

## Monitoring abrupt changes in bacteria within biological stable RAS water

**Rojas-Tirado, Paula Andrea; Pedersen, Per Bovbjerg; Vadstein, Olav; Pedersen, Lars-Flemming**

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# 4<sup>th</sup> NordicRAS Workshop on Recirculating Aquaculture Systems Aalborg, Denmark, 12-13 October 2017 Book of Abstracts



**DTU Aqua Report No. 321-2017**  
By Johanne Dalsgaard (ed.)

NordicRAS



Technical  
University of  
Denmark



# **4<sup>th</sup> NordicRAS Workshop on Recirculating Aquaculture Systems**

***Book of Abstracts***

**Aalborg, Denmark**

**12-13 October 2017**

## Colophon

### **4<sup>th</sup> NordicRAS Workshop on Recirculating Aquaculture Systems Aalborg, Denmark, 12-13 October 2017 Book of Abstracts**

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**The workshop is organized by the Nordic Network on Recirculating Aquaculture Systems (NordicRAS) in cooperation with the Technical University of Denmark (DTU Aqua). In addition, the Aquacultural Engineering Society was in charge of organizing the session on "Saltwater RAS".**

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## Welcome to the 4<sup>th</sup> NordicRAS workshop

Despite the fact that it is only 6 years ago, much has happened since the first NordicRAS workshop in Helsinki in 2011. Large RAS facilities have been built around the world and there are many more to come. Until now, the major obstacle to RAS has been economy (costs of construction and costs of operation) but as the industry grows larger it seems like the gap to cage farming, facing increasing cost of e.g. sea lice treatment, is slowly narrowing.

A recent economic analysis of land based salmon farming in Norway, to be presented at the workshop, will discuss the issue from a comparative, economic point of view, and in the session «*On-growing of different species in RAS*» farmers commercially producing different species in RAS will tell about practical experiences.

To get the best production results from RAS the fish need optimal conditions. Water quality is essential and will be addressed in the opening keynote and in the session «*Water quality in RAS*». Furthermore, gases and in particular supersaturation is more or less inevitable in intensive RAS and will be addressed in the «*Gases and online monitoring*» session.

Fish health is a ubiquitous issue in RAS and as more practical experiences are gained the insight into the interdependency between fish health, system operation and water quality is improving. The topic will be addressed in a keynote and in the session «*Particles and fish health in RAS*».

RAS in itself entails the concentration of nutrients (waste or potential resources?) that may be removed in an end-of-pipe treatment set-up. From an environmental point of view, waste treatment is thus an essential final step of the RAS concept and is addressed in the «*Waste treatment*» session.

We are pleased to announce the collaboration with the Aquacultural Engineering Society (AES) who has organized a separate session at the workshop on «*Saltwater RAS*». The aim of AES is to provide a means by which its members can come together to discuss engineering problems related to aquaculture and the 4<sup>th</sup> NordicRAS is an obvious forum.

220 persons from 24 countries representing all parts of the industry (a complete vertical integration) are gathered at the NordicRAS workshop confirming the great interest in RAS. BioMar is again the main commercial sponsor of the workshop for which we are very grateful. Similarly, we are very grateful for the commercial sponsorship from Grundfos and the financial support from AG-Fisk/Nordic Council of Ministers. All in all the stage is set for a great event - let us together make it the best of the NordicRAS workshops yet.



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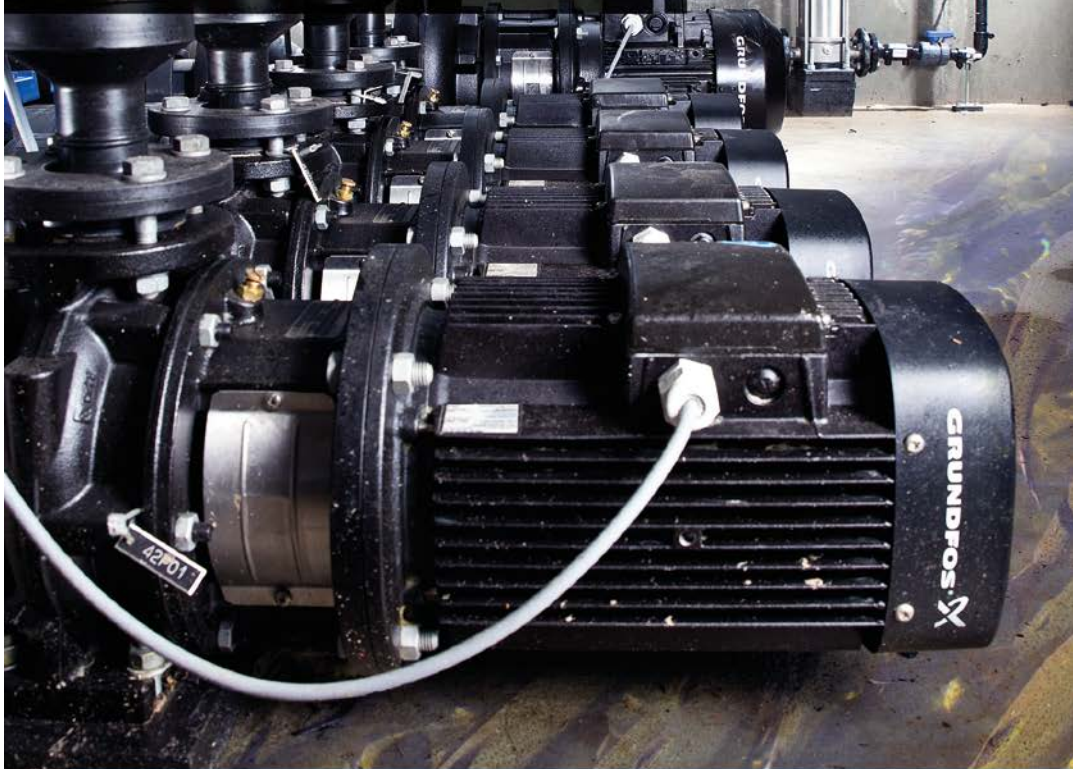
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**19<sup>00</sup>-23<sup>00</sup> Workshop dinner at Musikkens Hus**

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# **Abstracts of oral presentations**

**Presented at the  
4<sup>th</sup> NordicRAS Workshop on  
Recirculating Aquaculture Systems**

**Aalborg, Denmark**

**12-13 October 2017**

## Keynote on recirculation aquaculture systems and microbiomes

Willy Verstraete\*

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### **Abstract**

RAS technology is continuously evolving. In the conventional 'used water' treatment, the concept of using aerobic microbial fermentation for upgrading - which is central to the RAS system - is also becoming of interest. Indeed, instead of destroying nutrients one can incorporate them to microbial cellular biomass and use them for various applications. Some recent perspectives and examples of this are briefly evaluated.

Plenty of challenges remain and directly relate to a well-functioning microbiome within the RAS. The basic insights about what the former can be are presented.

Subsequently, key features of how to manage and control functional microbial associations are discussed. Some practical issues such as suspended solids and colloids and the formation of sulfides in RAS are addressed. Finally, the tools for now and for the near future to deal in a better way with microbiomes within RAS are highlighted.

Clearly, the consumer is carefully looking on how performant RASs are, not only in terms of economy but also with respect to issues such as contaminants and overall environmental wellbeing. We must deal with care with these concerns.

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## The relation between rearing environment on the development of gut microbiota in juvenile tilapia

Marc Verdegem<sup>1\*</sup>, Christos Giatsis<sup>1,3</sup>, Detmer Sipkema<sup>2</sup>, Hauke Smidt<sup>2</sup>, Hans Heilig<sup>2</sup>, Johan Verreth<sup>1</sup>

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### **Abstract**

The effect of rearing environment on water bacterial communities (BC) and the association with those present in the gut of Nile tilapia larvae (*Oreochromis niloticus*, Linnaeus) grown in either recirculating or active suspension systems was explored. 454 pyrosequencing of PCR-amplified 16S rRNA gene fragments was applied to characterize the composition of water, feed and gut bacteria communities.

Observed changes in water BC over time and differences in water BCs between systems were highly correlated with corresponding water physico-chemical properties. Differences in gut bacteria communities during larval development were correlated with differences in water communities between systems. The correlation of feed BC with those in the gut was minor compared to that between gut and water, reflected by the fact that 4 to 43 times more OTUs were shared between water and gut than between gut and feed BC.

Shared OTUs between water and gut suggest a successful transfer of microorganisms from water into the gut, and give insight about the niche and ecological adaptability of water microorganisms inside the gut. These findings suggest that steering of gut microbial communities could be possible through water microbial management derived by the design and functionality of the rearing system.

Acknowledgements: This research was funded by the European Community's Seventh Framework Program (FP7/2007-2013) under grant agreement no. 227197 Promicrobe "Microbes as positive actors for more sustainable aquaculture".

