Development of a single-sludge denitrification method for nitrate removal from RAS effluents: Lab-scale results vs. model prediction

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Abstract

A lab-scale activated-sludge type reactor was used to induce denitrification by using the organic solid waste of a typical Recirculating Aquaculture System (RAS) as the electron donor (‘Single-sludge denitrification’). The results were compared with the predictions of a stoichiometry-based model. As predicted by the model, reactor’s performance was found to be strongly related to the mean solids retention time (SRT) employed. Measured denitrification rates conformed very well to model predictions. High nitrate removal rates of up to 590 mg N (Lreactor d)−1 were recorded at a relatively low SRT of 4 d. Oxygen, that entered the reactor via both atmospheric diffusion and with the stream used to simulate the influent from a fish tank, reduced the amount of organic matter available for denitrification, resulting in lower denitrification rates. This interference was more significant when the system was operated at the longer SRTs. Most of the excess ammonia released to the aqueous phase through ammonification was oxidized (presumably by anammox bacteria) under the prevailing anoxic conditions, resulting in very low effluent TAN concentrations. Phosphate release to the aqueous phase was significantly lower than predicted, suggesting above-typical microbial P assimilation. Reaction kinetics was found to be zero order with respect to nitrate at concentrations of above 1.5 to 2.0 mg N L−1. Taken together the findings indicate that intensive single-sludge denitrification for treating RAS effluents is technically feasible, and that the process appears to be a cost-effective solution to reducing both the nutrient and the organic loads generated by intensive fish farms. Reaction kinetics was found to be zero order with respect to nitrate at concentrations of above 1.5 to 2.0 mg N L−1. Taken together the findings indicate that intensive single-sludge denitrification for treating RAS effluents is technically feasible, and that the process appears to be a cost-effective solution to reducing both the nutrient and the organic loads generated by intensive fish farms. The main advantages of the method include minimal formation of undesired by-products, small reactor volume and simple control and operation. Furthermore, the process is well described by a conceptual mathematical model, allowing its application as a part of any RAS design.

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1. Introduction

Recirculating Aquaculture Systems (RAS) are being developed as an alternative to traditional aquaculture technologies. One of the main advantages associated with RAS operation is low water consumption, which in seawater based RAS, results in reduced pumping costs. Low effluent flow-rate also allows a more cost-effective pollutant removal scheme. Most commercial RAS, however, do not include a nitrate removal unit because nitrate is relatively non-toxic to most aquatic species (Van Rijn, 1996), and because its removal is, by and large, not obligatory. However, since the release of large nitrate fluxes has been
shown to adversely affect aquatic ecosystems, its discharge is anticipated to become strictly regulated in the near future in various states, including Israel. As a result, the main goal of the work presented here was to develop an economic solution for minimizing the discharge of nitrate with RAS effluents.

Nitrate removal from wastewaters is commonly achieved by employing the bacterial process of denitrification, in which nitrate is reduced to innocuous nitrogen gas (N₂). The process requires an electron donor to supply electrons (energy) to the bacteria. The electron donor is typically supplied in the form of a soluble organic substance, such as methanol. This is a costly procedure, which, however, results in high denitrification rates and low reactor volumes. Alternatively, the electron donor may be supplied from within the system itself, i.e., an intrinsic energy source. This method is known in the environmental-engineering terminology as ‘single-sludge’ denitrification. By implementing this method, the need for the relatively expensive external carbon source is minimized. Additionally, a significant reduction in the mass of solid wastes produced by the fish farm can be achieved. In marine RAS application, this advantage is of considerable importance, because solutions for the disposal of high salinity organic wastes hardly exist.

Studies on denitrification in RAS using the intrinsic solids as the energy source are scarce. Two completely-closed recirculating systems (i.e., with no exchange of water, except for compensation for evaporation losses) that applied a single reactor for both nitrification and denitrification, and that used the intrinsic solids as the electron donor for the denitrification step, were described by Jewell and Cummings (1990) and by Knosche (1994). Although high nitrogen removal efficiencies were reported, the high level of control over dissolved oxygen concentration that was required seems to limit the feasibility of this approach. Additionally, nitrate accumulated in these systems to relatively high concentrations, which are unacceptable in RAS that are not completely-closed (i.e., systems from which a certain volume of water is continuously discharged to the environment). Arbiv and van Rijn (1995) described a fluidized bed denitrification reactor that operated within a semi-closed RAS. Solids generated in the RAS were pre-digested under anaerobic conditions in a ‘sedimentation basin’ to produce volatile fatty acids (VFA) that served as the electron donor in the following denitrification stage. Recent developments on the same system (e.g., Shnel et al., 2002; Gelfand et al., 2003) indicate that the bulk removal of nitrate actually takes place during the sludge digestion stage, and the fluidized bed reactor is currently used for removing the sulfide which is generated under the anaerobic conditions that prevail in the sedimentation basin. All of the three systems described above require a relatively high level of control and daily maintenance, which are undesirable in a competitive fish farm. Furthermore, no general process model has been laid out to direct the design of either system. Klas et al. (2006-this issue) proposed a conceptual stoichiometry-based model to describe the performance of a single-sludge denitrification reactor, used for treating RAS effluents. The proposed methodology was aimed at predicting the performance of a single activated-sludge (AS) type denitrification reactor, based on a comprehensive analysis of the intrinsic organic matter extracted from the back-wash of the RAS’s solids filter. The inputs to the model are the generic chemical formula of the Back-Wash Organic Matter (BWOM), which represents the substrate, the formula of the bacterial cells generated, and the non-biodegradable (in practical terms) organic matter fraction of the BWOM (termed X_{sb}). Klas et al. (2006-this issue) showed that the BWOM of the investigated system could be described by the generic formula C_{7}H_{13.4}O_{3.3}P_{0.3}N. Based on this formula and assuming that the bacterial cells can be represented by the formula C_{6}H_{12}O_{2}P_{0.1}N (Metcalf and Eddy, 2003), the following denitrification reaction was developed as a function of the apparent yield constant, \( Y \) (for \( 0 < Y < 0.53 \) g COD (g COD)\(^{-1}\)).

