Nitrate removal from aquaculture effluents using woodchip bioreactors improved by adding sulfur granules and crushed seashells

This study examined the effects on nitrate removal when adding sulfur granules and crushed seashells to a woodchip bioreactor treating aquaculture effluents. Using a central composite design, the two components were added at three levels (0.000, 0.125 and 0.250 m³/m³ bioreactor volume) to 13 laboratory-scale woodchip bioreactors, and a response surface method was applied to find and model the optimal mixture ratios with respect to reactor performance. Adding 0.125 m³/m³ sulfur granules improved the total N removal rate from 3.27±0.38 to 8.12±0.49 g N/m³/d compared to pure woodchips. Furthermore, the inclusion of crushed seashells together with sulfur granules helped to maintain the pH above 7.4 and prevent a production (i.e., release) of nitrite. According to the modeled response surfaces, a sulfur granule:crushed seashell:woodchip mixture ratio containing about 0.2 m³ sulfur granules and 0.1 m³ crushed seashells per m³ reactor volume would give the best results with respect to high N removal and minimal nitrite release. In conclusion, the study showed that N removal in woodchip bioreactors may be improved by adding sulfur granules and seashells, contributing to the optimization of woodchip performance in treating aquaculture effluents.
Nutrient removal in a constructed wetland treating aquaculture effluent at short hydraulic retention time

We examined the longitudinal and seasonal removal of dissolved and particulate nutrient components in a free water surface (FWS) constructed wetland treating all the effluent from a commercial recirculating rainbow trout Oncorhynchus mykiss farm. The wetland consisted of a meandering, 0.7 m deep channel with a total FWS area of 5811 m², a total
hydraulic loading rate (HLR) of 2.23 m d\(^{-1}\), and a total hydraulic retention time (HRT) of 0.32 d. Bi-weekly, 24 h composite samples were obtained along the wetland for 1 yr and analysed for dissolved and particulate nutrient components. Furthermore, a short sampling campaign assessed the sedimentation of particles (5 to 200 \(\mu\)m). A first order kinetic plug flow model was fitted to the longitudinal data, and a first set of area-based removal rate constants (k(A)) for this wetland type was derived. Sedimentation led to particulate nutrient removal, but there was no annual net removal of dissolved nutrients aside from an infinitesimal removal of phosphorus. Microbial removal processes were substrate-limited, and removal rate constants followed an annual cycle presumably coupled to available plant surface area and temperature. Denitrification was limited by low carbon availability and high oxygen concentrations, and the wetland became a net producer of nitrate at times due to oxygenation of ammonia. In summary, dissolved nutrients were largely not removed and the wetland was over-dimensioned for particulate nutrient removal. This new insight should be taken into account in future efforts to improve the treatment performance of similar types of aquaculture wetlands operated at short hydraulic retention times.
Particle surface area and bacterial activity in recirculating aquaculture systems
Suspended particles in recirculating aquaculture systems (RAS) provide surface area that can be colonized by bacteria. More particles accumulate as the intensity of recirculation increases thus potentially increasing the bacterial carrying capacity of the systems. Applying a recent, rapid, culture-independent fluorometric detection method (Bactiquant®) for measuring bacterial activity, the current study explored the relationship between total particle surface area (TSA, derived from the size distribution of particles >5 μm) and bacterial activity in freshwater RAS operated at increasing intensity of recirculation (feed loading from 0.043 to 3.13 kg feed m⁻³ make-up water). Four independent sets of water samples from different systems were analyzed and compared including samples from: (i) two individual constructed wetlands treating the effluent system water from two commercial, freshwater rainbow trout (Oncorhynchus mykiss) farms of different recirculation intensity; (ii) an 8.5 m³ pilot scale RAS; and (iii) twelve identical, 1.7 m³ pilot scale RAS assigned one of four micro-screen treatments (no micro-screen, 100, 60, or 20 μm mesh size micro-screens) in triplicate. There was a strong, positive, linear correlation (p < 0.05) between TSA and bacterial activity in all systems with low to moderate recirculation intensity (i.e. feed loading ≤1 kg feed m⁻³ make-up water). However, the relationship apparently ceased to exist in the systems with highest recirculation intensity (feed loading 3.13 kg feed m⁻³ make-up water; corresponding to 0.32 m³ make-up water kg⁻¹ feed). This was likely due to the accumulation of dissolved nutrients sustaining free-living bacterial populations, and/or accumulation of suspended colloids and fine particles less than 5 μm in diameter, which were not characterized in the study but may provide significant surface area. Hence, the study substantiates that particles in RAS provide surface area supporting bacterial activity, and that particles play a key role in controlling the bacterial carrying capacity at least in less intensive RAS. Applying fast, culture-independent techniques for determining bacterial activity might provide a means for future monitoring and assessment of microbial water quality in aquaculture farming systems.
New approaches to improve the removal of dissolved organic matter and nitrogen in aquaculture

