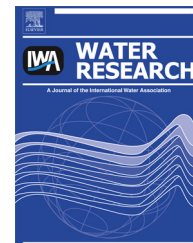




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# Bacterially mediated removal of phosphorus and cycling of nitrate and sulfate in the waste stream of a “zero-discharge” recirculating mariculture system

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## ABSTRACT

Simultaneous removal of nitrogen and phosphorus by microbial biofilters has been used in a variety of water treatment systems including treatment systems in aquaculture. In this study, phosphorus, nitrate and sulfate cycling in the anaerobic loop of a zero-discharge, recirculating mariculture system was investigated using detailed geochemical measurements in the sludge layer of the digestion basin. High concentrations of nitrate and sulfate, circulating in the overlying water (~15 mM), were removed by microbial respiration in the sludge resulting in a sulfide accumulation of up to 3 mM. Modelling of the observed S and O isotopic ratios in the surface sludge suggested that, with time, major respiration processes shifted from heterotrophic nitrate and sulfate reduction to autotrophic nitrate reduction. The much higher inorganic P content of the sludge relative to the fish feces is attributed to conversion of organic P to authigenic apatite. This conclusion is supported by: (a) X-ray diffraction analyses, which pointed to an accumulation of a calcium phosphate mineral phase that was different from P phases found in the feces, (b) the calculation that the pore waters of the sludge were highly oversaturated with respect to hydroxyapatite (saturation index = 4.87) and (c) there was a decrease in phosphate (and in the Ca/Na molar ratio) in the pore waters simultaneous with an increase in ammonia showing there had to be an additional P removal process at the same time as the heterotrophic breakdown of organic matter.

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## 1. Introduction

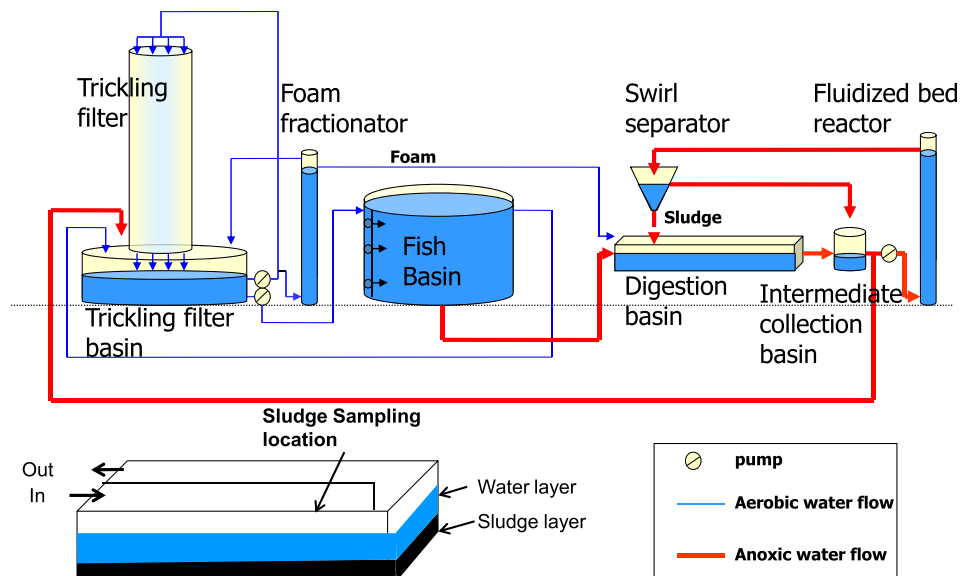
Fish cages, a widely used industrial mariculture technology, typically discharge up to 80% of the nitrogen and phosphorus that is supplied in the feed into the environment (Naylor et al., 1998; van Rijn, 2013). Land based mariculture offers more control of the waste, but is often limited by the shortage of coastal sites and the cost of inland pumping of seawater and its discharge. The “Zero-Discharge System” (ZDS) is a recently developed sustainable mariculture system (Gelfand et al., 2003) which uses natural microbial processes to control water quality (Cytryn et al., 2003; Gelfand et al., 2003; Neori et al., 2007). The system operates in a completely sealed way, meaning that only a small amount of freshwater is used to replace losses by evaporation. There is no continuous or even intermittent discharge of aqueous effluent to the environment as exists in other mariculture systems. Although the advantages of ZDS mariculture systems in terms of waste output are clear, the mechanisms behind the nitrogen, sulfur and phosphorus cycling in such systems are not well understood.

The ZDS consists of two water treatment loops (Fig. 1). The aerobic loop converts toxic ammonia produced by fish to nitrate by means of a trickling biofilter. In the second loop, an anaerobic loop, consisting of a digestion basin (DB) and fluidized bed reactor, particulate waste organic matter (principally fish feces) and other nutrients are metabolized to environmentally harmless forms. Previous studies on this and similar systems revealed that the major processes affecting the overall water quality are nitrification in the aerobic treatment loop and bacterial breakdown of organic matter by processes including heterotrophic nitrate and sulfate reduction as well as autotrophic nitrate reduction coupled to sulfide oxidation in the DB and fluidized bed reactor (Gelfand et al., 2003; Cytryn et al., 2005; Neori et al., 2007; Sher et al., 2008; Schneider et al., 2011). However the relative contribution of

these anaerobic bacterial processes was not known. Around 70% of the C and N supplied is lost as carbon dioxide and gaseous nitrogen species, presumed to be the result of heterotrophic bacterial respiration (Neori et al., 2007). Of the phosphorus supplied with the fish feed, 21% is taken up for fish growth. Only 5% of the remaining phosphorus accumulates in the water column while the rest is present as solid and pore water phosphorus, mainly in the DB sludge accumulating in the anaerobic treatment loop. It was not known in what form this P accumulates in the sludge nor what processes are controlling this accumulation.

Simultaneous removal of nitrogen (N) and phosphorus (P) by microbial biofilters has been used in a variety of water treatment systems to treat nutrient-rich waste streams. These include systems that use alternating aerobic-anaerobic conditions to trap phosphate as polyphosphate under aerobic (van Loosdrecht et al., 1997) or denitrifying conditions (van Loosdrecht et al., 1998) and release it in a controlled way during the anaerobic cycle. The DB of the ZDS system has free oxygen in the overlying water while the sludge itself is anaerobic with the precise location of the redox boundary depending on the balance of recycling processes within the system. In the DB examined in this study, N and P were found to be simultaneously removed from the waste stream by the accumulation of P in denitrifying organisms under entirely anoxic conditions (Barak and van Rijn, 2000a, 2000b; Barak et al., 2003; Neori et al., 2007).

Similar microbial processes to those in the digestion basin, may occur in natural marine systems particularly in sediments underneath the upwelling regions of the world such as the Benguela current off Namibia and off Oman in the Arabian Sea. These locations have high concentrations of organic matter in the sediment (up to 40%), much of which is labile causing high rates of heterotrophic bacterial activity including sulfate reduction and methane production (Schulz et al., 1999). Phosphorite (diagenetic apatite) nodules often form in



**Fig. 1** – A diagram of the system as a whole including a more detailed diagram of the digestion basin showing the location of the sludge sampling.

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