

Newsletter

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1. About the BONUS CLEANAQ project

The BONUS CLEANAQ project investigates novel water treatment technologies to further reduce the environmental impact from fish farming in recirculating aquaculture systems (RAS) in the Baltic area.

The project aims at making advances within cost-efficient nitrogen removal techniques such as single-sludge denitrification, woodchip denitrification as well as in non-microbial nutrient removal methods (N & P). Focus is on treatment of fish farm effluents at the different salinities prevailing in the Baltic Sea area.

2. Work performed during the second year of the project

Nitrogen (N) can be removed from RAS effluents via denitrification reactors using internal carbon sources (fish waste) as a carbon source. The effect of salinity on the production (hydrolysis and fermentation) of internal carbon sources for denitrification purposes was evaluated using rainbow trout (*Oncorhynchus mykiss*) fish waste. All treatments produced acetic, propionic, butyric and valeric acids, which can be used for denitrification. In the 0 ppt treatment, carbon sources accumulated throughout the experimental period, while the opposite was found for the salinity treatments (15, 25 and 35 ppt). The consumption of carbon sources in saline conditions relates to hydrogen sulfide (H₂S) production. Hydrogen sulfide production is an unwanted process as it is a toxic gas and the sulfate-reducing bacteria compete against the denitrification bacteria for the carbon sources.

According to the results of the BONUS CLEANAQ project, for a maximal carbon production, maximum seven days of hydrolysis/fermentation can be applied for the 0-10 ppt systems, while batch production for maximum four days are recommended for salinities between 15 and 35 ppt to avoid H₂S production.

The capacity of different carbon sources produced from fish waste as acetate and propionate for denitrification purposes was compared against ethanol, an external carbon source commonly used for this purpose. The results showed that acetate and propionate are better carbon sources than ethanol. Acetate (vinegar) doubled the speed of the process



FIGURE 1. EXPERIMENTAL SET-UP TO INVESTIGATE THE HYDROLYSIS PROCESS AT DIFFERENT SALINITIES.

COURTESY: CARLOS OCTAVIO LETELIER GORDO

compared to ethanol and in addition, acetate is nonflammable as compared to ethanol, thus less personal safety is required for its handling and storage. The final denitrification capacity showed that a denitrifying reactor of 1 m³ can remove 4.1 kg NO₃-N/d using acetate, 2.7 kg NO₃-N/d using propionate, 1.8 kg NO₃-N/d using ethanol, 1.0 kg/d using fermented fish waste and 0.5 kg/d using fish waste without prior fermentation.

A fairly new, alternative way to remove N from RAS effluents is using woodchips as a carbon source for denitrification. The microbial processes taking place in woodchip bioreactors are, not yet, fully understood. In the BONUS CLEANAQ project, we characterized the microbiology of denitrifying woodchip bioreactors by investigating three full-scale woodchip bioreactors operated at commercial RAS in Denmark. The composition of microbial communities

of these bioreactors revealed that they all have a high abundance of denitrifying microbes and that the incoming and nitrate removing microbial communities varied between the three farms, reflecting the differences in the conditions on the RAS. Never the less the same core proteobacterial groups were found to drive denitrification across all sites. Nitrogen gas (N_2) producing denitrification was the main nitrate reduction route in the woodchip bioreactors.

To investigate autotrophic denitrification in woodchip bioreactors of brackish and fresh water, an experiment in the experimental RAS facilities in Laukaa, Finland, was performed with and without additions of bicarbonate.



FIGURE 2. EXPERIMENTAL WOODCHIP BIOREACTOR SET-UP IN LAUKAA, FINLAND. COURTESY: JANNI PULKINEN

Freshwater bioreactors had the highest nitrate removal rates. The addition of bicarbonate slightly improved nitrate removal in saltwater bioreactors, but suppressed nitrate removal in freshwater bioreactors. Bicarbonate addition changed the microbial community in freshwater reactors by decreasing the diversity and abundance of denitrifying microbes. In saltwater treatments, microbial communities were similar.