\[
\begin{align*}
\text{C}_{7}\text{H}_{13.4}\text{O}_{3.3}\text{P}_{0.3}\text{N} + (6.6-6.3Y)\text{NO}_{3}^{-} \\
+ (5.8-6Y)\text{H}^{+} \rightarrow (3.3-3.2Y)\text{N}_{2} \\
+ (7-7.7Y)\text{CO}_{2} + (1-1.54Y)\text{NH}_{3} \\
+ (8.1-6Y)\text{H}_{2}\text{O} + (1.54Y)\text{C}_{5}\text{H}_{7}\text{O}_{2}\text{P}_{0.1}\text{N} \\
+ (0.3-0.15Y)\text{PO}_{4}^{3-}
\end{align*}
\]

To run the model, additional operational parameters such as the organic matter concentration in the reactor (COD\(_{r}\)), the reactor volume (\( V_{r} \)), the mean solids retention time (SRT) employed, the flow-rate (\( Q \)), the dissolved oxygen concentration in the inflow (DO) and the organic matter concentration in the effluents (COD\(_{e}\)), are required as inputs. Based on these, the daily flux of excess sludge (FCOD\(_{sl}\)) is computed, as well as the apparent yield coefficient (\( Y \)). Once the apparent yield coefficient is known, the flux of various by-products of the process, including ammonia, carbon dioxide, phosphate and alkalinity can be determined, based on Eq. (1).

Eq. (1) also enables the prediction of the parameter “COD to nitrate consumption ratio” (COD/N). This parameter allows the computation of the volumetric
and specific denitrification rates \( R_D \) and \( R_D' \) respectively). By knowing the ratio between the recoverable COD to the produced nitrate in a given RAS, the total nitrate removal efficiency can also be predicted. **Fig. 1** shows schematically the inputs and outputs of the model proposed by Klas et al. (2006-this issue).

The purpose of the work presented in this paper was to compare the predictions of the conceptual model described above with results that were derived from the continuous operation of an experimental lab-scale reactor. The conclusions enabled a preliminary assessment of the potential of the process to be applied in commercial RAS farms.

2. Materials and methods

2.1. Analytical methods

Particulate COD concentration in seawater was measured according to APHA (1995) after the sample had been rinsed by distilled water and was consecutively centrifuged for chloride ion removal. TSS and VSS were determined according to APHA (1995). Filtered samples were washed with distilled water in order to wash out salt residues. Nitrite (NO\(_2\)\(^-\)) analysis was based on spectrophotometric measurement through coupling of diazotized sulfanilamide with \( N\)-(1-naphthyl)-ethylenediamine dihydrochloride (NED-dihydrochloride) (APHA, 1995).

The combined nitrate (NO\(_3\)\(^-\)) and nitrite concentration was measured by an automated cadmium reduction of nitrate to nitrite, and then nitrite concentration was measured as described above (APHA, 1995). Phosphate (PO\(_4\)\(^3-\)) was determined by an automated ascorbic acid method (APHA, 1995). Ammonia (NH\(_4\)+) was determined by Indophenol formation with Sodium Salicylate method (Verdouw et al., 1977).

2.2. Operation of an activated-sludge denitrification experiment

**Fig. 2** shows a scheme of the activated-sludge denitrification reactor that was operated continuously for 136 days. The denitrification reaction was carried out in a 15 L continuously stirred rectangular tank (oxidation tank) maintained at a temperature of 25 (±1) °C. Simulated fish tank water, made of fresh Mediterranean seawater to which NaNO\(_3\) was added, was continuously pumped into the oxidation tank. The overflow of the oxidation tank flowed gravitationally to a 7-L, 13.8-cm internal-diameter settling cylinder, which was mixed at approximately 0.15 rpm in order to prevent formation of a sludge cake at the bottom. Settled sludge was pumped back to the oxidation tank by a peristaltic pump, at a constant flow-rate of approximately 60-L d\(^{-1}\). Effluents leaving the settler flowed gravitationally to a 100 L storage container.

In order to minimize fresh seawater supply, the inflow into the reactor was maintained at a maximum rate of 24-30 L d\(^{-1}\), leading to a relatively long hydraulic retention time of between 12 and 16 h in the oxidation tank. Nitrate concentrations in the inflow were adjusted to produce the required loads. The organic feed (BWOM) was obtained from the back-wash water of the solids filter of an experimental RAS. The 5 m\(^3\) low-head experimental RAS was stocked by approximately 250 kg Gilthead seabream (Sparus Aurata) and was located at the Israeli National Center for Mariculture (NCM) in Eilat. The solids were shipped to the lab at the Technion, Haifa under cooling conditions and than stored in a sealed refrigerated (4 °C) tank for up to 10 d. BWOM feed flux (FCOD\(_{in}\)) was normally supplied to the reactor once a day in a batch manner. FCOD\(_{in}\) values were derived from TSS measurements, based on an approximate ratio of 1.07 g COD per g TSS, which characterizes the BWOM solids (Klas et al., 2006-this issue). In order to control the SRT, an appropriate volume of surplus sludge was drawn out every day prior to the daily BWOM addition.
OUR (Oxygen Uptake Rate) tests were conducted in a batch manner once in each operational mode (i.e. once for each SRT employed), using sludge from the reactor, to determine respiration rate profiles versus time. BWOM was added to the sludge at the same ratio as it was added into the AS system. The sludge was diluted (4:1) only at the SRT of 4 days because oxygen respiration rates were too high to enable accurate reading.

