Content archived on 2024-06-18

The development of a deammonification treatment to remove nitrogen from recirculated water used in aquaculture

Reporting

Project Information

DEAMMRECIRC

Grant agreement ID: 262183

Project website

Project closed

Start date
1 January 2011

End date
30 April 2013

Funded under
Specific Programme "Capacities": Research for the benefit of SMEs

Total cost
€ 1 368 493,97

EU contribution
€ 1 090 267,05

Coordinated by
CYKLAR-STULZ GMBH
Switzerland

Final Report Summary - DEAMMRECIRC (The development of a deammonification treatment to remove nitrogen from recirculated water used in aquaculture)

Executive Summary:

The EU FP7 Research for SME project DeammRecirc , aimed to remove nitrogen from recirculated water used in aquaculture through development of a deammonification treatment. The 28 month project ended in April 2013.

The project has been coordinated by Cyklar-Stulz GMBH, a Swiss company specialising in deammonification technology to remove nitrogen in the municipal and industrial waste water sector. The consortium also consisted of the following Industrial partners: Formoplast Kunststoffbehalterbau und – vertrieb GMBH (Germany), ARAconsult GMBH (Austria), Tecnologias y Equipos para el Medio Ambiente, s.l. (Spain), Hardingsmolt AS (Norway), Anglesey Aquaculture Ltd (UK) and Marine Finfish Farmers Association of South Africa (S. Africa). In addition, the consortium included four RTD partners: Teknologisk Institutt AS (Norway), Aqua Consult Ingenieur GMBH (Germany), Institute of Sanitary Engineering and Waste Management (ISAH) at Gottfried Wilhelm Leibniz Universität Hannover (Germany) (LUH) and Centre for Sustainable Aquaculture Research (CSAR), Swansea University (UK).

Deliverable 1.2 was made public on the project website and is a very comprehensive overview of naturally occurring deammonification in marine and freshwater systems. Deliverable 1.3 is also a very comprehensive review of the deammonification process in waste water and crucial factors for its transfer to recirculation technology. DEMON® sludge from a reject water treatment plant was adapted to typical conditions in RAS farms. Four lab-scale SBRs were seeded and - after stable anammox conversion performance was reached with initial conditions (25 °C, reject water with NaNO2,) - the boundary conditions were adjusted slowly to investigate the influence of low temperatures (25 - 15 oC), high salt concentrations (freshwater to saltwater) and coupling anammox to partial denitrification to degrade NO3-N. The Cyklar DEMON® sludge was adapted successfully to cooler temperatures (15oC) as used in salmon smolt RAS farms. However the DEMON® sludge could not be adapted to a salinity > 10 ‰ and therefore “home grown” inoculum had to be produced for the marine pilot plant test. The project has successfully grown its own sea salt adapted inoculum from European sea bass (Dicentrarchus labrax) sludge. A methodology has been developed so fish farms can grow their own inoculum in the future. This inoculum has then been used in the DeammRecirc prototype, which has been developed within the project. This consists of a pre-treatment reactor, where RAS system water and sludge are mixed and partial denitrification occurs, transforming nitrate to nitrite. This effluent then is pumped to an anammox reactor (the main treatment) containing the inoculum sludge, enriched in anammox bacteria. Here nitrite and ammonium react to form nitrogen gas (the anammox process) and denitrification also occurs. The DeammRecirc prototype is semi-automated for use at the pilot plant but would be fully automated with a more comprehensive control system when transferred to commercial farms. Ammonium and nitrite have been dosed at higher than natural RAS rates in this project to keep enriching the anammox bacteria within the sludge. Therefore the post treatment has not yet been tested, which would complete the recirculation of water back to the fish tanks. Therefore more research is needed to complete development but the DeammRecirc project has taken major steps on the way to transfer the deammonification system to recirculation farms, allowing reduction in high system nitrate concentrations and using sludge, a waste product in the process.

Project Context and Objectives:

The DeammRecirc project is developing a deammonification treatment to remove nitrogen from recirculated water used in aquaculture. It is based on the recent experiences and success of using deammonification to convert ammonia to nitrogen gas in other waste water treatment applications. In DeammRecirc, this method will be adapted for use in recirculation aquaculture systems (RAS) for fresh- and marine water species.