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## Microbial water quality in a commercial Atlantic salmon smolt RAS

Julia Fossberg\*

Lerøy Midt AS, Norway

### **Abstract**

Lerøy Seafood is one of the world's largest producer of sea farmed salmon and trout. Lerøy Midt's recirculation facility in Belsvik, Norway opened in 2013 and is one of the largest smolt production plants with an annual production of 14 million smolts. It has a production area of 11000 m<sup>2</sup> comprising eleven separate RAS including three hatcheries and eight grow-out sections.

The presentation includes recent experiences with water quality and smolt production at Belsvik. Traditional and new approaches to assess the microbial abundance and composition in the water will be presented, and issues regarding biofilter maintenance and performance will be discussed.

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## Monitoring abrupt changes in bacteria within biological stable RAS water

Paula Rojas-Tirado<sup>1\*</sup>, Per Bovbjerg Pedersen<sup>1</sup>, Olav Vadstein<sup>2</sup>, Lars-Flemming Pedersen<sup>1</sup>

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### **Abstract**

Of the elemental composition of bacteria (carbon, nitrogen and phosphorus), carbon is often the growth limiting compound. Several studies have shown that a great part of the bacterial population in RAS water are heterotrophic, obtaining their energy from degradation of organic carbon compounds. In previous studies, dissolved carbon concentrations ranging from 5 to 12 mg C/L in RAS water have been recorded as a result of feed loading changes (0 to 3.13 kg/m<sup>3</sup>, respectively). According to literature, a dissolved carbon concentration of 1 µg C/L is enough to promote the growth 10<sup>3</sup> – 10<sup>4</sup> cells/ml. Therefore, further attention should be paid to development of methods for monitoring bacteria dynamic and thereby evaluate the effects additional substrate availability may have on bacterial load in RAS water.

This study evaluated how an abrupt change by addition of an easily biodegradable carbon source affected bacterial activity and abundance in a stable RAS. The experiment was carried out in twelve identical and independent RAS with a feed loading of 1.6 kg/m<sup>3</sup> make-up water, operated during 4 months under steady-state conditions to achieve stable water physical-chemical and biological conditions. The sudden changes were induced by adding acetate as carbon source. Changes in bacterial activity were assessed during 72 hours using BactiQuant®, hydrogen peroxide (HP) degradation assay, and an ATP bioluminescence method. Bacterial abundance was assessed by counting micro-particles and by flow cytometry. Before start-up of the trial, six RAS had their biofilter removed from which three were spiked with acetate, and the rest were kept as control. The same setup and procedure was applied for the other six RAS where biofilters were not removed. Acetate was spiked three times (0 h, 24 h and 48 h) and its degradation within the RAS was determined.

The results showed that the applied bacterial monitoring methods were capable of detecting abrupt increments in activity and abundance of water phase bacteria 24 hours after adding the acetate, followed by a rapid decay once acetate availability became limited. It was also seen that in the systems having biofilter, the acetate was consumed primarily by bacteria attached to the biofilter media, demonstrating the well-known buffer capacity biofilters have for degradation of organic compounds. Generally, the HP-degradation assay proved to be a cheap and easy method for monitoring changes in RAS microbial water quality.

Acknowledgements: This research was funded by ERA-Net COFASP through the project “Water treatment technology for microbial stabilization in landbased aquaculture systems – MicStaTech”.

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## Efficiency of biofiltration in aquaculture plants is reflected by a stable nitrifying community

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### Abstract

Nitrification is essential for nitrogen-removal in municipal wastewater treatment and in aquaculture plants to prevent accumulation of toxic ammonia and nitrite. Oxidation of ammonia to nitrite and further to nitrate is mediated by different microbial groups: ammonia oxidizing bacteria/archaea and nitrite oxidizing bacteria. During biofiltration, these chemolithoautotrophic microorganisms settle on biocarrier elements and form dense biofilms together with heterotrophic bacteria.

With the goal to gain a deeper insight into the community structure and niche differentiation of these highly specialized microorganisms, we analyzed the diversity of nitrifying bacteria in several biofilters of different recirculation aquaculture systems by FISH (fluorescence in-situ hybridization), electron microscopy, PCR (polymerase chain reaction) and Next Generation Sequencing-techniques. The nitrifying communities vary in dependence on operational conditions e.g. the temperature, salt content, N-load as well as pH-value. In addition, laboratory-based activity tests were performed to investigate the nitrifying potential for both, ammonia and nitrite oxidizers. The nitrifying capacity of a biofilter is restricted to special features of the inhabiting organisms and we isolated several strains of the key nitrite oxidizer *Nitrospira* from different recirculating aquaculture systems. Physiological stress tests were performed to uncover possible inhibition of nitrite oxidation in dependence on technical settings such as start-phases, disinfection events, accumulation of nitrate or chemical influences by fresh plastic material.

Maintaining a good water quality is most challenging for the rearing of fish larvae and the green-water technology is often used to stabilize the microbial community to prevent proliferation of opportunistic pathogens. Our strategy was to identify putative probiotic bacteria in microalgae communities on the one hand and to identify accompanying microorganisms in nitrifying enrichment cultures on the other hand. It is well known that pure cultures of nitrifying bacteria are much more sensitive compared to natural communities. In order to identify positive influences of heterotrophic bacteria on ammonia or nitrite oxidation, we performed co-culture-experiments with different *Nitrospira* isolates and heterotrophic bacteria, derived from the microalgae-phycosphere or from nitrifying enrichments. This approach revealed that the nitrifying capacity might be enhanced when supplemented with suitable heterotrophic bacteria.

Our results showed that a combined approach including nitrifying and heterotrophic bacteria is important to investigate recirculating aquaculture systems especially with the goal of stable larvae production.

Acknowledgements: This research was funded by the Deutsche Bundesstiftung Umwelt (DBU), State Research Centre of Agriculture and Fisheries Mecklenburg-Vorpommern (LFA), and the Bundesministerium für Bildung und Forschung (BMBF).

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## The effect of different cumulative feed burdens on performance of pikeperch (*Sander lucioperca*) and on water quality in RAS

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### Abstract

Reduced water exchange rates due to improved technology are one of the main advantages of recirculating aquaculture systems (RAS) but at some point fish performance is limited by nitrate accumulation and built up of dissolved and particulate matter. The cumulative feed burden (CFB) is used to describe the relation between feed applied and make-up water volume in a closed system.

Pikeperch are ammonotelic and excrete most of their nitrogenous waste as ammonia thus low water exchange rates result in high nitrate levels. The role of different classes of organic matter and the interactions between nitrogenous and carbon compounds on fish performance are hitherto not fully understood. This research focused on the changes in water quality (NH<sub>3</sub>-N, NO<sub>2</sub>-N, NO<sub>3</sub>-N, CO<sub>2</sub>, TSS, BSB5, pH, TOC) due to three different levels of CFB and the effects on fish growth, feed intake and fish health in a controlled intense RAS production setup. Water exchange rates were 140 L kg feed<sup>-1</sup> (high CFB), 500 L kg feed<sup>-1</sup> (medium CFB) and 1000 L kg feed<sup>-1</sup> (low CFB).

Nitrate nitrogen levels reached a steady state in all three CFB treatment tanks after five weeks at 36.9 ± 1.3, 69.3 ± 2.2 and 163.1 ± 4.2 mg L<sup>-1</sup>, respectively. Nitrite nitrogen levels were below 0.20 mg L<sup>-1</sup> during the course of the experiment in all treatments but peaked in week six (low and medium CFB) and seven (high CFB) at 0.66 ± 0.09, 1.05 ± 0.07 and 3.19 ± 0.85 mg L<sup>-1</sup>, respectively. CFB had a significant impact on the amount of total organic carbon (TOC) in the water with 20.43 ± 3.42 mg L<sup>-1</sup> in the low CFB treatment and 32.52 ± 1.84 and 47.03 mg L<sup>-1</sup> in the medium and high CFB treatment, respectively while there were only slight differences in inorganic carbon.

The average SGR was 0.82 ± 0.05 % and FCR was 1.08 ± 0.02 in all groups without significant differences. CFB had a direct impact on protein productive value (PPV) with significant differences between the low CFB treatment at 30.34 ± 0.13 % PPV and the high CFB treatment at 28.25 ± 0.43 % PPV. PPV for the medium CFB treatment was 28.80 ± 1.72 %.

The study indicates that different CFB levels have a complex effect on water quality parameters and directly impact pikeperch in terms of energy metabolism.

Acknowledgement: This research was funded by the Federal Ministry of Education and Research.

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## Experiences and future perspectives of RAS in Chile

Hugo Cajas Duran

Salmones Camanchaca S.A, Chile

### **Abstract**

The Chilean seafood company Camanchaca is one of Chile's largest salmon farming groups but has diverse activities in fisheries and processing as well. The group has more than 70 salmon farming licenses and a complete vertically integrated operation. They have their own broodstock program, hatchery and smolt operations in recirculation e.g. the Petrohue facility.

