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Evidence-based practice

Implementation of sulphur-based denitrification in a large-scale fully recirculated cold-salt water aquarium: A sustainability practice.

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Abstract

Autotrophic sulphur-based denitrification (ASD) was evaluated to control nitrate accumulation in the marine aquatic exhibit of the Biodôme de Montréal (Montréal, QC, Canada). Different substrates (two sulphur and two calcareous sources were tested), flow-rates and configurations (homogenous and low to high stratification levels) were evaluated that successively improved nitrate removal rates (g N-NO3- · day-1) and/or start-up time. Despite pronounced suboptimal conditions for anaerobic denitrifying bacteria, Thiobacillus denitrificans i.e. saline (24 PSU), cold (5-10°C) and aerated waters, our R&D efforts lead to the development of an efficient, simple, custom-made ASD unit adapted to a priori unfavourable conditions. The Rocky Shore exhibit (25 m^3) housing the most sensitive marine aquatic species of our live collections (mostly invertebrates) previously operated at ≥ 50 mg N-NO3- · L-1 is now at ~ 20 mg N-NO3- · L-1. Considering the significant economic and environmental gains achieved (e.g. sustainability) following the implementation of this technology at a small-scale on the Rocky Shore exhibit (100 kg of sulphur), a 10-fold upscaling (1000 kg of sulphur) ASD unit to be connected to the La Baie exhibit (2500 $m^{3)}$ is planned.

Background

In accordance with the commitment of zoos and aquaria to sustainable development, zoological institutions have a responsibility to reduce their environmental footprint by balancing economic and ecological factors (Townsend 2009). In line with this, developing and adopting strategies and/or systems to address environmental concerns and operate in a more sustainable manner must be considered and reinforced. Adhering to sustainability principles regarding aquatic life support systems requires minimisation of the use of resources such as water, energy and labour.

The Biodôme de Montréal's (BDM) marine ecosystem is an assemblage that replicates community dynamics of the Gulf of the St Lawrence, Canada. This exhibit houses 70 species inspired by the biogeography of the East Coast of North America (Mahon et al. 1998), including bird species and associated vegetation. The collection spreads over 1620 m2 to include a marsh and two basins: the first is a 2500 m^3 basin operated at 9-10°C, and the second is a 25 m^3 rocky, wave-beaten shore operated at 5-6°C. From its inauguration in 1992 to 2009, the marine exhibits were operated at 30 PSU. Le François et al. (2015) implemented a strategy of salinity reduction (now 24 PSU). This resulted in financial savings of ≈ CAD $14,000 annually which was invested in the development of an autotrophic sulphur-based denitrification (ASD) system.

Ammonia, nitrite and nitrate are the nitrogenous end-products of processing animal wastes or feeds through the action of nitrifying bacteria Nitrosomonas and Nitrobacter. Tolerance to nitrate depends on species, life-history stages and duration of exposure (for marine species see Morris et al. 2011; Van Bussel et al. 2012). In natural marine habitats, N-NO3- levels rarely reach 1 mg · L (Yeats 1990). In marine aquaria, chronic nitrate concentration values often exceed 50 mg · L (Grguric et al. 2000; Parent and Morin 2000) and Pierce et al. (1993) described values as high as 500 mg N-NO3- · L, as the accumulated nitrates cannot be removed by common life support components. Given that long-term operation of closed aquaria results in substantial accumulation, operators must frequently change water at great expense. Nitrate removal by biological treatment offers an economical alternative (Sauthier et al. 1998; Grguric et al. 2000; Tal et al. 2003). Whereas nitrifying biofilters are incorporated in most recirculating systems, denitrifying systems are still under development.

Parent and Morin (2000) computed an N budget of the BDM's marine exhibit in order to examine how nitrate increase could be controlled. This study contributed to 1) the identification and quantification of the role of each system component in nitrate; 2) improvements in operating practices and 3) predicting the impact of these changes on the water quality. Food for the animals was the primary input of N (58%), the second (35.5%) was seabird guano, whereas filtration and cleaning removed 20%. Consequently, the following strategies were adopted: 1) optimization of feeding levels; 2) reduction of the animal populations and 3) increase of the level of annual seawater changes (from 7-9 % to 16%). Previously values >180 mg N-NO3- · L were frequently observed, whereas after these changes, [N-NO3] stabilised to 100-120 mg N-NO3- · L. Early in the 2000's, heterotrophic denitrification was evaluated at our facilities in collaboration with l'École Polytechnique de Montréal (see Labbé et al. 2003; Labelle et al. 2005; Dupla et al. 2006). Heterotrophic denitrifyers derive electrons and protons for nitrate reduction to elemental nitrogen from organic carbon compounds (carbohydrates, organic alcohols, amino acids and fatty acids). The unit was dismantled in 2008 due to complexity of operation and technological transfer issues.