So far RAS farms have been very slow to adopt denitrification methods. Present practice is to convert the toxic metabolic waste product ammonia via nitrite to nitrate in a nitrifying bio-filter. This microbial process demands oxygen and reduces the pH, requiring expensive buffering treatment. The accumulating nitrate is generally flushed from the farm into recipient waters without further treatment.

By using a deammonification reactor within RAS, ammonia will be converted to nitrogen gas. Deammonification bacteria enriched in granules will be adapted to RAS water qualities. The expected benefits compared to currently used treatments include reduced oxygen use and cost for buffering chemicals, lower carbon footprint, reduced levels of nitrate rich effluent released to the environment, reduced pumping cost through reduced demand for new exchange water.

The scientific objectives for DeammRecirc include:

• To increase knowledge on recirculation systems relevant to enable incorporation of deammonification process (WP2, 3 and 4), including: definition of typical water qualities to be treated in marine and freshwater recirculation farms; Description of current RAS technology and required water qualities for the 2 end-user sites; screening of deammonification inoculums for fish pathogens.
• To increase knowledge of natural deammonification processes occurring in marine and freshwater environments with particular focus on conditions, rates and bacteria involved and use this knowledge in laboratory testing of bacteria under RAS conditions (WP2)
• To create an enhanced understanding of the deammonification process applied to waste water treatment in general and which factors will be crucial for its technological adaption to aquaculture RAS (WP 3)

During the development of the DeammRecirc technology, the project aims to reach the following technological objectives:

• To adapt enriched deammonification bacteria culture to defined RAS conditions (defined in 1.1) for marine and freshwater testing in WP 4.
• To describe, design and develop pre-treatments and post-treatments necessary to run the deammonification unit.
• To design and develop a deammonification unit, which maintains Total Ammonia and Ammonium Nitrogen (TAN), nitrite and nitrate concentrations at levels suitable for seabass in marine and salmon smolt in freshwater RAS systems.
• To validate during pilot-scale testing that oxygen and buffering chemical use to maintain biofilter operation has decreased by 60% and 100% respectively and exchange water (pumping & heating costs) has decreased by 50 %.

The main objectives are listed below for each work package (WP) and task within WP.

WP1 - Enhanced understanding to enable application of the deammonification technology transfer to RAS conditions (complete)

The main objectives of WP1 were to enhance scientific knowledge to enable bacteria adaption and deammonification technology transfer to RAS (Recirculation Aquaculture Systems) conditions.

Task 1.1 - The main objective of task 1.1 is to enhance the scientific knowledge on RAS relevant to incorporate deammonification. (complete)

Task 1.2 -The main goal was to increase knowledge of natural deammonification processes occurring in marine and freshwater environments with particular focus on conditions, rates and bacteria involved. (complete)

Task 1.3 - The main goal was to create an enhanced understanding of the deammonification process applied to waste water treatment in general and which factors will be crucial for its technological adaption to RAS. (complete)

WP2 - Development of start-up and operational strategies for the deammonification process under RAS conditions (compete)

The main objectives of WP2 were to develop and test suitable start-up and operational strategies for deammonification under RAS conditions in lab-scale reactors, evaluate the influence of boundary conditions on the biocenosis and determine design parameters for process up-scaling.

Task 2.1 The main goal is to operate lab-scale reactors with the deammonification process under RAS-specific boundary conditions. (complete)

Task 2.2 - The main goal is to identify inoculum sources for deammonification in RAS systems. (complete)

Task 2.3 - The main goal is to perform accompanying counselling during construction, start-up and operation of the pilot plant. (complete)

WP 3- Development of deammonification technology for RAS (complete)

The main objectives of WP3 were to design and build a deammonification reactor, components within and its control system for a RAS setting. For the incorporation of deammonification technology into RAS, to design and build the necessary pre and post treatments to allow deammonification.

