Active sludge and intelligent disease control in recirculated aquaculture systems (RAS)

Improving aquaculture's sustainability credentials

To reduce nitrogenous and phosphorous waste in recirculated aquaculture systems (RAS) new technologies are warranted. The development of novel methods to control disease will further reduce the environmental impact as well as improve the economics of fish farming. The aim of this project is to reduce the amount of nitrogenous and phosphorous waste using active sludge technology and to further reduce the occurrence of disease by developing a surveillance system using only water samples from RAS.

Farmed production of fish for consumption is on the increase worldwide and to control and reduce the environmental impact, RAS systems are becoming more popular. In these systems, a large proportion of the water is recirculated, and larger particles, proteins and nitrogenous waste must be handled. The production of fish in Denmark is regulated based on the environmental imprint of the farm and therefore there is an environmental and economic interest in reducing the emission of nutrients. In RAS, like in any other farming system for animals, there are risks of outbreaks of disease and because most of the water is recirculated in RAS, outbreaks can be severe and can affect the entire production at the time. This creates economic losses, increased nutrient waste (e.g. feed that has been wasted on fish that die) and welfare issues (fish suffer when they die from diseases). The latter are becoming more and more important for consumers.

To accommodate these problems, the project *Active sludge and intelligent disease control in Model 3 and FREA/RAS farms* funded by the Fisheries Agency under the Danish Ministry of Food, Agriculture and Fisheries focuses on the use of active sludge technologies to reduce the release of nutrients from both fresh- and saltwater fish production. Furthermore, the project seeks to develop a tool for surveillance of disease using only water samples.

Figure 1. A small-scale active sludge system. In tank 1 wastewater from RAS is added and denitrification begins. In tank 2 a carbon source such as sludge or methanol is added to increase the efficiency of denitrification. The water and sludge are then let to tank 3 where phosphorous is precipitated and aeration is added to release N_2 to the air. Finally, in tank 4, water and sludge are separated, and the sludge is harvested or returned to tank 1. The water is then discarded with much lower content of nitrogenous and phosphorous waste.

Active sludge technology reduces nutrients in waste water

Active sludge technology is not new, it has just not been implemented in full scale recirculated systems yet and that is what the partners in this project wish to do. The partners have constructed a small-scale active sludge facility and are testing this on salt- and freshwater.

Using this technology, the wastewater/discharge outlet from a drum filter within the internal water treatment system of the fish farm, is led into the active sludge treatment system, where the nitrogen and phosphorous content is reduced. Without an active sludge system, ammonia will be transformed into nitrate by nitrification in the aerobic biofilter systems, which are installed in the RAS fish farms. Within the active sludge treatment system an anaerobic environment will be maintained, which is required for the development of denitrifying bacteria. Subsequently, nitrate is transformed into free nitrogen. The denitrification process requires an organic carbon source, which ideally is provided using the sludge from the fish farm. The sludge which develops within the system, is to a large degree composed of denitrifying bacteria.

The active sludge system consists of 4 different compartments (Figure 1). The outlet from the fish farm is led to the first compartment, and then the water is led into tank 2 where an additional carbon source needs to be added to intensify the denitrification process. Then the water and sludge are led into tank 3 where phosphorous is precipitated with iron chloride and the matter is aerated to release N_2 to the air. Finally, the matter is led into tank 4 where the water is separated from the sludge (Figure 2). A proportion of the sludge is then harvested while another proportion is led back to tank 1 where the process starts all over again. The water is then discarded with much lower content of nitrogenous and phosphorous waste.

Figure 2. In this image the sludge in tank 4 can be seen. Because the active sludge was used with salt water from the RAS, the sludge is floating. Other methods to take out the sludge are needed compared to treatments for fresh water where the sludge settles at the bottom.