Autotrophic denitrifyers may use inorganic compounds, such as reduced sulphur as electron donors. The most convenient method to remove nitrate from contaminated water employs autotrophic sulphur-calcareous reactors based on the denitrifying capacity of Thiobacillus denitrificans, a bacterium that requires anaerobic conditions to remove nitrate. The bacteria bind to the substrate (e.g. thiosulphate, ferrous sulphide or elemental sulphur), which donates electrons to achieve a redox reaction. Nitrate (NO3) is reduced to nitrite (NO2), which is then reduced to N2. As a result, sulphur is oxidised to sulphate (SO42-), which leads to water acidification. Addition of a calcareous substrate to restore pH near neutrality is necessary. The optimal operating conditions for ASD are known to be 25-35°C, pH 7.0-8.0 and freshwater. In fact, denitrification efficiency declines when salinity rises (Koenig and Liu 2004). Early use of ASD in the public aquarium is reported at the Aquarium La Porte Dorée (Paris, France), the Aquarium Général de Saint-Malo and is described by Hignette et al. (1997). More recently, the National Aquarium of Baltimore (Aiken 2012) and Simard et al. (2015) at the Aquarium de Québec reported using ASD.

Early in 2009 a nitrate concentration threshold of 60 mg N-NO3- · L was fixed thus triggering a systematic renewal of 25-35% of the system water. Water from the cleaning/filtration of the bottom substrate by diving operations are now often discarded instead of directed toward a $150 m^3$ reservoir dedicated to recovery of the backwash water from the sand filters. In 2010, new targets were proposed, 10 mg and 30 mg N-NO3 · L for the Rocky Shore and the La Baie exhibits respectively. Consequently, a research programme exploring the efficiency of ASD was launched.

The characteristics of the water in the exhibit at the BDM are clearly outside standard ASD conditions: water temperature is cold (5-6°C and 9-10°C), well aerated and saline. However, Trouvé et al. (1998) isolated strains of *T. denitrificans* that are able to remove nitrate at 5°C and to adapt to varying levels of pH (6-8) and substrates (S2O32-> FeS > FeS2 > So). In contrast to Simard et al. (2015) and Trouvé et al. (1998) who used inoculants from various sources, we assumed that we would harvest an indigenous, locally adapted and efficient strain within our aquatic system.

We considered the Rocky Shore exhibit ($25 m^3$) for the introduction of ASD because of its small volume and because it holds the most sensitive animals (e.g. anemones, urchins, sea stars etc.). Many configurations of a fixed bed ASD were tested to evaluate the efficiency of N-NO3- removal (NRR) under our operational conditions.

Action

In order to test our assumptions about substrate quality and the effect of increased level of stratification, a small denitrification unit consisting of 3 X 4.41 L columns (Experiment 1 : Experimental scale autotrophic sulfur-based denitrification) was built to test different types of sulphur substrate (laminar (A1) vs granular (A2)) and calcareous substrate (oyster shells (B1) and limestone (B2)) and the effects of stratification level on nitrate removal rate (NRR) (see Figure 1). We ran our preliminary tests at higher temperatures, to achieve faster bacterial colonization and accelerate conversion. The unit was first connected to 250 L isothermal containers filled with water from La Baie exhibit and operated at room temperature (> 20°C). In a second series of tests the experimental ASD was connected to the La Baie exhibit operated at 9-10°C. The outcomes could then be safely applied to a pilot-scale system (Experiment 2 : Pilot-scale autotrophic sulfur-based denitrification) connected to the cold-SW Rocky Shore exhibit (5-6°C).

Data collection

Parameters monitored on a routine basis at the inlet and outlet of the system were: temperature, pH (Accumet® XL-50), nitrite (Ultrospec 3100 Pro), ammonia (Dual Start®, Thermo Scientific inc.), nitrate, chloride, bromide, phosphate and sulfate concentrations in the water using ion chromatography (881 Compact IC pro 1, Metrohm with a seawater compatible high performance separation column Metrosep A Supp 5 250/4).