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## Experiences with Atlantic salmon grow-out in RAS

Arndt von Danwitz\* and Kim Hieronymus Nielsen

Danish Salmon A/S, Hirtshals, Denmark

### **Abstract**

Danish Salmon started in 2012 and is a pioneer and so far among the world's largest, land-based RAS for growing market-size Atlantic salmon all the way from egg to slaughter at 3-5 kg. The annual production is approximately 1000 ton/year and is based on a high degree of recirculation (~ 500 l/kg feed applied).

The presentation will focus on the experiences and knowledge gained so far on farming large salmon in seawater RAS.

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## Experiences with commercial cleaner fish production in RAS

Dan Kristian Larssen\*

Atlantic Lumpus AS, Sleneset, Norway

### **Abstract**

Atlantic Lumpus AS started in 2016 and produces cleaner fish (*Cyclopterus Lumpus*) that are applied as a biological treatment alternative to therapeutic agents for de-lousing Atlantic salmon in net cages. Up to 60% of all Atlantic salmon producers in Norway use cleaner fish in their production.

Cleaner fish is a new aquaculture species and the presentation will focus on experiences and challenges with rearing this species in RAS including water chemistry requirements and other requirements specific for this species and for the production.

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## Experiences with rainbow trout production in FREA

Egon Folmer\*

FREA A/S, Kibæk, Denmark

### **Abstract**

FREA, situated near Kibæk in Denmark, produces rainbow trout fingerlings/juveniles (10 million/year) as well as portion sized fish in large RAS. The presentation will focus on the production as well as experiences and challenges with producing rainbow trout in fully recirculated aquaculture systems (FREA).

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## A closed circuit system for rearing of white fish

Aki Honkanen\*

Sybimar Ltd, Uusikaupunki, Finland

### **Abstract**

Sybimar Ltd specializes in aquaculture and the use of side streams. Applying a closed circulation concept, the company aims at combining food and energy production by recycling nutrients, water, heat and CO<sub>2</sub> back to production.

The company was founded in 2005 applying fish guts from the fish processing industry to produce biodiesel. This led to the development of modular concept, combining RAS with greenhouse and biogas based energy production. The company started farming fish in RAS in 2012 with an annual capacity of 250.000 kg white fish (*Coregonus lavaretus*).

The presentation will focus on the modular concept including pros and cons of the closed circuit system, and differences of rearing white fish compared to salmon/trout will be discussed.

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## Challenges in ongrowing vs fingerling production

Tahi Fu and Martin Vestergaard\*

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### **Abstract**

Within the last 10 years, Aquapri has made a substantial investment into developing Pike perch into a commercially farmed aquaculture species, and is now the biggest commercial producer of Pike Perch in Europe. Our new Pike Perch facility has led us to understand the challenges of RAS systems for grow-out in comparison to RAS systems which focus solely on fingerling production.

Ongrowing RAS systems experience the same challenges as with hatcheries, with the additional challenge of providing the level of product quality important to the final customer. This includes navigating diverse markets / end users, and cost and sales prices contra growth rate of the fish.

Scaling up RAS systems also results in the upscaling of daily routines such as cleaning tanks, removing mortalities and grading. Upscaling calls for unique and innovative approaches to tackling these resource demanding problems. Low manpower requirement and efficiency are key drivers of finding the most economically optimal solution. This requirement places a responsibility to those involved in system design to incorporate technical solutions for low maintenance or accomplishing tasks passively, reducing the need for extra staff.

The removal of off-flavors from the final product is a challenge that is specifically important to the grow-out phase, especially in a production of any high value species. Purging is the most common approach but is costly in terms of water expenditure and weight loss of the fish. Furthermore purging can have its own complications if there are pre-existing conditions with the fish or issues with water quality from the incoming water source. Finding the correct management solution is often difficult as it requires a balance between ensuring quality and maximizing production.

The economy of fingerling production is usually tied to a company's own fingerling requirements or through well-established contracts with external producers, while fish for consumption is traded in a free market with tough competition adding extra costs for sales and fluctuating prices.

One of our challenges with pike perch is competing with wild fisheries in a seasonal but sporadically unstable market. The challenge lies with remaining competitive against low cost production systems whilst also managing farm biomass respectively; to prevent sales at a loss but to also have the ability to take advantage of periods of insatiable demand. It is therefore important to continually strive for lower production costs (with no compromises in quality) and to find untapped markets.

These are but a few of the challenges that many commercial scale RAS operations will find themselves experiencing. Tackling these issues will require a substantial (and committed) economic investment, collaboration of creative minds, and time for further development of RAS operations such as our Pike perch production facility, with the hope of the economic fruition of commercial scale on growing RAS facilities – if we don't run out of money before we can make our first profit.

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## Kingfish in land based RAS

Claus Rom\*

Sashimi Royal A/S, Hanstholm, Denmark

### **Abstract**

Sashimi Royal has recently established a RAS production facility near the port of Hanstholm, Denmark, on a 30,000 m<sup>2</sup> plot. In collaboration with the associated company Maximus A/S, producing fry/juvenile kingfish, the facility in Hanstholm aims at producing 1200 ton/year of yellowtail kingfish in one RAS unit.

The production in the grow-out unit has just started and the presentation will be on experiences gained so far with the species and with the production of yellowtail kingfish in recirculation.

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## Experiences with Atlantic salmon grow-out in RAS

Jon-Birger Løvik\*

Atlantic Sapphire, Hvide Sande, Denmark

### **Abstract**

Langsand Laks started in 2011 and is a pioneer in the farming of market-size Atlantic salmon in land-based RAS. The facility in Hvide Sande, Denmark is currently expanding to reach an annual production of 2000 ton/year.

Langsand Laks is a subsidiary company of Atlantic Sapphire A/S that has just started the construction of a large RAS facility in Florida, USA. The facility is intended to initially produce 9000 ton Atlantic salmon/year. Later, a production of 90,000 ton/year is foreseen.

The presentation will be on experiences with Atlantic salmon grow-out in RAS and on future plans for production.

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## **The experimental aquaculture facility – showcasing southern hemisphere Atlantic salmon research using RAS**

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<sup>1</sup>Institute for Marine and Antarctic Studies, University of Tasmania, Hobart, Tasmania, Australia

### **Abstract**

Atlantic salmon farming in Tasmania has undergone significant expansion from 53 to almost 55,000 tonnes annual production in the last two decades. The industry injects \$626.9 million to the Tasmanian Gross State Product and is the leading farming activity in Tasmania.

The initial introduction of Atlantic salmon to Tasmania from the northern hemisphere meant that salmon were grown in some of the warmest waters in the world for Atlantic salmon. The industry now faces the climate change challenge to adapt farming practices to cope with higher summertime water temperatures.

Along with industry, the Institute for Marine and Antarctic Studies (IMAS), University of Tasmania, recognised the urgent need for a purpose built research facility for large Atlantic salmon to provide practical solutions to manage environmental change and sustainability of production. The A\$6.5 million Experimental Aquaculture Facility (EAF) was officially opened in 2015 using combined federal, state, industry and University funds. The University and the two industry partners, Skretting Australia and Huon Aquaculture, jointly manage the research and cover operational costs equally.

We believe the EAF is the only saltwater RAS facility in the Southern Hemisphere capable of conducting research on large salmon (up to 5 kg). The facility comprises 14 saltwater RAS: 12 x 3200L individual RAS; 1 x 72,000L RAS (2 x 13,000L fish tanks); 1 x 178,000L RAS (12 x 7,000L fish tanks each with separate temperature control). EAF commissioning provided important empirical evidence of refinements necessary for Saltwater RAS. Primarily the elimination of nitrogen supersaturation as a result of air injection at a depth >1 m and the need for carbon dioxide removal to reduce chemical addition for pH control and the subsequent deleterious effects to fish because of high alkalinity.

Each RAS system is equipped with sophisticated control including, gas control (oxygen, nitrogen and carbon dioxide), light control (photoperiod, intensity, fade up and fade down), parameter control (temperature, pH), feed control (delivery and recovery) and electronic system control (logging, remote control, alarm control, in tank live video feed). The high level of system control means the facility has the capacity to: mimic environmental variables that salmon would experience on farms, examine single or multivariate interactions on salmon growth and survival, develop Atlantic salmon RAS technology for optimal growth performance and model effluent outputs. The EAF has already achieved benefits with research outputs immediately integrated into commercial application.

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## Removal of off-flavor compounds based on combined adsorption and biodegradation in recirculating aquaculture system

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### **Abstract**

The microbial secondary metabolites geosmin and 2-methylisoborneol (MIB), two muddy-earthly taste and odor compounds, are most commonly associated with off-flavor in fish. To date, methods aimed at reducing geosmin and MIB levels in RAS lack efficiency and robustness. For instance, purging, the most widely used abatement method, requires vast amounts of fresh water and often results in weight loss and quality deterioration of the pre-marketed fish.