Reducing the environmental impact of aquaculture requires that waste treatment practices are further improved. Currently applied treatment technologies achieve good solids removal and nitrification. Yet discharge of nitrogen (N) and organic matter (OM) from fish farms is still often an important issue constraining aquaculture development, especially in sensitive areas. Possibilities for efficient end-of-pipe treatment exist for large intensive recirculating aquaculture systems (RAS), while smaller and especially the technically less advanced fish farms, struggle to reduce nutrient discharge further due to the lack of cost-effective and easy applicable treatment methods for removing dissolved N and OM. The purpose of this PhD thesis was to assess the problem of removing dissolved N and OM in the context of the large differences in system intensity between farms, and to devise new, simple methods for removing dissolved N and OM from aquaculture effluents of technically less advanced farms in particular. The work split in two parts. The first part focused on the turnover of dissolved N-compounds (Paper I) and dissolved organic matter (DOM) (Paper II) and in aerobic biofilters operated at increasing long-term waste loadings. The second part examined the potential of using anoxic denitrifying woodchip bioreactors for removal of nitrate from aquaculture effluent (Paper III-V). Investigations within the first part showed that the effectiveness of biofilters, as determined by their areal removal rates, for removing DOM and degrading ammonia, nitrite and urea, increases with increasing long-term waste loading. The findings sustained/suggested that DOM to (some extend)? can be removed by biofiltration, and that biofilters therefore may be applied for removing DOM from aquaculture effluents. The studies furthermore showed that degradation of urea contributes to the ongoing nitrification activity in aquaculture biofilters, and that the transition zone from first order (substrate dependent) to zero order (substrate independent) degradation of ammonia and nitrite was elevated with increasing long-term biofilter loading up to a certain threshold. The latter indicated that the removal capacity of biofilters operated at lower loadings is easily exceeded, and that they may not respond very well to sudden increases in total ammonia nitrogen (TAN) concentrations. In the second part of the thesis, a field study documented the start-up performance of a pilot-scale, denitrifying woodchip bioreactor at a commercial outdoor fish farm (Paper III). Nitrate removal was immediate after bioreactor start-up and was accompanied by short-term leaching of nutrients and organic matter from the woodchips. The study demonstrated that woodchip bioreactors are able to remove nitrate from dilute aquaculture effluents under commercial conditions. The obtained nitrate removal rate (7.06±0.81 g NO3-N/m3d at ~8°C) was, however, relatively low, signifying that a quite large reactor would be required for complete removal of NO3-N at commercial farms. Laboratory studies were therefore carried out to test whether removal rates in woodchip bioreactor could be improved. Paper IV demonstrated that simultaneously changing the hydraulic retention time and adding bicarbonate to the inlet water of laboratory-scale woodchip bioreactors improved N removal. Moreover, the study indicated that sulfur-based autotrophic denitrification is potentially important to the overall N removal in woodchip bioreactors. A subsequent laboratory study demonstrated that higher N removal rates could be achieved in mixotrophic denitrification reactors containing mixtures of woodchips, sulfur granules and seashells (Paper V).

12 Altogether, the woodchip studies sustained that denitrifying woodchip bioreactors may represent an alternative and simple method for removing nitrate from dilute-low-organic-strength aquaculture effluents for which application of, for example, heterotrophic denitrification reactors needing input of organic carbon sources is generally not feasible.