Reaction kinetics was measured in batch experiments. A sludge sample from the reactor was diluted by fresh seawater in a 2 L sealed container to which a low nitrate concentration was added together with a large excess of BWOM solids (over 5 times the organic load of the AS system, in relative terms). The dilution rate depended on the SRT and on the total time available for the test — usually 4 to 5 h. The container was mixed and maintained at a constant temperature of 25 \( (^\circ \text{C}) \). 20 mL samples of the supernatant were taken, at 15 to 30 min intervals, after the solids had been settled for 2 min. The extracted supernatant was exchanged by fresh deoxygenated seawater. Nitrate removal rate was calculated for each sampling interval and was plotted against the average nitrate concentration. Apparent nitrate half saturation constant \( K_{SN, NO_{3}^-} \) values were derived under the assumption of Monod-type reaction kinetics.

Temperature, pH, and oxidation–reduction potential (ORP) of the AS system were systematically recorded at the end of each 24 h cycle, i.e. 24 h after BWOM addition.

### 2.3. Anoxic ammonia–oxidation batch experiments

Two sets of experiments were conducted. In the first experiment, two 2 L containers were filled with 350 mL sludge (TSS=10 g L\(^{-1}\)) from the denitrification reactor which operated at a SRT of approximately 11 days. 200 mL BWOM water with TSS concentration of approximately 13 g L\(^{-1}\) were added to each container and the rest of the volume was filled with fresh deoxygenated seawater. Sodium nitrate was added in excess to both containers (initial concentration of 740 mg NO\(_3\) N L\(^{-1}\)). One container, which acted as a control, was then sealed. To the other container two types of nitrification inhibitors, ATU (C\(_4\)H\(_8\)N\(_2\)S) and Pirydine (C\(_6\)H\(_3\)NCl\(_4\)) were added at concentrations of approximately 20 mg L\(^{-1}\) each. The containers were sealed and stirred for 16 days at a temperature range of 25–29 °C.

In the second experiment, two 3.5 L containers were operated in a similar manner to the first experiment, however with a relatively high initial ammonia concentration of 40 mg N L\(^{-1}\). Constant positive pressure was maintained in the gas phase of the containers to enable gas release while preventing oxygen in-diffusion. Two additional containers were operated in a similar manner, with the exception that air was added to maintain DO concentrations at above 5 mg L\(^{-1}\). The substances added to each of the containers in the second experiment are summarized in Table 1.

### 3. Results and discussion

#### 3.1. Performance of the laboratory denitrification AS reactor

In order to evaluate the predictions made by the conceptual model presented by Klas et al. (2006–this issue), an activated-sludge type reactor was chosen as a case study. It is emphasized that the conceptual model was developed based on stoichiometric considerations, and therefore any other acceptable wastewater treatment method could have been applied, presumably with similar results.

The experimental denitrification reactor was operated at three major modes characterized by long (>27 d), medium (11 d) and short (4 d) solid retention times (SRT). SRT was calculated according to Eq. (2) (Klas et al., 2006–this issue):

\[
\text{SRT} = \frac{\text{COD}_{r} \cdot V_{r}}{\text{FCOD}_{\text{in}}}
\]

Where SRT = the mean solids retention time [d], \( \text{COD}_{r} \) = the COD concentration of the organic solids
maintained in the oxidation tank \([g \text{ L}^{-1}]\), \(V_r = \text{oxidation tank volume [L]}\), \(\text{FCOD}_{\text{in}} = \text{the COD daily organic (BWOM) load input [g COD d}^{-1})\].

System operation was aimed at attaining steady state conditions at each SRT in terms of nitrate removal rates, excess sludge removal and the solids concentrations in the oxidation tank (COD). It is noted that during the first stage of operation (SRT>27 d), sludge was not removed at all. The purpose of this stage was primarily to determine the performance of the system at the maximal practical SRT. This can be theoretically achieved when steady state is maintained without deliberate sludge removal, i.e. when the sum of organic matter feed and cell formation rate approximates the organic matter (cells and other organic matter) degradation rate.

Table 2 summarizes the major results recorded from the operation of the system along with the operational parameters applied at each mode.