In earlier studies conducted on a zero-discharge RAS, it was found that geosmin and MIB were reduced in the digestion basin of the system. Here we report on the mechanisms underlying this removal. It was found that MIB, the more recalcitrant of the two compounds, was removed by a combination of adsorption and biodegradation within the sludge. Based on Langmuir kinetics, a model for MIB removal was developed. As experimentally verified, the model enabled an accurate prediction of MIB removal by the sludge over a wide range of experimental conditions. Long-term operation of reactors filled with sludge and fed with a MIB-rich influent showed that under steady-state conditions, biodegradation accounted for >99% of the overall MIB removal process.

Two treatment compartments of an on-campus recirculating aquaculture system were examined in order to study which members of the microbial community are actually affected by – and thus potentially remove – geosmin and MIB. Sludge derived from the digestion basin and biofilms obtained from the trickling of the system were incubated under laboratory conditions and enriched with geosmin and MIB during 14 days. By means of next-generation sequencing, it was found that geosmin and MIB in RAS treatment units elicited selective changes in the microbial community structure. These changes were shown to take place in both the aerobic TF and the anaerobic DB sludge, previously demonstrated to harbor, respectively, producers and degraders of geosmin and MIB. Overall, the results presented in this study emphasize the potential for bacterial geosmin and MIB removal in recirculating aquaculture facilities.

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## **First experiences from full-scale denitrifying woodchip bioreactors operated end-of-pipe at commercial RAS**

Mathis von Ahnen\*, Per Bovbjerg Pedersen, Johanne Dalsgaard

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### **Abstract**

Sustainable development of aquaculture requires that the technologies for reducing the environmental impact of fish farming systems are further improved. The aquaculture industry currently lacks simple but effective methods for removing nitrate from RAS effluents. A potential alternative solution for removing nitrate is denitrifying bioreactors, which are typically trenches filled with woodchips that serve as a solid carbon source for denitrifying bacteria. These bioreactors are nowadays a well-established method for treatment of nitrate-laden drainage water but could potentially also be applied to treat RAS effluents.

After woodchip bioreactors have been proven to be successful at removing nitrate from RAS effluents in laboratory as well as pilot-scale set-ups, a few trout farmers in Denmark have constructed full-scale woodchip bioreactors at their recirculating farms during the beginning of this year. Different reactor designs have been realized to tackle issues related to hydraulic conductivity and clogging.

Regular monitoring of these full-scale reactors confirmed that nitrogen removal functions well also under commercial conditions. Some considerations need to be taken into account before constructing these bioreactors to avoid environmental problems during reactor start-up and to facilitate a maintenance free bioreactor operation over a longer period. The first experiences of these full-scale bioreactors in regards to treatment performance, reactor design and hydraulics and further observations are presented.

Acknowledgements: This research was funded by the Ministry of Environment and Food of Denmark and by the European Union through the European Fisheries Fund (EFF).

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## **RAS waste treatment: challenges and opportunities within the circular economy paradigm**

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<sup>2</sup>Faculty of Biosciences, Fisheries and Economics, The Arctic University of Norway (UiT), Tromsø, Norway

### **Abstract**

A circular economy appreciates that global resources are finite, and aims to better control and manage material stocks by (re)circulation of materials and products. The European Commission has adopted circular economy as a concept to develop stronger and more resource efficient industry. Following principles ascribed to the circular economy; i.e., to manage natural material stocks for optimized resource yield and system effectiveness, waste treatment should enable the realization of material and energy resource potential of residuals at minimal impact.

With recycled aquaculture systems (RAS), formerly highly diluted effluents from aquaculture become available for recycling of nutrients, biomass or energy. Even if they often are treated by stabilization, or used to low-utility purposes, the sludge wastes contain significant amounts of bio-based residuals and critical nutrients. In this paper, we discuss sludge waste treatment for closed aquaculture concepts within the paradigm of circular economy. We extract best practices considering resource management, utilization yield, and the avoidance of negative impacts, based on a survey of the life cycle assessment literature for RAS.

The conclusions are relevant for the development of the aquaculture sector in light of general growth in the aquaculture sector and in markets for RAS solutions. Increased resource utilization may add to the commercial robustness as well as sustainability of the aquaculture sector.

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## Performance of a marine activated sludge system for N removal using external and internal carbon sources

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### **Abstract**

More land based salt water recirculating aquaculture systems (RAS) are being constructed with increasing production capacities between 1,000 up to 90,000 tons of fish per year. This new scenario requires the development and evaluation of in situ technologies to treat saline effluents for the removal of nitrogen (N), phosphorus (P) and organic matter at the end-of pipe treatment phase. In the case of N, a series of media-laden reactors working in brackish or marine water have been reported in aquaculture, but scarce information exists on the application of activated sludge systems (ASS) in marine aquaculture. Contrary to media-laden reactors, activated sludge systems maintain suspended bacteria in the reactor, having a potential advantage over media-laden systems for less maintenance, the avoidance of filter channeling, clogging and posterior failure with eventual release of unwanted H<sub>2</sub>S. The purpose of the current study was to assess the performance of an ASS by treating the effluent of a 26 ppt marine trout RAS using acetate as external carbon source, and posteriorly fermented fish organic matter as internal carbon source.

An ASS was connected to the effluent of a rainbow trout (*Oncorhynchus mykiss*) RAS with a rearing intensity of 0.56 m<sup>3</sup>/Kg feed, a constant nitrate discharge concentration of 50 mg NO<sub>3</sub>-N and operated at a salinity of 26 ppt and a temperature of 16°C. The ASS was comprised of 3 independent 50 L reactors with individual 30 L settlers. Each reactor was fed from two sources: the overflow effluent from the RAS and a solid waste tank, supplying respectively N and carbon sources (C). The trial was divided in two phases; phase I where acetate was used as external carbon source in three different levels (40, 30 and 20% of the total amount of TCOD discharged from the system) and phase II where the organic matter produced by the RAS was used as internal carbon source previously fermented at different days.

The performance of the ASS showed that if 40% of the total amount of TCOD discharged from the system was recovered and converted to acetate, 16.29 g NO<sub>3</sub>-N/d or 73% of the total NO<sub>3</sub>-N discharged from the system could be removed per day, giving an overall volumetric removal of 326 g NO<sub>3</sub>-N/m<sup>3</sup> reactor. As the second and third levels were applied the removal capacity of NO<sub>3</sub>-N in the system was proportionally reduced. With the results from Phase I a baseline was established to further compare the system performance against Phase II. The results from phase II shows; a) how much N can be removed when different days of pre-fermentation are applied b) how using internal carbon sources reduces the cost of buying external carbon sources. If an ASS is used to treat the effluent of a 1000 ton salmon production per year with a constant discharge of 400 Kg NO<sub>3</sub>-N/d, a smaller volumetric footprint, less investment and operational costs are obtained if compared to media-laden systems. Thus the application of an ASS to marine aquaculture end-of-pipe treatment appears as a strong candidate for marine end-of-pipe treatment.

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## Development and test of an automated control system for denitrification reactors

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### **Abstract**

Nitrification is well known and easy to handle, unlike denitrification where several parameters, nitrate, ORP, pH, the flow and carbon, have to be monitored and controlled. Manual adjustments can have a large effect on the denitrification filtration, thus precise control and monitoring is needed.

Goal of the presented study was to develop an innovative and novel fully automated monitoring and control system for denitrification in recirculating aquaculture systems. To measure nitrate an optical sensor was used offering a direct control of nitrate in the filter and total system. In several tests conducted on a denitrification filter connected to a commercial like RAS, the interactions between controlling processes (regulation of water inflow and addition of carbon source) and effects on water parameters ( ammonium, nitrite, nitrate, pH and ORP) were tested. By this iterative development process parameter were adjusted to achieve flexible and robust control logics which were tested under different stress scenarios (fluctuating water condition, filter start phase, strong changes of target nitrate concentration). Finally, control logics were implemented in a novel measurement and control unit that was connected to a denitrification filter in a RAS.

Results demonstrated, that the developed control logics are suitable to run a denitrification filter and to achieve constant and favoured nitrate concentrations in the RAS. The logic controlled denitrification filter reacted flexible to different stress tests and was controllable at any time. First tests with the new measurement and control unit demonstrate that it is suitable for an autonomous operation of a denitrification filter. Additionally the system enables long term test under constant nitrate levels.

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## Replacement of methanol by biodegradable polyhydroxyalkanoate (PHA) plastics in a new biological denitrification-reactor for an efficient and safe use in recirculating aquaculture systems

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### Abstract

An efficient and safe nitrate elimination by biological denitrification is still one of the most promising but challenging alternatives in RAS development. Existing conventional denitrification processes are often accompanied by further challenges, such as the accurate dosage of methanol, which is necessary as carbon source, but a potentially hazardous substance.

