining 75 % should have disappeared as CO₂. This were interpreted as an indication of a high and efficient mineralization process. It was also found that the tanks

with the smallest amount of leftover organic carbon also were the ones with most aeration and the highest growth rate in the fish culture. Avnimelech *et al.* (1986) noticed that during their experiment it developed locally anaerobic sites, despite aeration. It developed a thin layer of sediment especially in the miniponds or in the tanks that were not drained. It turned out that the metabolites from the anaerobic conditions had negative effects on the culture and should be prevailed by better aeration and daily drainage.

Discussion

Aquatic animals are dependent on feeds with high digestibility (Hepher, 1988). That have resulted in a high consumption of fishmeal pellet all over the world, even to omnivorous fish species. Studies that applied BFT have got very promising results (e.g. (Avnimelech, 2007; Azim & Little, 2008; Crab *et al.*, 2010; Emerenciano *et al.*, 2012). In ponds with BFT, the culture shows better survival and better growth than control ponds fed with 30 % CP pellet. The maintenance of a stable water balance is of high priority, to prevent accumulation of toxic TAN (Anthonisen *et al.*, 1976) and keep a satisfactory level of dissolved oxygen and suspended particles (Avnimelech *et al.*, 1986). The advantage that aquatic animals have, with their ability to degrade nucleic acids from bacteria to less toxic products (Rumsey *et al.*, 1992), is something the industry should exploit.

To be able to grow other omnivorous fish species in Sweden, like tilapia, it has to be in conditions that is favourable to warm water species. When keeping a high C:N ratio the biofloc can provide stable conditions in ponds with low or no water exchange. The addition of an organic carbohydrate e.g. glycerol (Crab *et al.*, 2010) that is upgraded to protein will reduce the need of other protein sources which will lead to lower feeding costs. Though the system will need constant aeration and movement to maintain a good environment. This will need lots of surveillance and control. When the system is working there is no need for an external water treatment system (Crab *et al.*, 2007) which is an advantage to the traditional aquaculture in tanks where water treatment is needed. However, earlier reports believe that arctic charr and eurasian perch have a more promising future in swedish aquaculture because of their growth in cold water (Jordbruksverket, 2016). Arctic charr and eurasian perch are carnivorous species but studies with SCP from yeast (Langeland *et al.*, 2014; Vidakovic *et al.*, 2015) shows that there may not be necessary to use fishmeal. In the trials the diets were made from protein meal and not a living mass like the biofloc, because not all species is adjusted for that kind of system. Fish that comes from colder and clearer water have difficulties to cope with the number of particles that the biofloc contains (Kiessling, 2016). No trials with carnivorous fish species and BFT was seen in literature which can be interpreted as being unable. When feeding arctic charr and eurasian perch with different protein feeds they were fed twice a day (Langeland *et al.*, 2014) but according to Brännäs & Linnér (2000) feeding should be given more often to increase growth rate and reduce aggressive behaviour. The arctic charr can probably be used both in land-based indoor system as well as in more traditional system in lakes. Even if the BFT may not contribute to growth, it may have other positive effects, if the fish is not sensitive to turbid water. It could perhaps protect against pathogens (Crab *et al.*, 2010) or maintain water quality which makes BFT to continue to be an interesting future research field. Further, BFT may reduce the use of drugs if opportunistic

pathogens have difficulties to grow in the biofloc that rapidly consume the added carbohydrates (Kiessling, 2016). An indoor system will also be more protected against contamination that disturbs the cycles or dangers the held culture. But BFT will also need more surveillance and technology to maintain the favourable conditions that is good to the held culture and keeps the pathogens at a low growth rate. This will need expensive investments and facilities. BFT could function together with other systems like RAS and aquaponics systems (where wastewater provides nutrients to plants) that have not been mentioned in this review. These techniques take care of the wastewater and the nutrients will not become a load to the environment. Though it can be difficult to get a good balance in those systems when harvest takes place at different occasions.

To connect the aquaculture with the current situation in Sweden, where many dairy farmers is closing up, those isolated facilities could be used to grow fish. With renovation and a new inside, these old stables could perhaps come to good use. Excess sedimentation in the tanks can be used as fertilizer on cereal fields. Cereal meal could contribute to the C:N ratio when used as input in the tanks (Hargreaves, 2006). Locally produced fish is ensuring access to good food, and will provide jobs on the countryside.