### Table 2: Performance of the AS denitrification reactor at the three operation modes

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Mode</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1st (d)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2nd (d)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3rd (d)</td>
</tr>
<tr>
<td>SRT</td>
<td>d</td>
<td>~ 37</td>
</tr>
<tr>
<td>Duration</td>
<td>d</td>
<td>15</td>
</tr>
<tr>
<td>FCOD&lt;sub&gt;in&lt;/sub&gt;</td>
<td>g COD d&lt;sup&gt;−1&lt;/sup&gt;</td>
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<td>TSS&lt;sub&gt;r&lt;/sub&gt;</td>
<td>g TSS L&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>8–9</td>
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<tr>
<td>Max. nitrate removal rate</td>
<td>mg N L&lt;sup&gt;−1&lt;/sup&gt; reactor d&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>25</td>
</tr>
<tr>
<td>Av. nitrate removal rate</td>
<td>mg N L&lt;sup&gt;−1&lt;/sup&gt; reactor d&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>15</td>
</tr>
<tr>
<td>Specific NO&lt;sub&gt;3&lt;/sub&gt; removal rate</td>
<td>g N g TSS&lt;sup&gt;−1&lt;/sup&gt; d&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>0.003</td>
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<tr>
<td>COD to NO&lt;sub&gt;3&lt;/sub&gt; N removal ratio</td>
<td>g COD (g NO&lt;sub&gt;3&lt;/sub&gt;N&lt;sub&gt;\text{reduced}&lt;/sub&gt;)&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>6–11</td>
</tr>
<tr>
<td>% Sludge production</td>
<td>g TSS&lt;sub&gt;sludge&lt;/sub&gt; (g TSS&lt;sub&gt;\text{substrate}&lt;/sub&gt;)&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>–</td>
</tr>
<tr>
<td>% NH&lt;sub&gt;4&lt;/sub&gt; production</td>
<td>g NH&lt;sub&gt;4&lt;/sub&gt;N (g NO&lt;sub&gt;3&lt;/sub&gt;N&lt;sub&gt;\text{reduced}&lt;/sub&gt;)&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>1.4–2.7%</td>
</tr>
<tr>
<td>% P production</td>
<td>g PO&lt;sub&gt;4&lt;/sub&gt;–P (g NO&lt;sub&gt;3&lt;/sub&gt;N&lt;sub&gt;\text{reduced}&lt;/sub&gt;)&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>3.2%</td>
</tr>
<tr>
<td>Alkalinity production</td>
<td>eq (mol NO&lt;sub&gt;3&lt;/sub&gt;N&lt;sub&gt;\text{reduced}&lt;/sub&gt;)&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>1.1</td>
</tr>
<tr>
<td>VSS/TSS in sludge</td>
<td>mg L&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>0.57–0.65</td>
</tr>
<tr>
<td>COD/TSS in sludge</td>
<td></td>
<td>0.80</td>
</tr>
<tr>
<td>Effluents average TSS</td>
<td>mg L&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>0–5</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td>7.15</td>
</tr>
<tr>
<td>ORP</td>
<td>mV</td>
<td>+67</td>
</tr>
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</table>

At the beginning of the 1st mode of operation, steady state operation could not be attained while working with an organic load of 4 g COD d<sup>−1</sup>. It appears that the bacterial population that developed under these conditions was not large enough to oxidize the daily organic load added, as evidenced by a continuous increase of the solids concentration in the oxidation tank (data not shown). In order to attain steady state, the organic load was thus reduced (from 4 to 2.5 g COD d<sup>−1</sup>), resulting in SRT increase from roughly 27 to 37 d. As a result, the nitrate removal rates declined from around 45 to values below 25 mg N L<sup>−1</sup> d<sup>−1</sup>. Such removal rates are not acceptable in intensive systems, and therefore the attempts to find the maximal practical SRT were discontinued.

As shown in Fig. 3, the volumetric nitrate removal rates during the second mode of operation (SRT of ~11 d) increased to around 100 mg N L<sup>−1</sup> d<sup>−1</sup> with two occurrences of 180 mg N L<sup>−1</sup> d<sup>−1</sup>. These maximal removal
rates were recorded when the system was operated in a batch manner in order to determine the daily nitrate concentration profile (results not shown), in which oxygen interference was reduced to a minimum by stopping the water inflow and sealing the reactor’s lid. It can be thus concluded that the potential nitrate removal rate at this loading was at least 180 mg N L$^{-1}$ d$^{-1}$. The lower daily removal rates recorded during the first weeks of operation were probably a result of oxygen interference, which consumed a considerable part of the available organic substrate. To minimize this interference, the settler was better sealed towards the end of the second operation mode. The sealing of the settler led to an increase in the average removal rates to approximately 120–150 mg N L$^{-1}$ d$^{-1}$. However, due to time limitation, operation was not continued for enough time to properly quantify the effect of O$_2$ in-diffusion on the denitrification rate. It is emphasized that at all times neither the oxidation tank nor the settler were completely sealed, and thus some atmospheric O$_2$ diffusion was unavoidable.

During the third mode of operation (SRT of $\sim$ 4 d) nitrate removal rates increased steeply and proportionally to the increase in the organic load, up to a maximal removal rate of approximately 590 mg N L$^{-1}$ d$^{-1}$. Such a high rate allows installing a relatively small reactor, i.e. of a volume less than 4% of the entire RAS volume. The COD removal rate was 39 (g COD d$^{-1}$)/15 (L$_{reactor}$)*1000 (mg g$^{-1}$)$\approx$2600 mg COD L$^{-1}$ d$^{-1}$, which is on the same order of magnitude as the typical COD removal rates obtained in municipal wastewater AS reactors. For comparison, acceptable removal rates in such systems are around 1600 mg BOD$_L$ L$^{-1}$ d$^{-1}$ at SRT of 5 d, where BOD$_L$ is defined as the total BOD initially present in the water (Metcalf and Eddy, 2003). The solids concentration in the oxidation tank increased to values close to 13 g TSS L$^{-1}$ (VSS concentration of approximately 10.4 g L$^{-1}$). This concentration appeared to be close to the highest possible operational concentration for the investigated system, as sludge began to accumulate in the settler.

Further important observations can be made regarding other parameters in the AS system: the VSS to TSS ratio in the sludge increased as the SRT decreased, from 0.60 g g$^{-1}$ at SRT of 37 d to 0.68 g g$^{-1}$ at SRT of 4 d, compared with an average value of 0.74 g g$^{-1}$ in the raw BWOM. The average pH values in the oxidation tank increased as SRT was decreased: from 7.15 at the long SRTs to approximately 7.7 at SRT of 4 d. This increase in pH was probably a result of the increase in the flux of alkalinity released in the denitrification reaction. Correspondingly, ORP values decreased from positive values of 40 to 60 mV at the long SRTs down to $-70$ mV at SRT of 4 d. It should be noted that during a 24-h cycle ORP values decreased sharply immediately after BWOM addition and then increased slowly to the maximal levels that are reported for each operational mode.