In the present study, the new Self-cleaning Inherent gas Denitrification reactor (SID-reactor) was optimized for an efficient and safe use in RAS. Aim of this study was to evaluate the possible application of biodegradable plastic granulate made of Polyhydroxyalkanoate (PHA) as biocarrier and as internal carbon source to replace methanol.

An experiment with three RAS (1m<sup>3</sup> each) was conducted. Each RAS was stocked with pike perch (*Sander lucioperca*) at a density of 30 kg/m<sup>3</sup>. The first RAS (RAS-C) was operated as a control without a denitrification system. The second RAS (RAS-M) was operated with a SID-reactor fueled with methanol as external carbon source, and the third RAS (RAS-B) was operated with a SID-reactor filled with biodegradable plastic granulate functioning as biocarrier and internal carbon source at the same time. During the course of the experiment (110 days) several hydro-chemical parameters were determined daily for all three RAS.

The results reveal that RAS-C accumulates nitrate during the experimental period as expected. In both RAS-B and RAS-M the nitrate values decreased, whereby the SID-reactor with biodegradable plastics showed slightly lower denitrification rates compared to the methanol fueled SID-reactor. Initial nitrate values in all three RAS were 140 mg NO<sub>3</sub><sup>-</sup>-N/L. Nitrate in RAS-C continuously increased up to 500 mg NO<sub>3</sub><sup>-</sup>-N/L without reaching a plateau phase. In contrast, the holding water of RAS-B reached a plateau phase at 160 mg NO<sub>3</sub><sup>-</sup>-N/L at the end of the trial, while RAS-M reached the plateau phase at 50 mg NO<sub>3</sub><sup>-</sup>-N/L in the holding water.

Alkalinity and pH indicated that there was a high demand of 2.6 kg NaHCO<sub>3</sub> per kg feed for the control RAS to guarantee a stable pH of 7.5. In contrast, there was only a need for 0.8 and 0.9 kg NaHCO<sub>3</sub> per kg feed for the methanol fueled RAS and the RAS with the biodegradable plastics, respectively.

Even though the denitrification rate of the methanol fueled SID-reactor is higher compared to the SID-reactor filled with biodegradable plastics, the latter shows advantages concerning its maintenance effort and stability, reduced risks by usage and dosage of the respective carbon source.

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## Denitrification in saltwater recirculating aquaculture systems (RAS) using an up-flow sludge bed reactor (USB)

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### **Abstract**

Adequate solutions to eliminate nitrate from marine aquaculture effluents are required as more marine land-based recirculating systems are being implemented. Biological denitrification is a suitable process in which, heterotrophic bacteria (under anoxic conditions), use a carbon source as electron donor converting  $\text{NO}_3^-$  into nitrogen gas, being the latter released to the atmosphere. In order to perform such a process, several types of reactors exist, being the up-flow sludge blanket (USB) of special interest. In USB reactors as compared with other reactors, larger amounts of bacterial biomass can be concentrated in less volume due to the formation of granular sludge, spherical aggregates with high density and excellent settling properties. This characteristic allows a higher denitrification rate per reactor volume reducing space requirements.

The purpose of the current study was to evaluate the performance of an USB as a candidate for denitrification in seawater RAS effluents. Three 10L reactors (replicates) were used for; a) evaluating the formation of granules using waste from rainbow trout saltwater RAS as inoculum b) evaluate denitrification loading rate under different up-flow velocities. The reactors were fed with 250 mg/L of  $\text{NO}_3^-$ -N and sodium acetate (1500 mg/L of chemical oxygen demand (COD)) as a carbon source, representing a C:N ratio of 6.

The obtained granules showed a variable diameter reaching a maximum value of 9.6 mm at 60 days and a minimum sludge volume index (SVI) of 5.4 mL/g. A maximum denitrification rate of 322 mg  $\text{NO}_3^-$ -N g/VSS per day at the third tested up-flow velocity (0.97 m/h) was achieved. After this point, biomass progressively started to fluidize until the seventh tested velocity, moment in which no granules were visible and bacterial flocs predominated.

The results indicate the feasibility for developing granular sludge under marine conditions with a potential denitrification capacity of 14.9 Kg  $\text{NO}_3^-$ -N/m<sup>3</sup> per granule per day.

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## Economic analyses of land based farming of salmon

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### **Abstract**

Salmon farming has been one of the fastest growing industries in Norway for more than three decades. Nevertheless, in recent years the environmental sustainability of the industry has been questioned. This is mainly due to two reasons. First, the incidence of sea lice has increased tremendously, causing diseases and very high treatment costs. Second, escaped salmon mix with wild salmon with undesirable genetic effects. This situation has led to increased interest for development of new technology and new ways of growth for a promising industry.

The study analyses land based farming of salmon – an emerging technology which also represents a way for the industry to grow without requiring new sea based licenses. The aim of the analysis is to investigate the economics of land based farming of salmon and to compare the competitiveness of land based production to the conventional, sea based production model, focusing on cost of production. Using local and national data, the study is designed to compare farm-gate cost of production in a Norwegian context.

The paper considers a farm with annual steady state production of 5,000 tonnes salmon. The results indicate that cost of production in full cycle land based salmon farms are higher than in sea based farms. However, increased flexibility with regard to location closer to markets for land based farms, with corresponding logistical and value chain advantages, could present new opportunities for salmon production globally.

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## Keynote on fish diseases and a health management focus in RAS

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### **Abstract**

Recirculating aquaculture systems of considerable size and scale are now becoming commonplace features with the production of freshwater and marine species with growing levels of intensification.

The demand for system performance and production imposes significant demands on water treatment. The quality of the water supply and maintenance of system water quality is paramount. New and emerging water quality-related issues such as H<sub>2</sub>S, metal and other contaminant toxicity are imposing challenges beyond the conventions of nitrification and pH management. Moreover, as systems become more intensive, growing larger fish, even to market size in fresh, brackish and marine systems, disease and pathogens are emerging and re-emerging.

Management of diseases such as Yersiniosis, skin disorders, and systemic bacterial infections in recirculation systems become challenging when one considers the components and dynamics of the system. The biofilter and other system components may harbour potential pathogens, providing a source for re-infection under less favourable conditions.

The use of improved disinfection technology as well as prophylactic and treatment medication along with new approaches to health management offer hope for continued and sustained growth and use of RAS into the future.

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## Biostability in RAS - How do we measure it?

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### **Abstract**

In response to an increase in the use of recirculation technology in Aquaculture, it has been acknowledged that microbiological factors have a strong influence over the success or failure of fish production in RAS. In 2006, Aquapri embarked on a development project with the goal of establishing Pikeperch production with RAS technology. Four years later, we acquired equipment that allowed us to measure bacterial activity in our water on site (Bactiquant®). This was the beginning of our realization that over physical and chemical water quality parameters, the microbiology in the system also needed to be understood and managed.

In recent years, we have tested a variety of different quantitative methods to monitor bacterial activity of the water in our pikeperch facilities. Both the advantages and the disadvantages of these methods will be discussed.

Working with the quantitative monitoring of bacteria has contributed to our insight into the management of the water treatment processes of RAS, e.g. effective use of UV treatment, the extreme effects of food waste, how to operate and maintain optimal biofiltration (nitrification and denitrification) etc.

There has been a rapid development in the methods of monitoring microbiology, including their development into commercially available products. In the coming years, there will be a shift from quantitative to more qualitative analyses. Recently more focus has been placed on microbiological stability as a method of addressing problems, especially health issues, in RAS: It is clear that many problems in RAS (and aquaculture in general) arises from a distortion of equilibrium, e.g. outbreak of diseases by specific pathogenic microorganisms or a propagation of opportunistic microorganisms, resulting in a general negative impact of the system and the fish. Theoretically, it makes sense that increased microbial diversity, and support of "positive" bacteria, should be encouraged in order to minimize system fluctuations. This hypothesis is supported in various studies focusing on specific microbial parameters, and is also reflected in our practical experience. However, our experience has been that this is not a "wonder drug", and one should still anticipate potential problems, mainly because no RAS system is run to be microbiologically stable. Furthermore, a RAS system includes only the minor most essential parts of a natural ecosystem (enough to be able to support a production facility) thereby lacking a whole array of buffers found naturally.

It is also our experience that system stability cannot solve situations where there is tank instability. Therefore, it is crucial to monitor fish behaviour and health on a tank level and take the necessary precautions/treatment, even though this by definition will interfere with the ultimate goal of supporting a stable, self-adjusting microbiological environment. The use of chemicals as water disinfectants e.g. chlorine based products, or ozone and physical installations, e.g. UV, should be seen as tools to be able to return to equilibrium, and should be thought of as a toolbox of value, to be used in a consequent manner.