Conclusion

BFT is promising as a complement feed, as it can minimize feed costs and can be used to maintain water quality when having little or no water movement. Application should be possible indoors, because of shifting outdoor temperatures and the un-stable environment that photoautotrophic algae provides during sunny weather. It would probably work with several herbivorous and omnivorous fish species. To induct modern aquaculture with BFT further research is needed to adapt with native species as well as tropical species, like tilapia. I believe that this technology together with other systems could function in Sweden to supply the inhabitants with locally produced fish that not have a negative impact on the environment.

References

- Anthonisen, A.C., Loehr, R.C., Prakasam, T.B.S., Srinath, E.G. 1976. Inhibition of nitrification and nitrous acid compounds. *Water Environment Federation*. 48, 835–852.
- Avnimelech, Y. 1999. Carbon/nitrogen ratio as a control element in aquaculture systems. *Aquaculture*. 176, 227–235.
- Avnimelech, Y. 2007. Feeding with microbial flocs by tilapia in minimal discharge bio-flocs technology ponds. *Aquaculture*. 264, 140–147.
- Avnimelech, Y., Diab, S., Kochva, M. 1992. Control and utilization of inorganic nitrogen in intensive fish culture ponds. *Aquaculture and Fish Management*. 23, 421–430.
- Avnimelech, Y., Mokady, S. 1988. *Protein biosynthesis in circulated ponds*. In: Pullin, R.S.V., Bhukaswan, T., Tonguthai, K., Ma-clean, J.L. (Eds.), *The Second International Symposium on Tilapia in Aquaculture*, ICLARM Conference Proceedings, vol. 15. pp. 301–309.
- Avnimelech, Y.; Mokady, S.; Schroeder, G. L. 1989. Circulated ponds as efficient bioreactors for single cell protein production. *Israeli Journal of Aquaculture*. 41:2, 58–66.
- Avnimelech, Y., Weber, B., Hefher, B., Milstein, a, Zorn, M., 1986. Studies in circulated fish ponds: organic matter recycling and nitrogen transformation. *Aquaculture and Fish Management*. 17, 231–242.
- Azim, M.E., Little, D.C. 2008. The biofloc technology (BFT) in indoor tanks: Water quality, biofloc composition, and growth and welfare of Nile tilapia (*Oreochromis niloticus*). *Aquaculture*. 283, 29–35.
- Azim, M.E., Little, D.C., Bron, J.E. 2008. Microbial protein production in activated suspension tanks manipulating C:N ratio in feed and the implications for fish culture. *Bioresource Technology*. 99, 3590–3599.
- Behrends, L.L., Kingsley, J.B., Bulls, J.B. 1990. Cold tolerance in maternal mouthbrooding tilapias: phenotypic variation among species and hybrids. *Aquaculture*. 85, 271–280.
- Bowen, H.S. 1987. *Composition and nutritional value of detritus*. In D.J.W. Moriarty and R.S.V Pullin (eds.) *Detritus and microbial ecology in aquaculture*. ICLARM Conference Proceedings 14. Manila, Phillipines. pp. 192–216.
- Brännäs, E., Linnér, J. 2000. Growth effects in Arctic charr reared in cold water: Feed frequency, access to bottom feeding and stocking density. *Aquaculture International*. 8, 381–389.
- Burford, M.A., Thompson, P.J., McIntosh, R.P., Bauman, R.H., Pearson, D.C. 2003. Nutrient and microbial dynamics in high-intensity, zero-exchange shrimp ponds in Belize. *Aquaculture*. 219, 393–411.
- Burford, M.A., Thompson, P.J., McIntosh, R.P., Bauman, R.H., Pearson, D.C., 2004. The contribution of flocculated material to shrimp (*Litopenaeus vannamei*) nutrition in a high-intensity, zero-exchange system. *Aquaculture*. 232, 525–537.