### 3.2. Comparison between empirical results and model predictions

The model was run with input values resembling as accurately as possible the conditions employed in the experimental system. Fig. 4 shows the specific denitrification rate curve as predicted by the model (as a function of SRT) along with the highest measured denitrification rates attained at each of the operation modes (see Table 2). The specific denitrification rates were obtained by dividing the average volumetric denitrification rates by the TSS concentration in the oxidation tank. The term used for denitrification rate prediction, expressed in Klas et al. (2006-this issue) per COD units, is expressed here per VSS units in order to allow an straightforward comparison between the results of the current work and corresponding data from the literature (typical COD to TSS ratio measured in the oxidation reactor was 0.8). Fig. 4 shows a very good correlation between the model predictions and the empirical results, especially at the short SRTs. The better accuracy is attributed to the relatively lower oxygen
interference at the short SRT (reducing the SRT led to higher nitrate removal rates whereas the rate of oxygen diffusion hardly changed).

Fig. 5 shows model-predicted and average measured values of the COD to NO$_3$-N utilization ratio as a function of the SRT. In contrast with the values predicted by the model, the measured values were higher at the higher SRT values. In all likelihood, this observation was due to oxygen interference, which was more dominant at the longer SRTs, as explained before. At the short SRT of 4 days, where oxygen interference was low relative to the nitrate reduction rate, the COD utilized to NO$_3$-N reduced ratio was between 4.5 and 4.9, i.e. close to the theoretical prediction.

The mass of organic matter that can be recovered and supplied to the denitrification reactor in RAS systems is expected to be limiting relative to the amount of nitrate that is produced, i.e. the COD to NO$_3$-N utilization ratio is expected to be in many cases the limiting factor for the process. Therefore, it is desired to operate at a low COD to NO$_3$-N utilization ratio in the denitrification reactor. Fig. 5 implies that in contrast with the predictions (the model does not account for O$_2$ diffusion), this can be only achieved at the lower SRTs. Fig. 5 also shows that because of oxygen interference and stoichiometry limitations, a COD to NO$_3$-N ratio lower than 4.5 g COD (g N)$^{-1}$ cannot be practically achieved in the investigated system, unless oxygen penetration is further reduced by sealing the reactors.

The daily mass of sludge removed from the system is shown in Fig. 6 as a percentage out of the mass of solids that entered the reactor daily. It is reminded that at the longer SRTs (>27 d) sludge was not deliberately removed. The actual sludge production exceeded the prediction of the model by 24% and 5% at SRT of 11 days and 4 days respectively. Possible explanations to this disagreement include oxygen interference, and the possibility that $X_{sb}$ and/or the maximal bacterial yield ($Y_{max}$) were underestimated in the model. The estimation of the amount of excess sludge is important both from an operational standpoint, as well as from an economic perspective, as salty sludge requires further treatment.

The alkalinity produced during the process was slightly underestimated by the model, as shown in Fig. 7. This can perhaps be explained by inaccuracies in the model, resulting from the use of generic substrate and bacterial cell formulas. notwithstanding this small deviation from model prediction, the mass of alkalinity produced in the process suggests that if denitrification effluents are recycled into the RAS, alkalinity compensation from external sources (i.e. make-up water and/or strong base addition) can be minimized and perhaps not required at all.

The production of phosphate as a result of degradation of the organic matter was significantly overestimated in the model. Such a disagreement, as shown in Fig. 8, can hardly be explained by a possible error in the estimation of the P content in the substrate generic formula. It seems more likely that the P content in the bacterial cells was underestimated. This hypothesis was not further investigated in this work. However, evidence of heterotrophic denitrifiers capable of phosphorus storage in excess of their metabolic requirements under either aerobic or anoxic conditions, without the need for alternating anaerobic/aerobic conditions, appear in the recent literature (Barak and van Rijn, 2000). From a practical standpoint,
the low phosphate release attained in the experimental system is most advantageous, as its removal from the aqueous phase is both required and difficult.

Similarly to phosphate, Fig. 9 shows that the ammonia concentration in the system also deviated significantly from the values predicted by the model. TAN concentrations in the effluents remained very low throughout the whole experiment. This phenomenon, that was also observed in biodegradation experiments conducted on the BWOM (Klas et al., 2006-this issue), gave rise to the hypothesis that beyond ammonification and ammonia assimilation, that were accounted for in the model, other mechanism/s had an effect on the ammonia mass balance in the system. This issue was further investigated as discussed below.

3.3. Anoxic ammonia oxidation

According to the results obtained from the conceptual model, TAN should have been released into the aqueous phase of the experimental system in significant amounts as a combined result of ammonification (release of ammonia to the aqueous phase as a result of organic matter degradation) and bacterial assimilation (uptake of ammonia from the aqueous phase for bacterial cell buildup). However, in reality the net TAN production was very low, as shown in Fig. 9. Several explanations to this observation were examined, including excessive biomass assimilation, NH₃(g) emission and even possible struvite (NH₄PO₄Mg·6(H₂O)) precipitation. None of these explanations, however, were found to be satisfying when quantifying their ammonia removal potential. It was thus hypothesized that ammonia was being oxidized under the constant anoxic conditions prevailing in the system. In order to evaluate this hypothesis, two experiments were conducted as described in Section 2.3.