Intelligent disease control gives advance warning of a possible outbreak

To develop a method to detect diseases before they become a severe problem or to just establish which pathogen is causing problems at a given time, the project will develop an intelligent disease control. With the use of a relatively new technology, the partners aim to detect pathogens using environmental DNA (eDNA) that is directly extracted from the water. eDNA is a term for the DNA traces, that every organism leaves in its surrounding environment. It is relatively stable and can be detected even after the organism has died. Therefore, the aim with the intelligent disease control is that, instead of sampling fish and examining for diseases, the farmer can just sample water and get that analysed. From the water the purified eDNA is applied on a Fluidigm chip. The chip can take up to 24 samples and analyse up to 24 different pathogens in one go (Figure 3). Since making such a chip is a huge task, this project will focus on 10 pathogens, all central to rainbow trout aquaculture production.

The pathogens that the project is focusing on are: **Parasites (3)**: *Ichthyophthirius multifiliis, Tetracapsuloides bryosalmonae* (proliferative kidney disease), *Myxobolus cerebralis* (whirling disease); **Bacteria (7)**: *Aeromonas hydrophila*, *Renibacterium salmoninarum* (bacterial kidney disease), *Aeromonas salmonicida* subsp. *Salmonicida* (furunculosis), *Photobacterium damselae* subsp. *damselae*, *Yersinia ruckeri* (red mouth disease), *Flavobacterium columnare* (columnaris disease), *Flavobacterium branchiophilum* (bacterial gill disease).

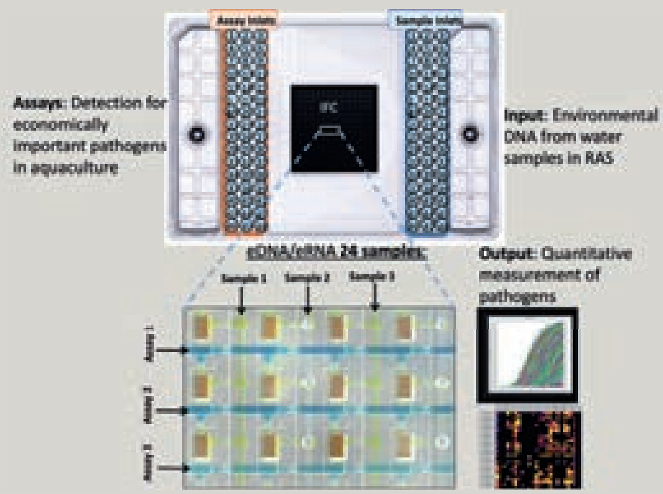

Figure 3. The Fluidigm chip (top) shown has 48x48 slots but the chip for this project will only have 24x24 slots. Input material for the chip is purified eDNA from water samples from RAS systems. Assays on the chip will be detections systems for up to 24 different pathogens. On the chip, classical real-time qPCR will be run giving quantitative data on the presence of the different pathogens.

Combining hardware and software for better prediction

Such a chip brings the vision of better disease control and less morbidity and mortality on a farm. Regular use of the chip would work as a surveillance system where the farmer has a chance to do something – like increase oxygen, add salt, stop feeding – before a disease outbreak occurs. Processing of the water sample should be rapid, and the farmer should get the data within 1-2 days ideally. When the chip has been fully developed the next step would be to write software or programmes to interpret the data and transform it into something that is easy to understand. Looking into the future, and assuming that all farmers use the chip on a regular basis, a database could be created where data would show the complex composition of organisms on the farms. These data could be used to interpret synergistic relationships and other co-occurrences that signal problems are on the way.

Aquaculture production should always aim to become more sustainable, reducing its impact on the environment and affording better welfare for the fish. With this project the consortium intends to achieve this goal with solid advances in water treatment and a front-line technological tool to detect and prevent diseases.

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Greening the fish farming sector

Title: Active sludge and intelligent disease control in Model 3 and FREA/RAS farms (*Aktivt slam og intelligent sygdomskontrol til Model 3 - FREA/RAS-opdræt*)
Funded by: Danish Fisheries Agency under the Ministry of Food, Agriculture and Fisheries
Value of support: EUR0.97m (DKK7.2m)
Period: 04.03.2021 – 31.12.2023
Partners: University of Copenhagen, Royal Danish Fish A/S, Aqua-partners ApS, Technical University of Denmark, and Eurofish International Organisation
Consultants: Aquahouse, Aquamind