- Charo-Karisa, H., Rezk, M.A., Bovenhuis, H., Komen, H. 2005. *Effects of rearing conditions on low-temperature tolerance of Nile tilapia, Oreochromis niloticus, juveniles*. In: Proceedings of the 6th international symposium on tilapia in aquaculture, Manila, Philippines, 12-16 September 2004.
- Colt, J., Ludwig, R., Tchobanoglous, G. and Cech, J. 1981. The effects of nitrite on the short-term growth and survival of channel catfish, *Ictalurus punctatus*. *Aquaculture*. 24, 111-122.
- Crab, R., Avnimelech, Y., Defoirdt, T., Bossier, P., Verstraete, W. 2007. Nitrogen removal techniques in aquaculture for a sustainable production. *Aquaculture*. 270, 1–14.
- Crab, R., Defoirdt, T., Bossier, P., Verstraete, W. 2012. Biofloc technology in aquaculture: Beneficial effects and future challenges. *Aquaculture*. 356-357, 351–356.
- Crab, R., Kochva, M., Verstraete, W., Avnimelech, Y. 2009. Bio-flocs technology application in over-wintering of tilapia. *Aquacultural Engineering*. 40, 105–112.
- Crab, R., Lambert, A., Defoirdt, T., Bossier, P., Verstraete, W. 2010. The application of bioflocs technology to protect brine shrimp (*Artemia franciscana*) from pathogenic *Vibrio harveyi*. *Journal of Applied Microbiology*. 109, 1643–1649.
- Cruz, E.M. Ridha, M., 1994. Overwintering tilapia, *Oreochromis spilurus* (Günther), fingerlings using warm underground sea water. *Aquaculture and Fisheries Management*. 25, 865–871.
- Dalsgaard, J., Lund, I., Thorarinsdottir, R., Drengstig, A., Arvonen, K., Pedersen, P.B. 2013. Farming different species in RAS in Nordic countries: Current status and future perspectives. *Aquacultural Engineering*. 53, 2–13.
- Dan, N.C., Little, D.C. 2000. Overwintering performance of Nile tilapia *Oreochromis niloticus* (L.) broodfish and seed at ambient temperatures in northern Vietnam. *Aquaculture Research*. 31, 485–493.
- Ebeling, J.M., Timmons, M.B., Bisogni, J.J. 2006. Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia-nitrogen in aquaculture systems. *Aquaculture*. 257, 346–358.
- Ekasari, J., Rivandi, D.R., Firdausi, A.P., Surawidjaja, E.H., Zairin, M., Bossier, P., De Schryver, P. 2015. Biofloc technology positively affects Nile tilapia (*Oreochromis niloticus*) larvae performance. *Aquaculture*. 441, 72–77.
- Emerenciano, M., Ballester, E.L.C., Cavalli, R.O., Wasielesky, W. 2012. Biofloc technology application as a food source in a limited water exchange nursery system for pink shrimp *Farfantepenaeus brasiliensis* (Latreille, 1817). *Aquaculture Research*. 43, 447–457.
- Emerson, K., Russo, R.C., Lund, R.E., Thurston, R.V. 1975. Aqueous ammonia equilibrium calculation: effect of pH and temperature. *Journal of the Fisheries Research Board of Canada*. 32, 2379–2383.
- FAO, 2014. State of World Fisheries and Aquaculture. FAO, Rome
- Francis, G., Makkar, H.P.S., Becker, K. 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. *Aquaculture*. 199, 197-227.