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## Micro particles in Danish Model Trout Farms

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### **Abstract**

Increasing the intensity of recirculation in recirculating aquaculture systems (RAS) has been shown to cause a buildup of micro particles. Over the past 20 years, there has been a gradual shift in the Danish rainbow trout (*Oncorhynchus mykiss*) aquaculture industry from traditional flow through systems to more recirculating Model Trout Farms (MFTs). While multiple studies have been conducted on the chemical water quality in these systems, very little attention has been given to the buildup of micro particles.

The objective of this study was to establish the baseline distribution of micro particles in Danish Model Trout Farms and their potential implications on water quality. Furthermore, the objective was to assess the implication of various internal components in the recirculation loop on the distribution of micro particles.

A screening was carried out at seven different trout farms during spring 2017 including a total of 20 RAS units. Grab samples were obtained before and after different components (drum filters, biofilters, airlifts and production tanks) and analyzed for the number and size distribution of particles between 1 and 200  $\mu\text{m}$ . In addition, multiple water quality parameters were measured including the microbial water quality in form of  $\text{H}_2\text{O}_2$  degradation, biological oxygen demand ( $\text{BOD}_5$ ), chemical oxygen demand (COD), and turbidity.

Particle loads varied significantly from farm to farm but interestingly, they also varied significantly between RAS units within the same farm. Hence, variations of more than 3 times in particle numbers, volume and surface area were observed between different RAS units within the same farm despite very similar operation conditions and theoretical feed loadings.

An overall correlation of 85% was found between the surface area of micro particles and the microbial water quality, sustaining that the amount of surface area provided by smaller particles has a large impact on the load of bacteria in these systems. Intriguingly, the surface area provided by particles between 1 and 200  $\mu\text{m}$  was in several systems larger than that provided by the biofilter.

While the full implications of these results are still being assessed, the large variation between systems run in a similar way by the same operators indicates that the individual characteristics of each system may be fundamental in determining the system steady state while in operation. Furthermore, the strong correlation between micro particles surface area and bacterial loads might have significant impact on the rearing environment in Model Trout Farms.

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## **Combined effects of chronic exposure to suspended solid load and increased unionized ammonia concentrations on the physiology and growth performance of rainbow trout (*Oncorhynchus mykiss*)**

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### **Abstract**

Until recently, high levels of accumulating particles, and especially fine particles, were assumed to be harmful to fish health and performance in recirculating aquaculture systems (RAS). However, recent investigations with rainbow trout have shown that suspended solids by themselves do not result in detrimental impacts on fish health or performance in RAS when water chemical variables were held at uncritical levels. Nevertheless, combined effects of suspended solid load with other potentially confounding water parameters remain largely unknown.

The present chronic exposure study (13 weeks) analyzes combined effects of suspended solid load and increased unionized ammonia concentration to investigate the mode of interaction of both parameters. Rainbow trout were maintained in two replicate RASs. After an acclimatization period of three months, the particle load in one system was artificially increased, whereas the second system remained under normal conditions as a control. Furthermore, ammonium concentration and pH was increased both in the control and treatment system in course of the study to elevate the unionized ammonia concentration.

Relevant water parameters, such as nitrite and CO<sub>2</sub>, were continuously monitored and adjusted to levels with no impact on fish health or performance to ensure a sole investigation of particle accumulation effects coupled with increased ammonia concentrations. Health-related effects were examined by means of hematological parameters, fin condition and gill histology. Furthermore, the impact of increased particle load on bacterial activity was investigated using a patented method called BactiQuant® (Mycometer, Denmark), which is an indirect measure of microbial enzyme activity.

Overall, the results indicate no negative impact on health parameters despite increased unionized ammonia concentrations in both systems and particle concentrations of up to 70 mg/L in the treatment RAS. However, bacterial activity was significantly elevated in the treatment system.

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## Water quality, histopathology and nitrification bacteria using combinations of fixed bed and moving bed bioreactors in RAS

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### Abstract

A good effort has been made to compare nitrification efficiency in different types of bioreactor design but less is known for other impacts on water quality. Besides nitrification, submerged fixed bed bioreactors trap fine solid particles, whereas moving bed bioreactors tend to grind solids, thus they might increase solid and particle accumulation in the system.

In this experiment different combinations of fixed bed and moving bed bioreactors were compared in laboratory scale recirculating aquaculture system (RAS). System consists of 10 individual RAS, each consisting of bottom drained plastic rearing tank, waste feed collector unit, swirl separator, drum filter, two bioreactor tanks, trickling filter aeration tower, pump sump, pH control and oxygenation. Total water volume was 890 l per system.

The 14 week trial consisted of three treatments with triplicate tanks: 1. two consecutive fixed bed bioreactors (FF); 2. fixed bed bioreactor followed by moving bed bioreactor (FM); and 3. two consecutive moving bed bioreactors (MM). Water quality was monitored online and nitrogen products weekly in laboratory. Particle size distribution was analyzed once and solids balance in sludge outflows twice during the experiment. Histopathological samples and bacteria samples from the carrier media were collected four times during the experiment. Rainbow trout of initial size 110 g were stocked in the system. Water replenishment rate was 500 l per kg feed. pH in the pump sump was maintained at 7.2 using NaOH.

Fish performance was good during the trial with SGR between 1.72-1.78 % bw d<sup>-1</sup> and FCR between 0.98-1.01 in the treatments. Opposite to other findings, nitrite levels were higher in FF system compared to FM and MM system. Average NO<sub>2</sub>-N was 0.61, 0.45 and 0.4 mg l<sup>-1</sup>, respectively, whereas average TAN was not influenced by treatments (1.1, 1.0 and 1.0 mg l<sup>-1</sup> respectively). Larger particles (above 20 µm) were found to be more abundant in treatments with moving bed bioreactors. Total solids balance was similar in all treatment groups and drum filter was compensating for solid accumulation in groups FM and MM. In general, no significant differences between the different treatment groups or the control group were seen in histopathological analysis. Analysis of bacteria communities in different bioreactors is on progress.

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## **Water boarding on the modern fish farm: How do you know if your fish are inadvertently being throttled by rapid increases of carbon dioxide?**

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### ***Abstract***

On a modern fish farm, fish are stocked at much higher densities than in nature. Natural water alkalinities are simply not high enough to buffer the pH drops seen in a heavily stocked fish tank. Thus, feeding the fish often results in rapid pH drops, which in-turn leads to large free CO<sub>2</sub> spikes across the fish tank... a clear case of CO<sub>2</sub> water boarding!

Alternatively, where bicarbonate alkalinity, free CO<sub>2</sub> and pH are all measured across the fish farm, bicarbonate alkalinity can be safely managed upwards to provide a better buffer capacity against pH drops. As CO<sub>2</sub> is in chemical equilibrium with pH, toxic CO<sub>2</sub> increases are less severe when pH is prevented from dropping.

This presentation will show the CO<sub>2</sub> water boarding phenomena, and illustrate how measurement and management can eliminate the issue. The harmful effects of the CO<sub>2</sub> water boarding phenomena are also documented with greatly improved fish performance where water quality across the fish tank is stabilized.

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## Acute and long-term CO<sub>2</sub> exposure reduces the performance of Atlantic salmon in RAS

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### **Abstract**

A high-level of free CO<sub>2</sub> is a prevalent feature of intensive RAS, and chronic exposure is common for most species during the production process. Currently, standard operating procedures, regulations and “safe” levels of CO<sub>2</sub> are based on values that do not necessarily represent a point at, up to which, production and fish welfare are unaffected.

The high solubility of CO<sub>2</sub> in water and the large input from fish respiration also means that current practices for the removal of CO<sub>2</sub> are often inadequate for the scale of production.

High CO<sub>2</sub> levels can be addressed by the control of alkalinity, thereby creating a scenario where the majority of CO<sub>2</sub> exists as carbonate and bicarbonate. Any acute reduction in pH can shift the equilibrium towards a large and sudden release of stored CO<sub>2</sub> which can have a detrimental effect on fish welfare.

The current investigations aimed to determine the effects of both; acute increases in dissolved CO<sub>2</sub> on the physiological capacity of Atlantic salmon, as well the effects of chronic exposure to different CO<sub>2</sub> concentrations on production in freshwater.

Results show that acute exposure (up to 40 mg L<sup>-1</sup>) significantly reduces aerobic capacity and the rate of recovery from stress, and that these effects are driven primarily by CO<sub>2</sub> exposure, and to a much lesser extent by the associated reduction in pH. Growth and feed conversion experiments during chronic exposure suggest that there is no [CO<sub>2</sub>] where production performance, and likely welfare, are unaffected.

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## Optimum ozonation of freshwater pilot recirculating aquaculture system - Water quality

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### **Abstract**

The water quality in intense recirculating aquaculture systems (RASs) is characterised by an accumulation of pollutants, potentially allowing fish pathogens to grow. Ozone improves water quality, diminishing significantly both bacteria load and dissolved organic matter.