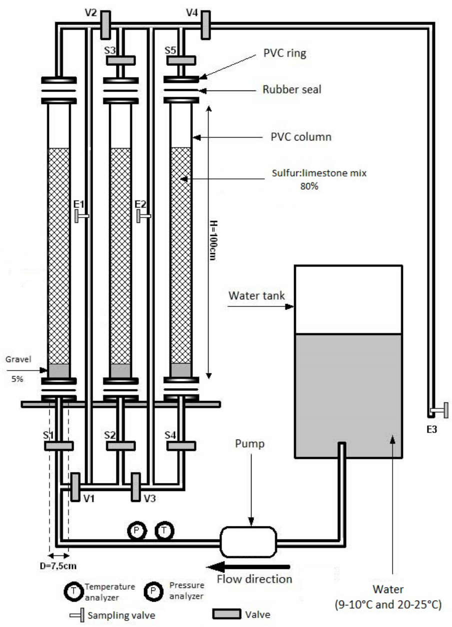


Figure 1. Schematic drawing of the experimental ASD unit.

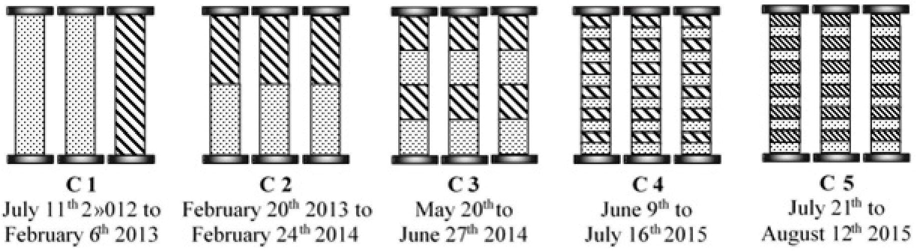


Figure 2. Five different configurations (C1-C5) for the experimental ASD unit connected to La Baie exhibit (9-10 ˚C). Granular sulphur (Substrate A2) (░), oyster shells (Substrate B1) (▧) and limestone (Substrate B2) (▨).

Only results of nitrate concentrations and pH will be shown. Water flow rate was measured at the end of the system. Nitrate removal rate (g N-NO3- · d-1) for each sampling period was calculated using the following formula:

Nitrate removal rate (NRR)
(g N-NO3- · d-1) = (Flow rate × (Water Nt-Water N0) × 24) / 1000

Where the flow rate units are (L · h-1) and water Nt and N0 represent N-NO3 concentrations (mg · L-1) after and before its passage through the ASD system, respectively.

Experiment 1. Experimental scale autotrophic sulphur-based denitrification

Sulphur and calcareous substrates quality/price ratio (closed system at 20°C)

Two types of sulphur source were compared, a laminar sulphur that contained heterogeneous sized granules and residual dust (Substrate A1: Buck Expert inc., CAD$47/25 Kg) (used by Simard et al. 2015) and granulated industrial grade elemental sulphur (A2) Tiger® (Tiger-Sul Inc. Calgary, Alberta: 99.5% purity, 260 SGN CAD$73/25 Kg). The latter is formed from pure molten sulphur using a drop-forming process that minimizes dust creation.

To compare the quality/price ratio of both sulphur substrates, we sieved the two sulphur substrates at 2 mm and then at 6.3 mm. Results indicated that the amount of loss of the laminar sulphur (Substrate A1) 62.0 ± SD 0.5 %, was much greater than for the granular sulphur (Substrate A2) (0.36 ± 0.5%), leading to a better price at $2.97/ Kg compared to $4.99/ Kg for substrate A1. Furthermore, substrate A2 allowed no previous sieving or rinsing.

As a calcareous substrate, oyster shells (Substrate B1) (Unicoop, QC, Canada) at CAD$40/25 Kg $1.60/ Kg) (used by Simard et al. 2015) was also evaluated by sieving at 2 mm and then at 6.3 mm. A loss of 36.9 ± 0.5 % was noted raising the effective price to $2.48/ Kg. A second type of calcerous substrate, limestone (Substrate B2: Graymont inc., Bedford, QC, Canada, http://www.graymont.com/fr) (Ø 5-10 mm), was also introduced and assessed in the evaluation of nitrate removal rate, but it did not require sieving prior to use and was provided free of charge by the regional mine operations.