In the first experiment, TAN concentrations were monitored in two 2 L containers, both operating under conditions similar to those prevailing in the AS system. Nitrification inhibitors were added to one of the containers. It is emphasized that material with electron donating capacity (i.e. BWOM) was added in large excess to both containers to ensure that even if oxygen penetrated the container through its sealed lid, it would be instantly consumed by the heterotrophic bacteria, ensuring practically negligible oxygen concentrations.

Fig. 10 shows clearly that in the presence of the nitrification inhibitors, ammonia accumulated in the water, whereas in the absence of inhibitors, ammonia did not accumulate, and after a day or two dropped to near zero concentrations. These results suggest that ammonia was indeed oxidized in the non-inhibited container even though the conditions were absolutely devoid of oxygen (i.e. anoxic).

The second experiment was repeated twice and resulted in similar results. In this experiment, the oxidation rate of ammonia under both aerobic and anoxic conditions was examined. Four containers where operated simultaneously with an initial ammonia concentration of approximately 40 mg N L⁻¹ (see Table 1). In two containers constant aerobic conditions were maintained (DO concentration >70% saturation), whereas anoxic conditions were strictly maintained in
the other two. Fig. 11 shows, in accordance with the first experiment, that the TAN concentration under the anoxic conditions and in the absence of nitrification inhibitors (container A), decreased with time at a constant rate of approximately 7 mg NH₄-N d⁻¹. In the aerated uninhibited container (container C), TAN concentrations decreased at a faster rate than in container A (>20 mg NH₄-N d⁻¹). This observation simply indicates that “regular” aerobic nitrifiers were present in the reactor in large numbers. The fact that normal aerobic nitrification occurred in container C was confirmed by an alkalinity decrease of approximately 2 eq per mol TAN oxidized in the un-inhibited aerobic container. In the aerated container, in the presence of nitrification inhibitors (container D), TAN concentrations rather increased with time. This was also expected as no aerobic nitrification could occur, and TAN was continuously produced as a result of ammonification. A bit surprisingly, however, TAN concentrations in the anoxic nitrification-inhibited container (B) did not accumulate as expected, but rather remained constant. This indicated that under the experimental conditions, TAN was consumed and produced at a similar rate. The reason for this observation is not clear. One hypothesis is that the bacteria involved are of the anammox group, which is capable of oxidizing ammonia under anoxic conditions, with nitrite being the preferred electron acceptor (Schmidt et al., 2002). The fact that the inhibitor chemicals used were not specific for anammox bacteria may explain the partial inhibition observed.

Taken together, the results suggest that the reason for the low TAN concentration observed in most of the denitrification experiments was, with high probability, a result of anoxic ammonia oxidation. It is very likely that BWOM waters contain a relatively high concentration of “regular” nitrification bacteria which originate in the nitrification biofilter and solids filter of the RAS, as evidenced by the results obtained in the aerated containers. Some of these bacteria have the ability to oxidize ammonia even under very low oxygen concentrations using nitrogen dioxide (NO₂) or dinitrogen tetraoxide (N₂O₄) as the electron acceptor (Schmidt et al., 2002). However, formation of NO₂ in natural environments takes place only under oxic conditions. Because oxygen concentrations in the anoxic containers were practically zero, it was concluded that the anammox process was very likely responsible for most of the anoxic nitrification observed. The presence of anammox bacteria in the investigated system was also established directly using FISH (results not shown). This finding conforms to other reports that have described the presence of anammox populations in marine RAS nitrification biofilters (e.g. Tal et al., 2003).

3.4. Electron acceptor removal-rate profiles and further biodegradation analysis

Klas et al. (2006-this issue) performed biodegradation batch experiments on the BWOM. These experiments suggested that roughly 84% of the BWOM is available for microbial utilization. This portion can be further subdivided into three fractions with different biodegradability properties, while the rest of the BWOM was assumed to be mostly bacterial biomass, that was generated either in the biofilter or in the solids filter itself (this fraction has a relatively low value as an organic substrate in the denitrification reaction). However, the biodegradability tests were conducted without accounting for the conditions prevailing in the AS reactor, namely high acclimatized biomass concentration. In order to further assess the biodegradability of the substrate (BWOM), as well as to corroborate the denitrification rate results, OUR (Oxygen Uptake Rate) batch experiments were conducted with sludge that was taken from the AS reactor at each of the SRTs employed. Because most of the heterotrophic denitrifying bacteria are essentially facultative (Rittman and McCarty, 2001), it was assumed that a constant ratio between OUR and NUR (Nitrogen Uptake Rate) exists in the investigated system. Thus, by measuring OUR under oxic conditions, the theoretical NUR, that represents the denitrification rate, can be calculated with reasonable accuracy. The conversion between NUR and OUR was attained simply by multiplying the OUR measured value by the molecular weight ratio of nitrogen and oxygen (14/32) and by the ratio of 4/5 (1 mol of nitrate accepts 5 mol electrons versus 4 mol electrons accepted by 1 mol of oxygen). The product was then divided by 0.8, which was used to represent the anoxic/aerobic yield ratio (Muller et al., 2003). Altogether, the approximate value of the NUR can be attained by multiplying the OUR measured value by a factor of (14/32·4/5)/0.8=0.4375.

The results of the OUR profiles and the calculated NUR profiles are presented in Figs. 12 and 13 respectively. It is

Fig. 12. Measured OUR profiles for the three SRTs applied.
noted that in contrast with the other two cases, the OUR profile for SRT > 27 d was not actually measured for a full 24 h, but only for 2 h (and also BWOM was not added in this particular experiment). However, for comparison purposes the peak of this experiment was artificially constructed, using the assumption that the “base-line” (i.e. the respiration rate before BWOM addition) remained constant during the 24 h, and that the peak representing BWOM addition was proportional to the other two curves, which are entirely empirical.