General information

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Optimizing nitrate removal in woodchip beds treating aquaculture effluents

Nitrate is typically removed from aquaculture effluents using heterotrophic denitrification reactors. Heterotrophic denitrification reactors, however, require a constant input of readily available organic carbon (C) sources which limits their application in many aquaculture systems for practical and/or economic reasons. A potential alternative technology for removing nitrate currently applied for treating surface and drainage water is based on using wood by-products as a carbon source for denitrification. Using lab-scale horizontal-flow woodchip filters, the current study investigated the potential of optimizing woodchip reactors for treating aquaculture effluent. A central composite design (CCD) was applied to assess the effects of simultaneously changing the empty bed contact time (EBCTs of 5.0-15.0 h; corresponding to theoretical hydraulic retention times of 3.3-9.9 h) and bicarbonate (HCO3-) inlet concentration (0.50-1.59 g HCO3-/l) on the removal rate of NO3-N, and additional organic and inorganic nutrients, in effluent deriving from an experimental recirculating
aquaculture system (RAS). Volumetric NO3-N removal rates ranged from 5.20 ± 0.02 to 8.96 ± 0.19 g/m3/day and were enhanced by adding bicarbonate, suggesting that parts of the removal was due to autotrophic denitrification. The highest N removal rate (8.96 ± 0.05 g/m3/day) was achieved at an EBCT and HCO3- combination of 15 h and 1.59 g HCO3-l. Bicarbonate inlet concentration as a single factor had the strongest effect on N removal rates followed by the interaction with EBCT, and EBCT2 (quadratic term). The study thus indicates that woodchip beds may be applied and optimized for removing nitrate from aquaculture effluents. Statement of relevance: This study is a relevant contribution to research in aquaculture as it presents an alternative method for removing nitrates from aquaculture effluents especially for less intensive fish farms. Furthermore, it shows how this method can be optimized to yield higher removal rates of nitrate.

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ISI indexed (2011): ISI indexed yes
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Start-up performance of a woodchip bioreactor operated end-of-pipe at a commercial fish farm—A case study

There is a need for simple, maintenance-free technologies for removing nitrogen (N) from aquaculture effluents. Denitrifying woodchip bioreactors have been used successfully to remove nitrate-N (NO3-N) from ground and surface waters and may potentially be applied to dilute aquaculture effluents as well. Real-life applicability in commercial, outdoor fish farms including practical start-up issues such as e.g. time till stable performance and potential leaching are, however, unknown to the industry. This case study consequently investigated the temporal performance of a woodchip bioreactor (12.5 m3) during start-up. The bioreactor was operated end-of-pipe at a commercial, outdoor rainbow trout (Oncorhynchus mykiss) farm in Denmark operated at low recirculation intensity. Applying an empty bed contact time (EBCT) of 5 h, the specific objectives of the study were to resolve: i) how fast the bioreactor would start to remove NO3-N; ii) how fast steady state was achieved; iii) which NO3-N removal rates could be attained at the relatively low effluent temperature (∼8 °C) and iv) to which extent any concomitant leaching of phosphorous (P), ammonia or organic matter would occur. In- and outlet grab samples were obtained every 6 h until the bioreactor was in steady state (2 weeks) followed by weekly 24 h pooled samples for another 3 weeks (5 weeks in total). Additional grab samples were obtained from 9 sampling ports within the bioreactor on 3 consecutive days during steady state. Samples were analyzed for dissolved nutrients (total N, nitrate, nitrite, ammonium, total phosphorous, ortho-phosphorous, BOD5 and COD). In addition, oxygen, temperature and pH were logged every 30 min while sampling and alkalinity were measured once a week. Removal of NO3-N started immediately and remained stable at 7.06 ± 0.81 g NO3-N/m3/d (n = 6) throughout the sampling period. Increased effluent NO2-N concentrations (peaking at 1.14 mg NO2-N/l after 4–5 days) were transiently observed during the initial 11 days. After that, the woodchip bioreactor was largely in steady state with respect to N-balances corroborated by a close match between filtered total-N (TNdiss) and NO3-N removal rates. Measurements within the bed showed that the majority of the influent dissolved oxygen (DO) was consumed within the first part of the bioreactor and that NO3-N removal thereafter proceeded gradually with distance within the bed. Leaching of non-structural, dissolved organic compounds were observed just after startup, causing a short-term (1 week) increase in effluent concentrations of COD, BOD5, P and ammonium. Additional measurements carried out until 147 days after start-up showed that the woodchip bioreactor continued to remove TNdiss at an average removal rate of 7.81 ± 0.82 g N/m3/d, and that the initial leakage of P stopped altogether. In summary, the study demonstrated that woodchip bioreactors can effectively remove NO3-N from dilute aquacultural effluents at low temperatures and commercial conditions and that stable performance is achieved within a
few weeks.