Excess of ozone (overdosing) is unwanted due to detrimental effects on the fish, and therefore, it is crucial to define the ozone demand of a specific RAS. Thus, this study aimed to develop a method to predict the ozone demand and to pursue a more direct approach to control the delivered ozone dosage in RASs.

The required continuous ozone dosage in a pilot-scale RAS, operated as an intensive commercial RAS, was predicted solely based on water quality parameters analysed in the laboratory including batch ozonation experiments.

The predicted ozone demand of the 1.7 m<sup>3</sup> system was 180 mg O<sub>3</sub>/h. This ozone dosage along with one higher and one lower dosage level, as well as a control, were applied to replicate pilot RAS systems with fixed feed loading (1.56 kg feed/m<sup>3</sup> make-up water). Selected water quality parameters were measured, assessing biofilter performances as well as nitrogen and carbon-based compound concentration changes during ozonation.

Overall, this study contributed to a better understanding of the challenges of an ozonated heavily loaded RAS, leading to the optimal design of such systems by monitoring the delivered ozone with a new method based on fluorescence.

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## The development of water parameters during live transport of fishes (with focus on gasses)

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### **Abstract**

The live transport of fishes is an everyday issue for farmers but knowledge about the development of physiological relevant water parameters is scarce. For animal welfare, as well as economic reasons, it is important that water parameters during transport stay within species specific ranges to ensure compliance with physiological requirements, and to avoid adverse effects in the stressful handling and transport situation. Until now the transport of fish is more or less based on the experience of the fish farmer and except for oxygen there is a lack of data for relevant parameters.

During several commercial transport operations of rainbow trout (*Oncorhynchus mykiss*) a comprehensive set of water parameters was monitored with focus on gas dynamics. A non-invasive sampling method was used to avoid external stressors. Stocking densities, duration and process of transports were in accordance with the typical routine of professional European trout transport. The parameters CO<sub>2</sub>, O<sub>2</sub> and total gas pressure were monitored continuously by probes. Additionally, ammonia, nitrite and pH were collected with a high temporal resolution.

The results reveal a complex interaction of different water parameters whereby CO<sub>2</sub> plays a central role: e.g. CO<sub>2</sub>-values increased steeply with the beginning of the transport quickly reaching values far above the optimum range, but decreasing the pH proportionally, which in turn protected against the parallel increasing ammonia values.

In a second trial, an aeration system was installed in the transport tank which efficiently removed CO<sub>2</sub> but seemingly further increased other relevant parameters like ammonia.

It can be concluded that certain water parameters during the live transport of fishes can pose a risk to fish welfare and health with implications for product quality.

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## Simulation of recirculating aquaculture systems in the OpenModelica environment

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### **Abstract**

Classical methods for design and analysis of RAS water treatment are often based on a limited number of static material balances. A RAS is, however, a very complex system where components interact in ways not captured by these equations, and as such it should be beneficial to use more complex models for design. Additionally, the slowly evolving nature of a RAS makes experimental improvement of design and operation difficult and tedious, and deviating from established operating conditions might be considered unsafe or infeasible. Against this background, simulation tools for recirculating aquaculture systems that use dynamic models could provide valuable information in all stages of RAS planning and operation.

Motivated by this, a dynamic simulator was previously developed and implemented in MATLAB and Simulink by Wik et al. (Aquaculture 287, 2009.) This software, dubbed FishSim, integrated a fish growth model with models for biological and mechanical water treatment. As a proof of concept it worked well, but for practical use it had severe limitations in terms of what could be simulated because of numerical issues.

Inspired by FishSim, we have developed a new software for simulation of recirculating aquaculture systems using integrated dynamical growth and treatment models. The free modelling and simulation suite OpenModelica was used for the new implementation, and the new simulator is capable of fast simulations of arbitrary system topologies. It is also robust with respect to different feeding schedules, growth parameters and control systems. The much improved performance of this simulation environment has also allowed added complexity in the modelled components, such as considering additional reactions and energy balances. Simulating a simple RAS loop over 30 days takes slightly less than half a minute on a standard desktop computer. Complex setups, which were not possible to run at all in the previous software, were simulated over a full year in 5-7 minutes in the new simulator.

We demonstrate applications of the simulator, such as investigating the influence of parameter perturbations and comparing different treatment system configurations and control strategies.

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## Marine finfish hatchery design: Design approach and resulting bead filter applications

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### **Abstract**

In 2015 HTH engineering was hired to design a phase-one marine finfish hatchery for red drum (*Sciaenops ocellatus*) and spotted seatrout (*Cynoscion nebulosus*) in the U.S. state of Florida. These species are among the most important recreational fishes in the Gulf of Mexico and have been the subject of decades of aquaculture and stock enhancement research. However, rather than simply reinventing the wheel, we instituted a *de novo* design process that operated within the context of Florida's Aquaculture Best Management Practices to achieve our client's objectives.

With no preconceived notions, the design process found an experience-based design approach in the work at the University of Bari Aldo Moro, the Florida Stock Enhancement Research Facility, and USM's Gulf Coast Research Laboratory which resulted in the application of floating-bead, bead filters. All of these R&D facilities focused on the application of the right marine technology for robust and efficient RAS based operations. The RAS approach was also strongly encouraged by Florida's Aquaculture Best Management Practices developed by various Florida Agencies to encourage the development of Aquaculture in the State and to streamline the environmental permitting process.

Performance objectives include: limited effluent discharge conducive to operation using artificial seawater, low maintenance costs, small footprint, efficient filtration of fine particles, resistance to corrosion, and low pressure – low energy operations.

The design includes seawater pumping, water treatment and recovery for operating quarantine, broodstock, live feeds, and larval rearing systems. The design incorporates Polygeyser Drop Bead Filters and Bubble Wash Bead Filters for mechanical and biological filtration in Pre-treatment, Quarantine and Broodstock Holding/Conditioning RAS. Bubble Wash filters are also used for bay seawater and recovered seawater filtration, and for larval rearing RAS upstream of moving bed bioreactors.

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## The effect of salinity and photoperiod on growth and performance of coho and Atlantic salmon in recirculating aquaculture systems

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### **Abstract**

Recirculating aquaculture systems (RAS) are currently being considered for the full life cycle rearing of salmon in aquaculture. Due to the high costs of operating RAS, however, fish must be reared under optimal conditions to enhance growth and maximize profitability.

The Initiative for the Study of Environment and its Aquatic Systems (InSEAS) at the University of British Columbia consists of 7 independent 15 m<sup>3</sup> RAS modules, each with 2 x 5 m<sup>3</sup> rearing tanks. In this study we reared Atlantic salmon and coho salmon under identical conditions at one of 4 salinities (2.5, 5, 10, 30 ppt) in combination with 2 photoperiods (12 h light:12 h dark (2.5, 5 and 10 ppt) and 24 h light (2.5, 5, 10 and 30 ppt). Smolts of each species were stocked in the 5 m<sup>3</sup> rearing tanks (initial weight: Atlantic salmon = ~100 g (mixed sex), coho salmon = ~170 g (all female)) and 100 individuals per tank were pit tagged.

Growth and physiological performance will be assessed throughout the 400 day growth trial which is on-going, but here we report on our findings for days 60, 120 and 200 following initiation of the respective salinity/photoperiod treatments. At 120 days, there was a significant increase in growth rate at 10 ppt in Atlantic salmon and a significant effect of photoperiod. In coho salmon there was no significant effect of salinity or photoperiod on growth, hypoxia tolerance, maximum swimming velocity, aerobic scope, or post exercise oxygen consumption.

We also investigated behavioural effects by placing individual coho salmon in an open field, novel approach, and light/dark test and found no effect of salinity or photoperiod on locomotion but did see some differences in zone preferences that warrant further investigation. Thermal tolerance in coho salmon was reduced in the 24 vs 12:12 photoperiod at all salinities at days 60 and 120, the basis for which is not known but could be significant in an intensive rearing environment.

Data for day 200 will be discussed and will include findings on growth and physiological performance as well as quantification of eye damage and early maturation.

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## **Integration of energy audits in the Life Cycle Assessment methodology to improve the environmental performance assessment of Recirculating Aquaculture Systems**

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### **Abstract**

In Recirculating Aquaculture Systems (RAS), water is continuously treated and recirculated as opposed to being discharged untreated into the environment as in other type of fish production systems; the design and production parameters will determine the overall energy consumption. This energy-intensive nature hampers their sustainability and cost-effectiveness. A combination of two methods (i.e. Life Cycle Assessment (LCA) with energy audits) to: improve environmental performance of RAS, identify energy consumption and thus, its environmental and monetary effects in order to seek cost reduction is proposed. Likewise, an online software analysing system's consumption patterns (principal and partial consumptions); abnormality detection and correction of habits; identification of opportunities for improvement; and assessment of potential economic progress.