Effect of sulphur substrate on nitrate removal rate at 20°C

The high temperature (20°C) was useful to maximize conversion rate and promote bacterial colonisation. At a volume load of 1.44 kg N-NO3 · m-3 · d-1, a first test lasting 36 days was conducted using two columns filled with Substrate A1 and one column of oyster shells (Substrate B1). The second test (30 days) included two columns filled with Substrate A2 and one column with Substrate B1. A slow flow rate of 0.18 L · h-1 was maintained in both trials to establish anaerobic conditions and facilitate sulphur colonization by bacteria. Preliminary tests indicated that the configuration Substrate A2: Substrate B1 (2:1) allowed a better NRR (0.103 compared to 0.021 g N-NO3 · day-1) resulting in a nitrate removal efficiency of 71.43% for the Substrate A2 compared to inconclusive results using Substrate A1 < 5%. Consequently, laminar sulphur was definitely discarded as a sulphur substrate for our needs.

Effect of stratification level on nitrate removal rate and calcareous substrate at 9-10°C (connected to the La Baie exhibit)

System pH strongly influences denitrification. The effect of stratification level on NRR was evaluated on the assumption that pH would be more stable by the proximity of a calcareous source over the distance from the entrance to the exit of the column. We evaluated five different configurations on the experimental ASD unit that was for these trials connected to the La Baie exhibit operated at 9-10°C (C1-C5) (see Figure 2).

C1-C4 were using granular sulphur and oyster shells, whereas C5 introduces the use of limestone (Substrate B2) in replacement of oyster shells requiring no sieving prior to use.

Experiment 2: Pilot-scale autotrophic sulphur-based denitrification

The outcomes originating from the experimental ASD unit were safely applied to a pilot-scale ASD system that was connected to the cold-SW Rocky Shore exhibit (25 m^3) operating at 5-6°C. It consists of six 47.1 L columns, offering flexibility of use with multiple by-pass combinations. Our efforts were concentrated on the maximisation of the flow rate by, the addition of a degassing unit to remove oxygen from inlet water (Pro-Cel®, Mini Module G541) and of a filter bag (FPX 100®, FSI Inc. @ 5µm) to reduce fine particle load. Our first trial (June 15 2012 until March 5 2014) was to operate three columns, filled with homogenous substrate e.g. substrate A2 and B1 2:1 (Figure 3).

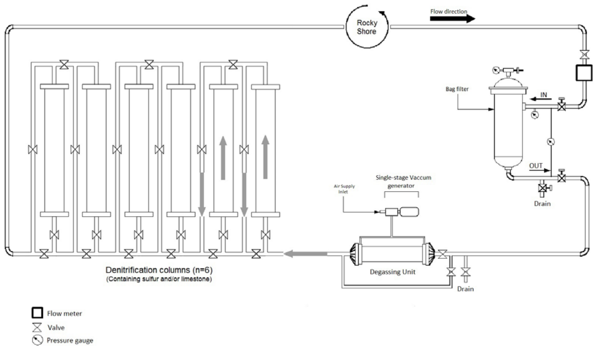


Figure 3. Schematic drawing of the pilot-scale ASD unit.

A second trial this time exploring stratification effect on NRR was planned with substrate A2 and B1 with altering layers for a total of 4 layers per column. This configuration was launched on January 21 2015 and is still in operation. The same sampling and measurements collected for the experimental unit trials were applied.

Consequences

Experiment 1. Experimental-scale autotrophic sulphur-based denitrification

C1 (217 days, 1 backwash): was launched at a low flow rate (0.26 L · h-1) for 28 days because it took this time for the bacteria to colonise the sulphur substrate and display noticeable NRR. Flow rate was then gradually increased to 2.77 L · h-1 until day 49. During this period, NRR started to decline. The maximum NRR observed was 1.31 g N-NO3- · d-1 at 1.7 L · h-1. NRR The mean NNR is 0.54 ± 0.29 g N-NO3 · d-1 (n=47).

C2 (368 days, 0 backwash): flow rate was set at 1.7 L · h-1 because noticeable NRR was achieved more rapidly. The pump was adjusted to 3.7 L · h-1 on day 64. At day 76 the system clearly outperformed the NRR of configuration no. 1. A maximum NRR of 4.11 g N-NO3- · d-1 was reached at day 188. Increasing flow rate to 3.9 L · h, caused a rapid decline in NRR. The pump was repositioned to 3.7 L · h-1 to allow the system to recover but the decline continued until it reached 0.94 g N-NO3- · d-1 and then stabilised at a mean of 1.24 ± 0.21 g N-NO3- · d-1 (n=7). A 2-fold increase in NRR was achieved compared to C1.