Task 3.1 - The main goal is design of pre-treatment of water required prior to entering deammonification tank and post-treatment of water required after leaving tank. (complete)

Task 3.2 -The main goal is to design deammonification reactor, components within and its control system (complete)

Task 3.3 The main goal is to construct the pre and post-treatment prototypes required (complete except for construction of post-treatment)

Task 3.4 The main goal is to construct the deammonification prototype (complete)

Task 3.5 Design of monitoring system (complete)

WP4 - System Integration and Industrial validation (complete)

The main objectives of WP4 were to integrate the adapted, enriched bacteria from WP 2 and the pretreatments, deammonification reactor, monitoring system and post treatments from WP3 to form a functional DeammRecirc prototype in an operating RAS system and to validate that the technology created is capable of performing to meet the specified reduction of recirculating nitrate concentrations and reduced operating costs as described in the technological objectives

Task 4.1 Integration of bioreactor prototype, enriched bacteria solution and monitoring system into unstocked RAS at SU (complete)

Task 4.2 Phase 1 validation of bioreactor deammonification performance in unstocked experimental RAS (chemical dosing) (complete)

Task 4.3 Phase 2 validation of bioreactor deammonification performance in experimental RAS stocked with a commercial marine aquaculture species (complete)

Task 4.4 Final validation of prototype bioreactor at commercial marine end user, Selonda, UK. This will require evaluation of prototype deammonification system using a side stream effluent supply from the farm with commercial loadings of ammonia under marine conditions. (complete but not at Selonda or AAL)

Task 4.5 Final validation of prototype bioreactor at commercial freshwater end user, Hardingsmolt, Norway. This will require evaluation of prototype deammonification system using a side stream effluent supply from the farm with commercial loadings of ammonia under freshwater conditions. (not done)

WP5 - Other activities-Dissemination, IPR and Training (complete)

The main objective is to develop plans to fully exploit the commercial potential of the technology and know how developed in the project to the benefit of the SME partners.

Task 5.1 - Protection of foreground (complete)

Task 5.2 - Dissemination activities (complete)

Task 5.3 - Absorption of results by SMEPs and Training (complete)

WP6 - Management (complete)

Project Results:

Results from Reporting period 1

DeammRecirc has had good cooperation between the different RTDs, SMEs, LE and other organizations involved. There have been 3 formal meetings in RP1: a kick-off meeting in Weesen,

Switzerland 10th-12th January, which was a combined management and technical meeting, and 2 technical meetings, 7th April in Zurich and 4th -6th May in Wales. The second management and technical meeting was held 4 days after the end of RP1 (4th October 2011). In addition there has been close cooperation through emails, Skype, phone calls and through basecamp, a website protected by password for all members of the DeammRecirc consortium. Here presentations and minutes from meetings, photos, reports and deliverables are made available to the consortium. Email correspondence and responses are also gathered here.

The RTD work has been focused on WP1 - WP3 in RP1. The main objective of WP1 is to enhance understanding to enable the application of the deammonification process in the recirculation environment. The scientific objectives (described above) were achieved and the first milestone reached at the end of month 4. This included the completion of 3 public deliverables to create a scientific basis for further work in WP2 - WP4. The main results from each deliverable are listed below.

D1.1 "Recirculation water quality definitions and overview of technology and farm data from the end-users"

Descriptions of the most important water parameters affecting farmed fish are presented and a table of these parameters has been compiled for the most common farmed species in Europe.

As the consortium has decided to develop the deammonification technology for the important commercial species sea bass and salmon smolt, more specific data on concentrations of the most important water parameters for these 2 fish species and mass balance data for the 2 farm systems have been collated. An overview of the technology used to treat water at the 3 fish farms or facilities, Swansea University, Selonda UK and Hardingsmolt have been presented to enable the development and validation work to take place in WP2 - WP4. Methods for pathogen screening of the Demon granules have also been considered.