The methodology was proved with a case study focused in a pilot-scale RAS unit used in codfish (*Gadus morhua*) production, located in the Basque coastal area (northern Spain). Feed and juvenile production/transportation, oxygen transportation and energy consumed during the whole experiment were considered as inputs for the assessment. Energy consumption was measured both continuously by an energy meter embedded in the RAS unit as well as with a portable energy analyzer to measure each of the energy-consuming devices independently. Although the system required an average of 29.40 kWh/kg fish for successful system operation, the energy consumption varied by season presenting maximum and minimum periods of 40.57 and 18.43 kWh/kg fish, respectively. Main consumers included the heat pump, followed by the main and secondary pumps, respectively.

Energy audit's results show the success in identifying the devices that consumed the largest amount of energy, and recorded data served to feed the Life Cycle Inventory and perform a more complete and precise LCA. Fossil fuel based on-farm electricity for the on-growing of fish was shown to be the most environmentally unfriendly input; it was the major impact producer in the assessed impact categories. It showed a temporal variability depending on the water temperature, which resulted to be the main factor linked to the energy use. This aided performing a precise assessment including system-specific scenarios.

The combination of LCA and on-farm energy audit represents a useful tool to secure a more complete assessment with a periodic assessment to design a less energy intensive, profitable and sustainable system; likewise, it increases the speed and transparency of governance and decision-making, taking into account the time-based fluctuation of the energy consumption throughout the production cycle.

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## Application of recirculating PolyGeysers® to aquacultural flows effluent flows

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### **Abstract**

The PolyGeyser floating bead filter has been established as a bioclarifier providing treatment for recirculating systems in freshwater and marine applications. Coupled with an airlift that provides for circulation, aeration and carbon dioxide stripping the units are capable of providing both clarification and biofiltration for a wide variety of applications. The “High Profile PolyGeyser” (HPPG) is primarily designed for single pass pumped application for heavy production applications as the unit’s height make the results in an aggressive pneumatic backwash. The “Low Profile PolyGeyser” (LPPG) is specifically design for airlift recirculation with tanks of approximately one meter in height. Configured with a matching tank hull, the “Filter in Tank” or “FIT” configuration, the LLPG is increasingly favored by the Aquaponic community as it simplifies the RAS installation and operation while providing a significant internal sludge mineralization capacity.

The “Recirculating PolyGeyser” (RCPG) is the newest PolyGeyser line designed to support wastewater treatment as a clarifiers, a bioclarifier, or biological filter. With a height over 3 meters, the unit has a robust backwash that is immune to biofouling under extremely heavy solids or biological loads. The bead bed is overlain with an airlifted recirculation tank that facilitates oxygen transfer in the face of high organic or ammonia loads. The units are configured with “Pneumatic Sludge Discharge” (PSD) that removes accumulated sludge from the unit as part of the backwash cycle. Thus, the units are able to operate for extended periods of time without manual or electrical intervention.

The RCPG configuration is currently being evaluated as a bioclarifier polishing the effluent from a lagoon/rock reed filter treating wastewaters from a domestic waste water source with stringent effluent standards of less 3 mg/L TSS, 3 mg/L CBOD<sub>5</sub>, and 1 mg/L TAN. These results are consistent with earlier studies suggesting that CBOD<sub>5</sub> conversions are proportional to the targeted effluent quality with, for example, a conversion of 1 kg-CBOD<sub>5</sub>/m<sup>3</sup>-day occurring at an effluent target of 1 mg/L. Ammonia conversion below 2 mg/L TAN is also proportional to effluent quality with a TAN conversion of 750 g-N/m<sup>3</sup> media per day being typically observed at a TAN concentration of 1 mg/L. Solids capture as with the other PolyGeyser filters is excellent, although some impairment was observed in early RCPG testing. Vortexing in the head tank was found to entrain bubbles that disrupted the capture of fine solids. Design analyses conducted on the effluent from a series of lagoons treating a catfish processing plant discharge and of a rotating microscreen drum filter backwash water treatment systems indicate the potential for employing the RCPG as an effluent polishing treatment device.

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## Denitrification in marine recirculating aquaculture systems

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### **Abstract**

Discharge of organic matter, dissolved inorganic nitrogen and phosphorus is a major cause of environmental pollution by recirculating aquaculture systems (RAS). While ammonia removal through incorporation of nitrifying biofilters is well established in RAS, active removal of nitrate, the end product of nitrification, is less common. Hence, in most RAS, nitrate levels are mainly dictated by the system's water exchange rate and to some extent by passive nitrate removal processes in the different system components.

Mainly due to stricter environmental regulations, an increased number of RAS are operated with incorporation of denitrification reactors in which nitrate is biological reduced to elemental nitrogen gas. Fixed film reactors, in which heterotrophic denitrification is fuelled by addition of external carbon sources, are most often used for this purpose. Alternatively, uneaten feed and fish faeces may be used as endogenous carbon and energy sources for the denitrifying organisms.

This latter strategy was used in a zero discharge system which produces marine fish with no pollutant discharge and minimal use of valuable fresh, makeup water. In this particular RAS, denitrifying activity takes place in a digestion basin which is fed with organic-rich effluents from the fish basins. In the digestion basin, denitrification is part of an array of interactive anoxic/anaerobic biogeochemical processes which collectively cause an effective reduction of carbon, nitrogen, phosphorus and sulphide in the treatment water. Nitrate reduction in these basins was found to take place by either one of the following processes: heterotrophic denitrification, autotrophic denitrification on sulfide or dissimilatory nitrate reduction to ammonia (DNRA). The relative contribution of each of these processes was found to depend on the carbon, nitrogen and sulfide concentrations in the various parts of the basins. At ample concentrations of available organic matter, heterotrophic denitrification was the dominant nitrate removal process. DNRA was pronounced at relatively high levels of available carbon and low levels of nitrate (high C/N ratios) or at high sulphide concentrations. Autotrophic denitrification, with sulfide as electron donor, was evident both in absence and presence of heterotrophic denitrification. Sulfide, accumulating in the sludge as a result of sulphate reduction and desulfurization during organic matter decomposition, influenced the relative contribution of the various nitrate removal processes. Results presented are illustrative for the complex biogeochemical processes underlying nitrate removal in organic-rich, marine systems.

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## Solid waste treatment for saltwater RAS: Microbial anaerobic digestion and biomethane production

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### **Abstract**

The recirculating aquaculture system (RAS) removes fish farming wastes such as ammonia and carbon dioxide allowing reuse of water for fish culture. Especially for saltwater RAS, environmentally responsible disposal of its solid organic wastes is critical for promoting marine RAS production, because salt-containing sludge cannot be used as landfill or fertilizer like freshwater aquaculture solid wastes. RAS removes solids from system water and collect them for discharge; therefore, it can be further treated to reduce pollution in its effluent. The bioconversion is an attractive organic waste treatment practice achieving both pollution control and bioenergy production, and the anaerobic digestion, well-established and widely used bioconversion technology in agricultural and industrial waste treatment, has been successfully adapted for marine RAS solid waste treatment reducing pollution and producing biogas (i.e. methane) using up-flow anaerobic sludge blanket (UASB) reactor, despite many concerns for the failure. Bioreactor containing methanogenic consortia of bacteria and archaea could digest high loads at low operating costs and with relatively low initial investment. However, the conversion efficiency of saline sludge to biomethane was challenging compared to freshwater sludge like agriculture wastes.

Understanding the microbial community composition is critical for system management in order to maintain or restore optimal microbiota during start-up and throughout the production processes. In this report, we describe the enrichment and characterization of a stable marine fermentative and methanogenic consortium developed from solid waste digester of marine RAS that is capable of reducing over 90% of digestible marine RAS solid wastes to methane at 15 ppt salinity. The halotolerant microbial consortium developed by sequential transfer is optimized for low COD:N ratios typical of RAS solid wastes and does not require supplemental materials such as organic carbon or nutrients for organic solid waste bioconversion. Five predominant phylotypes identified in the microbial consortium. Of which, two isolates are anaerobic fermentative bacteria *Dethiosulfovibrio* and *Fusobacterium* spp. Both species hydrolyze and ferment proteins, peptides and amino acids. The other three isolates are an acetate-utilizing methanogenic archaeon, *Methanosarcina* sp., and two hydrogen-utilizing methanogenic archaea, *Methanogenium* and *Methanoplanus* spp. Bioconversion rates of re-constituted microbial consortium containing all five isolates resulted in equivalent to the original enriched consortium suggesting those isolates can be used as seeding inoculum for marine RAS solid waste digester. A test of inoculum in the continuous flow system maintaining a high steady-state microbial population and high rates biomass conversion during intensive European sea bass RAS production will be discussed.

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