C3 (37 days, 2 backwashes): flow rate was maintained at 0.26 L · h-1 for hree days because it rapidly removed nitrates at < 5 mg NO3- · L-1 with a NRR of 0.29 g N-NO3- · d-1. The flow rate was therefore gradually increased to 3.0 L · h-1 until day 17 where NRR achieved 3.16 g N-NO3- · d-1. Finally, the flow rate was increased to 4.39 L · h-1 until day 37 but it did not result in a significant improvement of NRR, which has instead stabilized at a mean of 3.11 ± 0.17 g N-NO3- · d-1 (n=13) (i.e. a 5.75-fold increase compared to C1). Figure 4 presents the NRR's for C3-5.

C4 (37 days: 0 backwash): flow rate was initiated at 0.26 L · h-1 for the first two days and maintained a higher NRR than C3 until day 15, when the flow rate reached 2.74 L · h-1 and a NRR of 3.71 g N-NO3- · d-1. A collapse in NRR occurred when the flow rate was increased to 3.04 L · h-1 on day 16, so it was lowered back at 2.65 L · h-1 for two days before being increased again to 3.04 L · h-1. The system recovered and so the flow rate was kept between 3.04 and 4.49 L · h-1 for the last eight days, which achieved a mean NRR of 3.81 ± 0.33 g N-NO3- · d-1 (n=8) (Figure 4). A 23% increase compared to the C3 indicated that improvement of denitrification through stratification was achieved.

C5 (21 days: 0 backwash): flow rate was maintained at the lowest capacity, 0.38 L · h-1, for two days before gradually increasing it to 3.03 L · h-1 until day 12. On day 13, the flow rate was set to 3.48 L · h-1. The NRR decreased a little but was able to stabilise at a mean of 3.90 ± 0.26 g N-NO3- · d-1 (n=7). C4 and C5 resulted in somewhat similar NRR but C5 achieved a faster start-up phase (Figure 4).

In conclusion, data collected from C2-5 provided the confirmation that increasing stratification and using substrate B2 instead of B1 allowed quicker start-ups and improved NRR.

Experiment 2: Pilot-scale autotrophic sulphur-based denitrification

The first trial, with homogeneous loaded columns, ran for 625 days (9 backwashes). The flow rate was set up to 5 L · h-1 the first day, but was rapidly increased and readjusted during the first 110 days. Finally, the flow rate was maintained between 30 and 50 L · h-1, achieving NRR between 5.7 and 39.4 g N-NO3- · d-1 (mean 19.35 ± 5.76 g N-NO3- · d-1). The nitrate-loaded water was at 5-6 °C at the entrance and left the denitrification loop at 13-14°C with a significantly reduced concentration of nitrates that varied with flow-rate, stability of the system and water renewals events.

Results of the stratified pilot-scale trials that ran for 382 days (5 backwashes) show that start-up took 14 days during which the flow rate was increased to 99.6 L · h1. Relatively rapidly, NRR increased to a maximum of 63.99 g N-NO3- · d-1 until nitrate concentrations dropped at 20 mg N-NO3- · L and the flow rate was maintained at 78 L · h-1 until the end of the study. The difference between nitrate concentration at the entrance and the exit of the denitrification loop was significantly reduced, resulting in a mean NRR of 27.23 ± 15.58 g N-NO3- · d-1. Values for pH at the entrance and exit of homogenous and stratified trials are reported in Table 1 indicating that stratification enabled to operate in the optimal range of pH for T. denitrificans in line with measured improvement in NRR.

Concern about the undesirable rise in concentration of elements or metals (for ex.: manganese (Mn), magnesium (Mg), strontium (Sr), calcium (Ca), phosphorus (as P and PO4) dissolved solids, hardness (as CaCO3) liberated from calcareous material originating from quarries or mines in the water when submitted to pH reduction (like during the process of denitration based on sulphur) were recently addressed. Water samples taken at the inlet and the outlet of the pilot-scale system were sent for analysis (Exova inc., St-Augustin-de-Desmaures, QC, Canada 3rd of October 2016).