D1.2 "An overview on naturally occurring deammonification in marine and freshwater systems"

A short characterization of anammox bacteria and metabolism (= the anammox conversion process) provides the general microbiological framework. Moreover, methods used for the determination of different parameters are described briefly to ensure correct interpretation of presented data. As various environments with different characteristics have been found to be habitats for anammox bacteria, these are described and classified into "freshwater", "marine", aquaculture systems and wetlands. The main part of the report contains - for each environment- a collection of key publications including short abstracts. At the end of each chapter, information on the different anammox strains that are relevant, including their characteristics, conversion performance and kinetics are summarized and presented in graphs and tables. In the conclusions, an effort is made to link both abundance and conversion performance with the corresponding boundary conditions. Finally, relevant parameters determining the stability and performance of anammox conversions under natural conditions are identified and evaluated with regard to the application in RAS systems. The study points out the diversity of anammox bacteria and their wide-spread occurrence in different types of environments. Site-specific boundary-conditions lead to adaption and selection of specific anammox species and therefore, conversion rates and kinetics cannot be transferred to different conditions. However, there is strong indication that aquaculture systems are suitable habitats for anammox bacteria.

D1.3 "Deammonification process in waste water"

The general aim of the DeammRecirc project is to develop the water treatment technology, which will enhance the performance of the RAS technology and allow a decrease in the water exchange and make the technology more independent and sustainable. The technology will be developed for the nitrogen removal technologies applied at the RAS farms.

In order to understand the enhanced nitrogen removal processes, the established nitrogen removal technologies applied in the wastewater treatment were discussed in deliverable 1.3. Considering the knowledge and knowhow from wastewater treatment processes and the circumstances of the water treatment in the RAS technology, the general development of the enhanced nitrogen removal in RAS was described. The major factors and parameters affecting deammonification process have been described. The technological application of the process for RAS has been investigated.

In WP2, ISAH has constructed and is operating 4 Sequencing Batch Reactors (SBRs), each 20 l volume since March 2011. Each reactor is equipped with a substrate storage tank, an effluent storage tank, temperature regulation, influent and effluent pumps and pH and temperature measurements. The SBRs are fed with reject water from WWTP Guemmerwald (Hannover). The reactors were seeded with sludge (100 % inoculum) which was taken from the full-scale DEMON®-reactor at WWTP Plettenberg treating the reject water of anaerobic digested sludge.

Start-up and Steady state - SBR 1,2,3,4 (1.3.2011 - 30.6.2011)

In this phase all reactors were operated with the following strategy. The feeding media total N concentrations started at 200 mg/L and were increased gradually up to 600 mg/L with a molar ratio of NO2-N/NH4-N of 1,with operational temperature ranging from 24- 29°C. The observed Specific Nitrogen Removal Rate (SNRR) varied within 0.22 g N/g TSS*d up to 0.40 g N/gTSS*d. The operation setup in each SBR, in order to evaluate the effect of different boundary conditions (low temperatures, high salt concentrations and Enhancement of Anammox via Denitrification), was changed in 3 of the reactors after stable operation was achieved in all reactors.

Reference reactor - SBR 1 (1.7.2011 - 30.9.2011)

After the start-up phase the first reactor has been operated as a reference reactor with the temperature being kept at 27±2°C and no salt has been added. The nitrogen load has been increased gradually in order to determine maximum conversion capacities as well as critical loads and/or concentrations. The highest measured SNRR until now reached 0.78 g N/g TSS*d.

Influence salt concentration - SBR 2 (1.7.2011 - 30.9.2011)

After the start-up phase the second reactor has been fed with synthetic seawater in order to determine the adaptation velocities and capabilities of the anammox bacteria to high salt concentrations.

Surprisingly (literature suggests better adaption), trials up until now (90d) have shown that stable conversion performance could only be reached up to a NaCl concentration of 5.0 g/L with a respective SNRR of 0.24 g N/g TSS*d. Increment steps of NaCl had to be changed from originally 2.5g/L to 1g/L. The reactor temperature in this reactor ranged between 26±2°C.

Influence Temperature- SBR 3 (1.7.2011 - 30.9.2011)

After the start-up phase the temperature in the third reactor has been decreased gradually in 1 up to 2 week long steps by 1°C each. Until now, temperature in this reactor has reached 18°C showing a stable operation with a respective SNRR of 0.36 g N/g TSS*d. SNRR at the end of the start-up phase was 0.40 g N/g TSS*d at a temperature of 26°C.