- Hargreaves, J.A. 2006. Photosynthetic suspended-growth systems in aquaculture. *Aquacultural Engineering*. 34, 344–363.
- Hargreaves, J.A., Tucker, C.S. 2003. Defining loading limits of static ponds for catfish aquaculture. *Aquacultural Engineering*. 28, 47–63.
- Harris, R.H., Mitchell, R. 1973. The role of polymers in a microbial aggregation. *Annual Reviews in Microbiology*. 27:1, 27-50.
- Hepher, B. 1988. *Nutrition of pond fish*. Cambridge University Press, Cambridge, UK, 388 pp.
- Hertrampf, J.W., Piedad-Pascual, F. 2000. *Handbook on Ingredients for Aquaculture Feeds*. Kluwer Academic Publishers, Dordrecht, The Netherlands. 624 pp.
- Jauncey, K. 2000. *Nutritional requirements*. In: Beveridge, M.C.M., McAndrew, B.J. (Eds.), *Tilapias: Biology and Exploitation*. Kluwer Academic Publishers, London, UK, pp. 327–375.
- Jordbruksverket 2016-02-04. *Vattenbruk - en växande näringsgren på landsbygden*. Available: <https://www.jordbruksverket.se/amnesomraden/landsbygdsutveckling/branscherochforetagande/vattenbruk.4.e01569712f24e2ca0980008260.html> [2016-04-14]
- Kiessling, A., Askbrandt, S. 1993. Nutritive value of two bacterial strains of single-cell protein for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*. 109, 119–130.
- Kiessling, A. 2016. Department of Animal nutrition and management. Sweden : Swedish University of Agricultural Sciences. Unpublished material.
- Kungliga Skogs och Lantbruksakademien (KSLA)., 2004. *Fiskar och fiske i Sverige*. Available: <http://www.ksla.se/wp-content/uploads/2011/05/Fiskar-och-fiske-i-Sverige.pdf> [2016-05-02]
- Körner, S., Das, S.K., Veenstra, S., Vermaat, J.E. 2001. The effect of pH variation at the ammonium/ammonia equilibrium in wastewater and its toxicity to *Lemna gibba*. *Aquatic Botany*. 71, 71–78.
- Langeland, M., Vidakovic, A., Vielma, J., Lindberg, J.E., Kiessling, A., Lundh, T. 2014. Digestibility of microbial and mussel meal for Arctic charr (*Salvelinus alpinus*) and Eurasian perch (*Perca fluviatilis*). *Aquaculture nutrition*. doi: 10.1111/anu.12268
- Lewis W.M. Jr., Morris D.P. 1986. Toxicity of nitrite to fish: a review. *Transactions of the American Fisheries Society*. 115, 183-195.
- Luna, S.M. 2016-04-11. *Perca fluviatilis*. Available: <http://www.fishbase.org/Summary/speciesSummary.php?ID=358&AT=eurasian+perch> [2016-04-14]
- Michaud, L., Blancheton, J.P., Bruni, V., Piedrahita, R. 2006. Effect of particulate organic carbon on heterotrophic bacterial populations and nitrification efficiency in biological filters. *Aquacultural Engineering*. 34, 224–233.
- Moriarty, D.J.W. 1997. The role of microorganisms in aquaculture ponds. *Aquaculture*. 151, 333–349.

Neori, A., Chopin, T., Troell, M., Buschmann, A.H., Kraemer, G.P., Halling, C., Shpigel, M., Yarish, C. 2004. Integrated aquaculture: Rationale, evolution and state of the art emphasizing seaweed biofiltration in modern mariculture. *Aquaculture*. 231, 361–391.

Svenskt Vattenbruk. 2015-08-20. *Vattenbruket i Sverige*. Available: <http://www.svenskvattenbruk.se/amnesomraden/omvattenbruk/vattenbruketisverigeochvarlden/vattenbruketisverige.4.103f7b5a14cf721162be3250.html> [2016-05-02]

Reddy, K.R., Patrick, W.H. 1975. Effect of alternate aerobic and anaerobic conditions on redox potential, organic matter decomposition and nitrogen loss in a flooded soil. *Soil Biol. Biochem.* vol 7, 87–94.

Rumsey, G.L., Winfree, R.A., Hughes, S. 1992. Nutritional value of dietq nucleic acids and purine bases to rainbow trout (*Gncorhynchus mykiss*). *Aquaculture*. 108, 97-110.

Wajsbrot, N., Gasith, A., Krom, M.D., Popper, D.M. 1991. Acute Toxicity of Ammonia to Juvenile Gilthead Seabream *Sparus Aurata* under Reduced Oxygen Levels. *Aquaculture*. 92, 277-288.

Walsh, P.J., Wright, P.A. 1995. *Nitrogen Metabolism and Excretion*. CRC Press, Florida, USA. 352 pp.

Van Leeuwen, S.P.J., Van Velzen, M.J.M., Swart, C.P., Van der Veen, I., Traag, W.A., De Boer, J. 2009. Halogenated Contaminants in farmed Salmon, Trout, Tilapia, Pangasius, and Shrimp. *Environ. Sci. Technol.* 43, 4009–4015.

van Veen, J.A., Paul, E.A. 1978. *The use of tracers to determine the dynamic nature of organic matter*. Proc. 11th Int. Congr. Soil Sci. Vol.3. 1–43.

Vidakovic, A., Langeland, M., Sundh, H., Sundell, K., Olstorpe, M., Vielma, J., Kiessling, A., Lundh, T. 2015. Evaluation of growth performance and intestinal barrier function in Arctic Charr (*Salvelinus alpinus*) fed yeast (*Saccharomyces cerevisiae*), fungi (*Rhizopus oryzae*) and blue mussel (*Mytilus edulis*). *Aquaculture Nutrition*. doi: 10.1111/anu.12344