**General information**

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  Scopus rating (2010): SJR 0.546 SNIP 0.936
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  BFI (2009): BFI-level 1
  Scopus rating (2009): SJR 0.717 SNIP 1.393
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  Scopus rating (2008): SJR 0.732 SNIP 1.15
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Degradation of urea, ammonia and nitrite in moving bed biofilters operated at different feed loadings

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BFI (2013): BFI-level 1
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End-of-pipe removal of nitrogen using woodchip beds

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Room for all? - particulate surface area and bacterial activity in RAS

General information
Removal of urea, ammonium and nitrite in moving bed biofilters operated at different loadings

Projects:

Future growth in sustainable, resilient and climate friendly organic and conventional European aquaculture (FutureEUAqua) (39494)

The overall objective of FutureEUAqua is to effectively promote sustainable growth of resilient to climate changes, environmentally friendly organic and conventional aquaculture of major fish species and low trophic level organisms in Europe, to meet future challenges with respect to the growing consumer demand for high quality, nutritious and responsibly produced food. To this end, FutureEUAqua will promote innovations in the whole value chain, including genetic selection, ingredients and feeds, non-invasive monitoring technologies, innovative fish products and packaging methods, optimal production systems such as IMTA and RAS, taking into account socioeconomic considerations by the participation of a wide spectrum of stakeholders, training and dissemination activities. To achieve the objective and to relate to the work program, nine work packages will contribute to improvements of future aquaculture. To ensure sustainable and resilient production of fish, FutureEUAqua will work with tailor made fish and feed, and validate fish performance and water quality in cost effective production systems. Consumer demand and awareness of how to choose sustainable and climate friendly seafood. With the increasing production of seafood, we face space-conflicts, which, in combination with the current regulatory frameworks will be considered. Wireless sensor technology for health and welfare monitoring and novel technology for product quality and packaging to meet future demands, will be implemented. Stakeholders' knowledge and views will be important, and communication, dissemination as well as training sessions will be emphasized. The project is coordinated by NOFIMA, Norway and is funded by HORIZON 2020 Blue Growth Programme.

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Pedersen, L., Project Participant, National Institute of Aquatic Resources
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01/10/2018 → 30/09/2022

Keywords: Research area: Aquaculture
**Green switch in Danish Aquaculture by changeover to recirculation (GODAOR) (39462)**
The overall aim of the project is to disseminate scientific knowledge and practical experiences regarding optimum use of recirculation technology in land based fish farming. The main concrete aims are: 1. To promote green and economic sustainability in recirculation fish farming by optimum use of recirculation technology to minimize the specific discharge of nutrients (nitrogen, phosphorus and organic matter) from the fish production. 2. To strengthen green switch by increased use of recirculation technology by supporting the changeover from traditional pond farming to modern recirculation technology. This is based on knowledge and experience from research- and development projects. E.g. will optimum designed farms and management reflect less fish diseases, less mortality and improved feed utilization concomitant with better fish welfare. This project is coordinated by the Danish Aquaculture Organisation and is funded by Green Growth and Development Program (GUDP).

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01/07/2017 → 30/06/2021
**Keywords:** Research area: Aquaculture
**Collaborators:** University of Copenhagen, Private veterinarians, Danish Aquaculture Association

**Design and operation optimization of constructed wetlands at rainbow trout farms (39430)**
This project aims at improving the design and operation of constructed wetlands with respect to the removal of waste nutrients and organic matter deriving from model trout farm systems type I and III. The project contains five work packages: 1. Selection of representative fish farms to be part of a user group and where testing and measurements will be carried out 2. Mapping and characterization of selected wetlands 3. Measuring the effects of flow velocity, water column depth, and hydraulic retention time on the removal of nutrients and organic matter 4. Data analysis 5. Project management, administration and dissemination of results. The projects is coordinated by DTU Aqua. The project is funded by Ministry of Environment and Food of Denmark and the European Maritime and Fisheries Fund (EMFF).