Enhancement of Anammox via Denitrification SBR - 4 (1.9.2011 - 30.9.2011)

In order to examine the possibility of anammox bacteria using intermediate NO2 together with NH4 as substrate, the feeding medium was changed by adding NO3 instead of NO2. No external COD was dosed in this phase. The accuracy of the measured ammonia up-take, which suggests an activity of the anammox bacteria, was not high enough to make any conclusions (due to the small concentration differences). Therefore strategies which augment the production of NO2 within the denitrification pathway are now the focus of further trials. This work will be reported fully in D2.1.

In WP3, work has focused on task 3.1 and 3.2 the pre-treatment technology before the deammonification reactor and the deammonification reactor. These tasks are very closely linked and TI and ACI have worked closely on these tasks. A model proposed by Cyklar and simulated by ARA is suggested for these 2 technologies. It became clear after work in D1.1 and D1.3 that deammonification should be performed on a side-stream of the fish waste water. It became quickly apparent that more water and sludge samples were needed from Selonda (now AAL) to simulate different boundary conditions and a comprehensive sampling series and acidification experiment was planned. Fresh sludge from the drum filters is an important element to pretreat the side stream waste water, which has aerobic conditions for bacteria and is full of dissolved oxygen for the fish. The plan is to acidify the fresh sludge releasing organic acids, ammonia and COD, which will quickly use up any dissolved oxygen in this side stream. It then flows to a deammonification reactor where it is planned both annamox reactions and denitrification will occur.

WP4 begins in month 14 and is not reported under RP1.

In addition work in WP5, which encompasses other activities like IPR, dissemination and training has been on going since the start of the project. This has resulted in establishment of a project web-site, described in D5.1 and a draft plan for the use and Dissemination of the foreground, described in D5.2. Here a list of the dissemination in the project in RP1 is presented, which includes presentation of DeammRecirc at AquaNor in August 2011.

Results from Reporting period 2

The most important foreground from WP2 – enriched deammonification bacteria -are that DEMON® sludge could be successfully adapted slowly to cooler temperatures (15 oC) as found in salmon smolt RAS farms. However the anammox activity decreased rapidly at higher salinity than 10‰, so DEMON® sludge could not be adapted for use in marine farms, like AAL. Because of this sea salt adapted inoculum was grown from sea bass sludge from SU and AAL and a methodology developed for growing inoculum which can be used by fish farms in the future.

In WP2, task 2.1 LUH adapted DEMON® sludge from a reject water treatment plant to typical conditions in RAS farms. Four lab-scale SBRs were seeded and - after stable anammox conversion performance was reached with initial conditions (25 °C, reject water with NaNO2,) - the boundary conditions were adjusted slowly to investigate the influence of low temperatures (25 - 15 oC), high salt concentrations (freshwater to saltwater) and coupling anammox to partial denitrification to degrade NO3-N. From this work, it was found that DEMON® sludge could be successfully adapted slowly to cooler temperatures (15 oC) as found in salmon smolt RAS farms. However the anammox activity decreased rapidly at higher salinity than 10‰, so DEMON® sludge could not be adapted for use in marine farms, like AAL. This 4 month steady-state operation and 10 month adaptation to different conditions of the 4 lab-scale SBRs is reported in full in D2.1.

In task 2.2 the fish sludge was analysed for anammox bacteria with FISH and batch experiments were performed to characterize the acidification, denitrification and anammox conversion potential of the samples. The Hardingsmolt (fresh water) sludge showed a high potential for (complete) denitrification of nitrate to nitrogen gas from internal carbon sources (no external carbon source addition). Parallel to nitrate reduction, ammonium was released, indicating acidification processes. Due to the high denitrification potential, anammox conversion activity could not be detected in these experiments. No positive signal could be detected in FISH analyses with anammox-specific probes. Acidification tests showed that hydrolysis of this sludge was always connected to gas production (CO2 and N2), probably due to parallel denitrification. During these tests, ammonium and COD were released from the biomass.

Similar results were obtained for Swansea salmon (sea salt) sludge, except that this sludge produced significant amounts of H2S in the gas phase. Ammonium and COD were released from the biomass. Detailed results and interpretation were compiled in D2.2 in June 2012.