The areas (A) under the curves in Fig. 13, which cover 24 h cycles, represent the total equivalent mass of nitrate (as N) that should have been reduced (per liter reactor per day) under anoxic conditions. In other words, these areas represent the maximal possible denitrification rates at the specific conditions employed. These values can be compared with the actual nitrate removal rates that were measured directly in the AS reactor (Table 2 and Fig. 3). The results show that the nitrate removal rates, based on OUR measurements, are between 4% (at SRT = 4 d) and 19% (at SRT = 11 d) higher than the maximal directly-measured rates. It transpires that as the SRT is decreased, the calculated maximal nitrate removal rates (based on the OUR measurements) are closer to the direct measurements. The over-estimation of the calculated NUR is in all likelihood a result of oxygen interference in the denitrification reactor, an interference that, naturally, could not occur in the OUR experiments.

Fig. 12 can also be used in order to re-assess the biodegradability pattern of the intrinsic organic matter under the conditions prevailing in the AS reactor. The areas underneath each respiration curve (A) represent the total daily mass of oxygen consumed, or in equivalent terms, the fraction of the electrons that are transferred to the final electron acceptor (oxygen in this case). It is shown that this area is more or less inversely related to the SRT, or proportionally related to the substrate load, FCOD\textsubscript{in}. The constant ratio observed between A and FCOD\textsubscript{in} implies that the reduction in SRT did not lead to a major change in the portion of FCOD\textsubscript{in} that is capable of donating electrons. Therefore, it was hypothesized that the SRT may be further reduced to values even lower than 4 d, resulting in higher baseline values, and probably a longer duration of the high respiration rate.

In order to re-assess the BWOM composition with respect to its biodegradable fractions, the shape of the respiration rate curves was examined. The addition of the BWOM brings about an immediate increase in the removal rates, equal to 2.5 (at SRT = 4 d) and 4 (at SRT = 11 d) times the baseline respiration rate. This increase is a result of the readily biodegradable organic fraction present in the BWOM, that were shown, for a short period of time, to produce an equivalent nitrate removal rates as high as 1470 mg N L\textsuperscript{-1} d\textsuperscript{-1} (at SRT of 4 d). Such rates are typical of systems that operate with an external energy source, such as methanol. This fraction however, is relatively small, and represents only 3% to 5% of the total 24 h mass of oxygen (or nitrate) consumed. Following this phase, the respiration rate is reduced to approximately one half of its maximal rate, and lasts a longer time. The latter phase, which represents about 10% (at SRT = 11 d) and 20% (at SRT = 4 d) of the total area under the OUR curves, probably represents the less easily biodegradable COD present in the BWOM. When the latter fraction is consumed, it seems that there is another phase in which the respiration rates slowly decrease until they return more or less to the initial respiration rate (the baseline). This decrease might be a result of endogenous respiration of the biomass produced during the utilization of the relatively easily biodegradable COD. The important observation, however, is that approximately 70% of the area under the respiration rate curve exists under the baseline itself. This observation indicates that the ratio between the area confined by the baseline and the respiration rate curve, and the total area under the respiration curve, does not represent the true proportion of the relatively easily biodegradable fraction out of the organic matter in the BWOM, but rather a

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Values</th>
</tr>
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<tbody>
<tr>
<td>SRT</td>
<td>d</td>
<td>27</td>
</tr>
<tr>
<td>FCOD\textsubscript{in}</td>
<td>g COD d\textsuperscript{-1}</td>
<td>0.2*</td>
</tr>
<tr>
<td>Total O\textsubscript{2} uptake (\textasciitilde A)</td>
<td>g O\textsubscript{2} d\textsuperscript{-1}</td>
<td>0.136*</td>
</tr>
<tr>
<td>Y = 1 – (A/FCOD\textsubscript{in})</td>
<td>g COD (g COD\textsuperscript{-1})</td>
<td>0.1 – 0.3</td>
</tr>
<tr>
<td>Y\textsuperscript{**} = 1 – [A/(0.84 FCOD\textsubscript{in})]</td>
<td>g COD (g COD\textsuperscript{-1})</td>
<td>0.19</td>
</tr>
<tr>
<td>Theoretical Y\textsuperscript{***}</td>
<td>g COD (g COD\textsuperscript{-1})</td>
<td>0.19</td>
</tr>
</tbody>
</table>

* Based on proportion.
** Assuming that 16% of FCOD\textsubscript{in} is inert (Klas et al., 2006-this issue).
*** Based on Eq. (3), assuming Y\textsubscript{max} = 0.53 g g\textsuperscript{-1} and k\textsubscript{d} = 0.06 d\textsuperscript{-1}.
smaller fraction. This ratio is expected to grow as SRT decreases, until it is equal to the actual fraction of the relatively easily biodegradable matter in the raw BWOM, which was estimated by Klas et al. (2006-this issue) to be approximately 50%.

The area under the OUR curve represents the substrate fraction that donated electrons to the final electron acceptor in order to supply energy for the process. The remaining substrate is either used for cell synthesis, or is inert under the experimental conditions and therefore not involved in any biological transformation (an example for "inert matter" are the bacterial cells that are supplied to the denitrification reactor with the BWOM).