RAS farms need to remove both phosphorus and nitrogen in their discharge waters to comply with discharge regulations. In search for alternatives to conventional biological methods, the suitability of

applying abiotic methods for removing P and N was investigated.

Recirculating aquaculture systems require continuous in-line removal of total ammonium/ammonia-nitrogen (TAN) to avoid any adverse effects of ammonia on the livestock. Further removal of dissolved nitrogen (typically nitrate) often takes place as end-of-pipe (using external and/or internal carbon sources or woodchips) to reduce discharge and assure that RAS effluent adhere to environmental standards. Traditionally systems rely on biological removal in terms of two bacterial processes i) nitrification (an aerobic process where TAN is oxidized to nitrate via nitrite) coupled with concomitant denitrification (an anaerobic process where nitrate is reduced to and released as nitrogen gas (N_2) or nitrous oxide (N_2O)). These methods are not always ideal (slow and dynamic and require large foot-print) for RAS and there is an increasing interest in alternative methods of N removal among other electrolysis.

In the BONUS CLEANAQ project the abiotic, electrolytic removal of dissolved nitrogen from brackish RAS was investigated focusing on the impact of cathode material on ammonium/ammonia and nitrate removal. The results showed that TAN could effectively be removed by electrolysis whereas only negligible amounts of nitrate were removed. In reducing TAN a copper (Cu) cathode produced the highest N removal rate per m^2 electrode area: $0.438 \text{ kg N}/m^2/\text{day}$. Conversely, Sn was more cost effective reducing TAN with a power consumption of $26 \text{ KWH}/\text{kg N}$. The results indicate that the N removal rate could be increased with increased electric potential (in the range of 5-20V) and that an increase in electric potential would mean a reduction in cost effectiveness in the range of 5-20V.

Aside from removal of N also removal of phosphorus (P) from RAS effluents needs to be addressed to increase the sustainability of RAS and to comply with environmental regulations. Abiotic phosphorus removal typically consists of a coagulation phase to precipitate P with metal ions usually aluminum, and a

flocculation phase aiming at forming flocs that can concomitantly be mechanically removed.

In the BONUS CLEANAQ project, we have focused on screening organic flocculants due to their higher environmental safety compared to acrylamides, mostly used by municipal wastewater treatment plants. Phosphorus removal is regarded as a fairly standard operation, but to combine effective P removal with good N discharge control remains to be documented especially in brackish Baltic Sea conditions. We combined potato starch based P flocculation with N removal in woodchip bioreactors. Organic flocculants increased the availability of carbon in the sludge supernatant and pumping this to woodchip bioreactors improved nitrate removal in the RAS discharge. However, both P and N removal remained at only modest levels and further work on this is required to give advice for RAS farms using Baltic Sea water.

Furthermore, investigations were started regarding phosphorous removal from the production water (excluding the solid waste stream) with three reactive filter media calcium-silicate-hydrate (Sorbulite), calcium-silicate (Polonite), Polonite in combination with Vermiculite or Sorbulite with Vermiculite.



FIGURE 4. EXPERIMENTAL SET-UP INVESTIGATING REACTIVE FILTER MEDIA FOR P REMOVAL.
COURTESY: GUNNO RENMAN

Results of phosphorus removal from the first 3-week trial showed excellent removal of phosphorus by Polonite from an influent concentration of about 2.3

mgP/L to an effluent concentration below 0.01 mgP/L. Sorbulite has much slower reaction kinetics which was revealed from batch experiments using Lukaa fish farm water, and which also was visible in a packed bed reactor experiment.

3. Main results achieved during the reporting period

- Submission of a report, describing the hydrolysis & fermentation yields obtained from different fecal waste types and salinities including design guidelines for fermentation targeted to end-users.
- Submission of a report on electrolytic removal of dissolved nitrogen from brackish RAS water – Impact of cathode material on ammonium/ammonia and nitrate removal.
- Publication of a scientific article in 'Aquaculture' demonstrating how salinity affects nitrate removal and microbial composition of denitrifying woodchip bioreactors treating RAS effluents.

von Ahnen et al.: Aquaculture vol. 504, 2019, p. 182-189. Salinity affects nitrate removal and microbial composition - -"

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