These sludges were not used as an inoculum for anammox bacteria in this project, as the focus was on salt-water RAS. Even though the FISH analyses did not give positive results it is possible that anammox bacteria are also found in salmon waste sludge. This would need further investigation either with a PCR method or by FISH analyses after anammox enrichment. However, the high potential for heterotrophic conversion processes (denitrification and acidification) with release of ammonium proves that this sludge would be appropriate for the envisaged DeammRecirc concept of acidification/partial denitrification +

anammox.

In task 2.3 LUH provided invaluable counseling during construction, start-up and operation of the pilot plant, and developed a methodology for growing inoculum which can be used by fish farms in the future. Skype meetings were held at regular intervals (approx. 1/month) to discuss the results and eventually adapt the operational settings. In the beginning, the set-up, operation (dosing, mixing, water exchange) and analytical scheme for anammox enrichment buckets were discussed and a description of the enrichment strategy was delivered by LUH. This document was later redrafted based on the experience with the actual sludge and generalized as D2.3 “Compilation of design and operating parameters for starting-up bioreactor”.

In a second step and until the end of the project, the set-up, operational issues (mixing, artificial dosing, and operational settings) and analytical scheme for pilot-plant reactors (see tasks 4.1 and 4.2) were discussed. This included especially appropriate analytical methods for COD and nitrogen compounds in salt water matrix as well as timing and frequency of sampling. Analytical data was provided by SU before the Skype meetings allowing LUH, ACI and ARA to analyze the data and make recommendations for the adaptation of the operational settings of the pilot-plant in order to increase anammox conversion performance (feed of ammonium and nitrite from acidification reactor or artificial stock solution).

Additional experiments and special analytical methods such as FISH, PCR and 15N activity tests were proposed by LUH, ACI and TI, who established the contacts to external laboratories and research institutions, clarified the required characteristics and amount of samples and finally interpreted the raw data delivered by the external laboratories. Furthermore, a series of additional experiments on acidification and partial denitrification of fish waste sludge was designed by the RTDs and performed by SU, with data evaluation by LUH and ACI.

At the very end of the project, LUH performed concluding anammox activity tests for biomass from pilot-plant anammox SBRs with the standard method used for wastewater sludge. These tests revealed high conversion rates of the anammox process (0.1 g N/ g VSS • d) under perfectly anoxic conditions, sufficient substrate supply and continuous mixing, which is much more than measured in situ in the reactor. Furthermore, a COD fractionation assay was performed with Swansea fish waste sludge to provide further information on the biodegradability of this sludge as the input for the mathematical model and COD balances

The most important foreground from WP3 – Development of treatment system - is that the DeammRecirc technology was developed and built for integration in a small marine RAS system. The DeammRecirc prototype, consists of a pre-treatment SBR, two anammox SBRs (to allow different types of operation) and control system. It uses partial denitrification of nitrate to nitrite in the acidification reactor and ammonium production from aged sludge coming daily from acidification reactor. The effluent from each then flows to an anammox reactor, where ammonium and nitrite were transformed by anammox bacteria to nitrogen gas. So the final DeammRecirc technology for RAS, is based on partial denitrification and anammox, rather than deammonification as is common in the waste water industry.

In WP3, TI and ACI together with ARA and Cyklar developed the deammonification technology for RAS. A lot of experimentation with sea bass and salmon fresh sludge lies behind the pre-treatment development in task 3.1 ACI repeated a 3 day acidification experiment at AAL in November 2011 while also studying drum filter flow patterns and settlement characteristics. The experiment was repeated with vacuum filtration of the sludge water to avoid the oxygen mixing into the water sample which was believed to have taken place during first series of acidification experiments and with a more concentrated sludge solution. TI took sludge samples from Hardingsmolt in January and February 2012 and performed the same acidification experiment as ACI using normal sludge and concentrated sludge over a 3 day period, but at temperature typical for Hardingsmolt system water. The importance of working with fresh sludge rather than 3 day old – 1 week old sludge was very clear after this experimentation, as the N and C species react so quickly, even when cooled in fridge. After the data had been compiled and discussed, ACI together with Cyklar and ARA devised a pre-treatment concept for the marine RAS stocked with sea bass, using AAL and Swansea quarantine laboratory as models. It uses partial denitrification of nitrate to nitrite in the acidification reactor and ammonium production from aged sludge coming daily from acidification reactor. The effluent from each then flows to an anammox reactor, where ammonium and nitrite were transformed by anammox bacteria to nitrogen gas. So the final DeammRecirc technology for RAS, is based on partial denitrification and anammox, rather than deammonification as is common in the waste water industry.