Considering the COD input values used in each of the OUR experiments, the empirical yield coefficient $Y$, could be estimated using a simple calculation as shown in Table 3, and be compared with the theoretical yield, derived from Eq. (3) (Klas et al., 2006-this issue).

$$Y = \frac{Y_{\text{max}}}{1 + k_d \cdot \text{SRT}}$$  \hspace{1cm} (3)

Where $Y$ is the yield coefficient [g COD-cells (g COD-substrate)$^{-1}$], $Y_{\text{max}}$ is the maximum yield coefficient [g g$^{-1}$] and $k_d$ is the endogenous decay coefficient [d$^{-1}$].

The results shown in Table 3 are in agreement with the theory, according to which the observed yield increases as SRT decreases (up to a certain $Y_{\text{max}}$). The yield coefficient values derived from the OUR experiments by assuming that 16% of the substrate does not undergo any biological transformation seem to agree well with the theoretical values calculated by using Eq. (3). These results further support the previous estimations made by Klas et al. (2006-this issue) that approximately 16% of the BWOM would not undergo biological transformation under the conditions applied in the process.

3.5. Kinetics

Kinetic experiments were performed in order to establish the apparent range of the nitrate half saturation constant ($K_{S,NO_3}$) values in the system, for the case where RAS intrinsic organic solids are used as the organic substrate. The aim was to get an estimate of the minimal nitrate concentration range at which kinetics would be independent of the nitrate concentration (i.e. zero-order kinetics). The results of three kinetic experiments, conducted at different operational modes of the AS reactor, are shown in Fig. 14. The results of all three experiments conformed well to Monod’s reaction kinetics, with half-saturation values ($K_{S,NO_3}$) in the range of 0.3–0.7 mg NO$_3$–N L$^{-1}$. These values were obtained from the slopes of the linear regression of the inverse values of the Monod equation (Lineweaver–Burk technique), without normalizing them for the bacterial cell concentration.

Based on the kinetic experiments, it was concluded that maximal removal rates are expected to occur even at nitrate concentrations as low as 1.5–2.0 mg NO$_3$–N L$^{-1}$. This is a very important finding from a practical perspective, as it allows the operation of a single high-rate denitrification reactor at low nitrate concentrations, without a need for a further polishing stage.

4. Summary and conclusions

A single-sludge denitrification process based on the activated-sludge concept and aimed at the removal of nitrate from marine RAS effluents using the intrinsic organic matter as the electron donor, was developed and run continuously for 136 days at lab-scale. From an engineering standpoint, the process has proven steady, and capable of removing nitrate at high rates.

Theoretical predictions of several important operational parameters were proposed by a conceptual model presented by Klas et al. (2006-this issue). In this paper, the predictions of this model were compared with empirical results obtained from a lab-scale denitrification reactor. By and large, the empirical results were in accordance with model predictions, in particular those obtained when shorter SRTs were applied. Volumetric denitrification rates as high as 590 mg N L$^{-1}$ d$^{-1}$ were recorded at SRT of 4 d, and it would appear that even higher rates are feasible at shorter SRTs. Such high rates allow the operation of a denitrification reactor with a volume that is less than 4% of that of the total RAS volume.
Uncontrolled oxygen penetration resulted in some deviations from the model predictions, especially at the longer SRT values. Oxygen inhibition resulted in lower measured denitrification rates and higher measured COD to NO$_3^-$ N utilization ratios than those predicted by the model. Regarding the latter parameter, O$_2$ interference led to an opposite trend relative to the prediction, suggesting that shorter SRTs may actually be favorable if a low COD to NO$_3^-$ N ratio characterizes the input to the denitrification reactor (which is often the case). Furthermore, utilization ratios lower than 4.5 g COD per g NO$_3^-$ N could not be attained in the current experiments due to oxygen interference, though in theory values as low as 3.0 g COD per g NO$_3^-$ N are possible.

The results indicate that for the RAS used in this work as a case study, between 60% and 70% of the nitrate could be removed by using the intrinsic organic matter that accumulates in the solids filter. This value might be higher in RAS configurations that make use of drum filters, in which the availability of the organic solids supplied to the process is expected to be higher.

Alkalinity production generally exceeded model prediction by up to 10% and reached values in the range of 1 eq per mol of reduced nitrate. Sludge production also slightly exceeded model prediction and reached values in the range from 62% to 67% (in TSS terms) relative to the mass of solids added daily to the reactor. Phosphate production did not behave at all according to model predictions with typical values of only 1% PO$_4^{3-}$ –P generated to NO$_3^-$ –N reduced (mass to mass basis). Finally, TAN production was also seriously overestimated by the model. Low ammonia production rates of 0.2% to 2.3% with respect to the reduced NO$_3^-$ –N were recorded throughout the experiment, although predicted values were in the range of 10%. Anoxic oxidation of the ammonia was shown to be the mechanism that was very likely responsible for this observation, which was speculated to be carried out by anammox-type bacteria. Batch OUR experiments supported the denitrification rates measured. These tests, together with the results obtained from the operation of the denitrification reactor, suggest that most of the organic matter recovered from the solids filter of the experimental RAS is capable of donating electrons and participate in the denitrification reaction. Moreover, it seems that this capability does not change significantly between the various SRTs that were applied. The process is capable of removing 30% to 40% of the mass of solid wastes generated by the fish farm when operated under relatively short SRTs. Kinetic experiments showed that nitrate removal rates are zero order with respect to the nitrate concentration, as long as the nitrate concentration is maintained above 1.5 to 2.0 mg N L$^{-1}$. Taken together, the results indicate that single-sludge denitrification is a feasible and economical method for nitrate removal from RAS effluents. The unexpected low production of phosphate and ammonia may further contribute to the simplicity and cost-effectiveness of the concept.

References