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von Ahnen, M., Project Participant, National Institute of Aquatic Resources
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**Keywords:** Research area: Aquaculture
**Collaborators:** Danish Aquaculture Association

**Environmentally effective nitrogen removal in fish farming using sludge hydrolysis (wiN-wiN) (39119)**
Reducing nitrogen discharge is important to fish farms and their environmental performance. Removal of nitrogen can be done by applying denitrification filters end-of-pipe (i.e. before discharge) through an anaerobic de-nitrification process using organic carbon as energy source. Using external carbon is costly and introduces additional organic matter into the system. In contrast, sludge produced by the farmed fish might provide the organic matter given that a hydrolysis process can be controlled and optimised according to the needs of the denitrification process. The project strives to establish, optimize and demonstrate an integrated system in commercial scale able to hydrolyse generated sludge and subsequently use it as energy source for nitrogen removal in end-of-pipe denitrification filters. This project is coordinated by HME, Denmark. The project is funded by the Danish Ministry of Food, Agriculture and Fisheries through the Green Development and Demonstration Program (GUDP) and the partners involved.

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**Keywords:** Research area: Aquaculture
**Collaborators:** HME, Lundby Dambrug

**Traditional trout farms (38095)**
Nutrient removal is imperative for Danish fish farms and upcoming requirements for environmental performance though application of BAT (Best Available Technology) cannot be met by traditional fish farms since simple, low-cost technologies does not exist. Removal of dissolved nutrients in low concentrations and large water volumes is especially difficult. In the project, potential low-cost technologies for removing nitrogen and organic matter were tested and documented. Removal and turn-over of organic matter and nitrogen in biofilters was studied, and the performance of constructed wetlands on
traditional farms was also investigated during a full year. In these farms, wetlands efficiently remove particulate matter and associated nutrients (O and P) whereas dissolved matter is almost not removed due to the hydraulic load and short residence time. Depending on the concentrations in the incoming water, requirements for O and P net-removal could be met, whereas a need for simple, low-cost nitrogen removal was clearly demonstrated. This project was coordinated by the Danish Aquaculture Association. The project is funded by the Danish Ministry of Food, Agriculture and Fisheries and the European Fisheries Fund (EFF).

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Keywords: Research area: Aquaculture
Collaborators: Commercial trout farms, Danish Aquaculture Association
Project: Research

**IT-solutions for environmental control of trout farms (39094)**

In the project a IT-solution for direct reporting of environmental performance data from trout farms to the Municipality registration system was developed. Also, calculations of compliance with allowances etc. can be calculated and evaluated regularly by the farmer. DTU Aqua further developed a discharge prediction model, able to calculate the resulting discharge from a ModelTroutFarm of any given layout and dimensions. This model ("Dambrugsmodellen" i.e. "the Trout Farm Model") is based on the existing Produktionsbidragsmodel ("Waste Production Model") and data and monitoring results from all treatment devices added and incorporated into a prediktive model. Both models are now widely used by the authorities as well as in the industry. This project was coordinated by the Danish Aquaculture Association. The project was funded by the Danish Ministry of Food, Agriculture and Fisheries and the European Fisheries Fund (EFF).

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Keywords: Research area: Aquaculture
Collaborators: Trout farms, Aarhus University, DHI Denmark, Danish Aquaculture Association
Project: Research

**New approaches and methods to improve the removal of dissolved nutrients in aquaculture**

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Pedersen, P. B., Supervisor, National Institute of Aquatic Resources
Jokumsen, A., Examiner, National Institute of Aquatic Resources
Healy, M. G., Examiner
Schulz, C., Examiner
1/3 DTU-stip, 2/3 FUR/andet
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Award relations: New approaches and methods to improve the removal of dissolved nutrients in aquaculture
Project: PhD

**Development of sustainable technologies and modeling tools in aquaculture aiming at increasing overall production (UDTÆNK) (39030)**

The project aimed at developing methods and modeling tools that may assist the aquaculture industry in expanding its production while minimizing the environmental impact. To obtain this, the project included six work packages concerning: - Increased production of rainbow trout by providing methods for reducing the discharge of nitrogen and organic matter. - Increased production in net cages by providing academic guidance to social workers on concurrent production of trout and mussels. - Improved sustainability of the industry by providing guidance on optimal system design with respect to reducing nutrient discharge. The project was funded by the Danish Ministry of Food, Agriculture and Fisheries and the European Fisheries Fund (EFF).

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Steenfeldt, S. J., Project Participant, National Institute of Aquatic Resources
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Collaborators: Dansk Akvakultur
Project: Research