The DeammRecirc prototype, consisting of a pre-treatment SBR, two anammox SBRs (to allow different types of operation) and control system was developed, parts ordered and assembled by ACI in Estonia. The reactors are kept at a constant temperature by a thermostat controlled water jacket surrounding the tanks. Cycles consist of a long mixing phase, settling phase and decanting phase. D3.1 written by TI, describes the development and building of the pre-treatment prototypes, while D3.2 written by ACI describes the development and building of the anammox reactors. Due to the lack of drum filters in the small quarantine RAS, fresh fish sludge was instead planned fed manually from bag meshes with RAS system water to the pre-treatment tank. All other pumping and mixing was automated by PLC control system. The development of the control system, potential sensors which could be selected and the final selection of sensors and level of automation is described in D3.3 written by TI. Here the comprehensive sampling regime and chemical monitoring of the DeammRecirc prototype is also fully described.

The most important foreground from WP4 –integration and validation – is the knowledge and experience generated in operating the DeammRecirc pilot plant for 10 months and accumulating 10 months of chemical monitoring results. It also includes results on increasing anammox activity, methods of tracking anammox, anammox bacteria identification into groups, load calculations, modelling work to simulate what these pilot plant results would mean at a full-scale farm and partial denitrification work.

In WP4, the first task for SU was to stock their RAS quarantine system with European sea bass (Dicentrarchus labrax) obtained from AAL and settle them in to produce sludge. Due to the discovery that DEMON® sludge could not be adapted to high salinity in task 2.1 SU began to grow their own inoculum from sea bass sludge from their own fish and AAL in March 2012, following a protocol prepared by LUH. After 5 months of care, enrichment and monitoring of two enrichment buckets, the inoculum showed clear signs of anammox activity and the presence of anammox activity was confirmed by 15N enrichment tests. The DeammRecirc prototype was integrated into the SU 10m3 RAS system stocked with sea bass in late April 2012 and some inoculum from the buckets mixed with other sludge was added in early July 2012. This work including inoculum growth and enrichment and results from integration are described in full in

D4.1 written by SU.

The pilot plant has been operated for 10 months with continual chemical monitoring by SU, particularly during start-up phase. They have devised methods to collect the most sludge by bag meshes and filled manually the acidification reactor twice daily with sludge, RAS water and chemically dosing nitrate. All manual and then automatic dosing has been performed by SU. Intensive 24 hour monitoring of one cycle within the reactor have enabled the anammox activity to be measured and it shows a 10 fold increase over 8 months. The final anammox activity measured by LUH (task 2.3) showed a very high activity under optimum mixing and anoxic conditions. Despite problems with leaking pumps, a failure of the RAS quarantine system at New Year and transfer of sea bass between RAS systems, SU has managed to keep the DeammRecirc plant operating, which shows good risk management. Both load calculations, a N balance for the pilot plant and modelling has been performed from the chemical monitoring results.

Further experiments were performed by both SU and ACI to understand the factors effecting partial denitrification – C source and amount, pH and salinity in sea bass sludge. Also the production of ammonium and COD by sludge was further investigated. All experiments were designed to give improve knowledge to better control the production of nitrite and ammonium for the anammox process.

PCR-DGGE analyses of the inoculum have shown clear presence of closely related anammox species to those found in natural marine samples, uncultured Candidatus Scalindua sp., Planctomycetes phylum and Verrucomicrobia phylum (specific details in D4.2). FISH analysis however of the inoculum at start and August failed to show any sign of anammox and did not prove a useful method in this project for fish sludge inoculum analysis. All results from operation of pilot plant, anammox identification and activities and additional experimentation and results are described in full in D4.2.

DeammRecirc has had good cooperation between all RTDs, most SMEs, and all other organisations.

There have been 4 consortium meetings; M10 in Hannover, M16 in Swansea, M22 in Oslo, and M28 in Swansea. There have been 7 technical meetings involving travel and 23 skype technical meetings, which have most involved work in task 2.3 WP3 and WP4. Overall consortium management has been done well (WP6). Dissemination in different languages and publications (Africa, Europe and globally) has been performed and an operation manual and feasibility study produced (WP5).

Potential Impact:

The human population is growing fast (7.124 million in June 2013) and the wild caught fish harvest has stagnated in many parts of the world. Aquaculture is therefore vital to supply enough fish and take the pressure off capture fisheries. Increasing regulatory pressure focusing on discharges to natural water bodies is forcing the aquaculture sector to adopt methods that are environmentally friendlier. Recirculation Aquaculture System (RAS) technology can reduce the effluent waste stream by a factor of 500-1000 and reduce water use drastically. Equally importantly, application of RAS technology to hatcheries can provide biosecurity against diseases and hence secure the industrial scale production of juvenile crustacea and fish species required for on-growing. RAS technology has been identified as a key area for expanding research to promote sustainable aquaculture development by the European Union. RAS treat internally the water contaminated with dissolved organics and ammonia and reduce the amount of new water use and discharge from aquaculture operations. A central component of any RAS system is the aerobic biological filter or biofilter that oxidises the toxic unionised ammonia excreted by the stock to nitrate by the nitrification process. As most RAS do not have a denitrification system, the nitrate accumulates in the system. Increasingly research is showing that high concentrations of nitrate, which would not occur in natural water bodies is not healthy for fish (Hamlin, 2006; Guillette & Edwards, 2005; Hrubec, 1996). High nitrate water is removed and system water diluted by flushing the farm with new replacement water. The nitrate rich effluent can cause eutrophication in the receiving water bodies, by encouraging excessive algae growth, which then decomposes reducing dissolved oxygen level and killing many organisms.

Farmed fish are increasingly being fed less fish, to reduce fishing pressure on natural fish and more plant material, e.g. soya bean in their feed (www.Ewos.com). These plants have been fertilized with inorganic nitrogen fertilizers, where nitrogen from the air has been fixed by the Haber process to ammonia. This ammonia is then reacted to form salts which are included in the fertilizers. It is important that this nitrogen returns to the atmosphere rather than accumulating in water bodies, where if can cause water pollution and eutrophication.

The DeammRecirc project has adapted the deammonification technology used very successfully in the waste water treatment industry for the RAS aquaculture sector and in the first instance, an important commercial marine species in Europe, European sea bass (Dicentrarchus labrax). The project has grown its own sea salt adapted inoculum and developed a methodology to allow other fish farms to grow their own biosecure inoculum in the future. The project has developed, built and tested the DeammRecirc prototype in a small marine RAS system stocked with sea bass at Swansea University. The prototype uses a part stream of RAS system water, high in nitrate and fresh fish sludge. This mixture is pre-treated by an innovative new method to convert the nitrate to nitrite. The effluent then flows to the anammox reactor where nitrite and ammonium (from aged sludge) react to form nitrogen gas (the anammox process). Denitrification (nitrate to nitrogen gas) also occurs in this reactor. Thus the DeammRecirc technology is converting nitrate in water to nitrogen gas in the air both by anammox and denitrification processes, thus reducing the nitrate load to recipient water bodies from RAS farms and at the same time providing a treatment of a waste product, the fish sludge.

The advances, when the DeammRecirc technology is fully developed compared to current state of the art, include reduced cost for buffering chemicals, lower carbon footprint, reduced levels of nitrate rich effluent released to the environment, reduced pumping cost and treatment need of new exchange water. These environmental improvements will benefit the whole society and the environment while all the advances benefit the fish farmers and companies using RAS. The SMEPs, who represent technology suppliers/sellers, plastic tank supplier and consultants for the waste water industry will generate new markets through future development of DeammRecirc technology to target the unique requirements of the aquaculture sector. More research and development is required before commercialization but the DeammRecirc project has taken major steps in the transfer of anammox technology to aquaculture.

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Last update: 27 June 2014

Permalink: https://cordis.europa.eu/project/id/262183/reporting