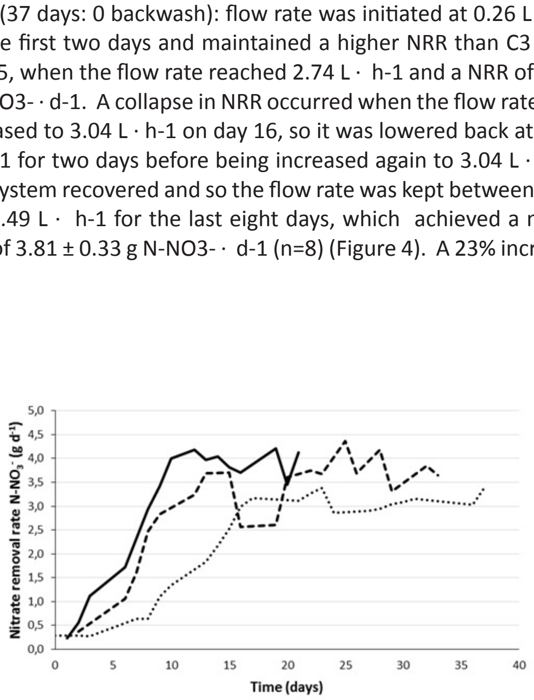
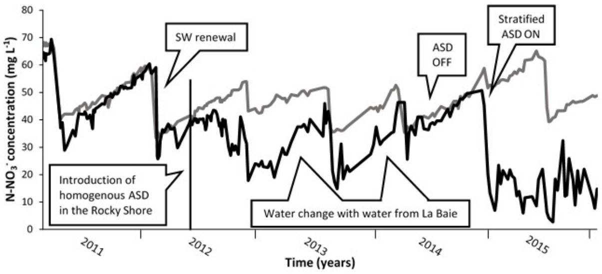


Figure 4. Nitrate removal rate (g N-NO3- · d-1) for the experimental ASD unit. Configurations C3 (˙˙˙) May 20th to June 27th 2014, C4 (‾ ‾ ‾), June 9th to July 16th 2015 and C5 (──), July 21th to August 12th 2015.

Table 1. Mean pH values ± SD during operation of pilot-scale ASD unit: homogenous (June-September 2012) and stratified (4 altering layers per column) (Jan 2015-May 2016) of 1) the incoming influent water, 2) at the exit of the first column (homogenous = sulphur only or stratified = Sulphur50%:Calcareous50%) and 3) at the exit of the calcareous substrate column (homogenous only).

Configuration	pH of the influent	pH exit 1st column	pH reduction (acidification)	pH out of oyster shell column
Homogenous	7.95 ± 0.07	6.35 ± 0.04	1.60 ± 0.05	7.11 ± 0.10
Stratified 4 altering layers $S_{50\%}$: $C_{50\%}$	8.04 ± 0.10	7.02 ± 0.10	1.02 ± 0.10	n/a

Figure 5. Variations of N-NO3- (mg · L-1) in La Baie (—) and the Rocky Shore (━━) between 2011 and 2016 in relation with ASD operation.



Apart from dissolved solids and Ca that respectively increased by 10-16.8% (36 100 to 43 400 mg/L) and 3.3% (271 to 280 mg/L) after passage in the DSS using mine calcareous material, results of water analysis do not indicate any increase in the concentration of Mg (713 to 683 mg/L) a reduction of 4.3%) and or P (6.97 to 6.89 mg/L a reduction of less than 1%). Hardness reduced slightly by 2.8% after passage in the ASD (3610 to 3510 mg/L) and Sr levels decreased by 6.8% (8.80 to 8.S4 mg/L). Mn levels were stable after passage in the stratified columns (< 0.001 to 0.001). Overall, after months of operation, seawater composition at 24 PSU of the exhibit connected to ASD were considered normal and within range of safe operation limits for aquatic life.

Figure 5, highlights the impact of ASD on the nitrate concentration of the Rocky Shore and the La Baie ecosystem (no ASD). The pilot-scale ASD unit made a great difference for the control of nitrate. Nitrate concentrations fluctuated in the 25m3 exhibit because water renewal uses “polluted” effluent water from the La Baie system to which it is connected. Noticeably, during a shut-down (ASD was off most of 2014), [N-NO3-] values quickly returned to the values of La Baie (see Figure 5). The restart of the ASD unit under C4 and C5 configurations enabled to reach N-NO3- < 20 mg · L very rapidly (14 days).

Based on the results of the stratified pilot-scale ASD unit, a predictive model estimating the effectiveness of a new large-scale ASD unit connected to La Baie (2 500m3) operating at 9-10°C was calculated. This unit adopted configuration C5 with 2m3 of a sulphur/limestone fixed-bed (50:50), and developed a mean volumic NRR of 0.64 Kg N-NO3 · m-3 S· d-1 at 900 L · h-1. Operating under progressively reduced [N-NO3] from 50 to 30 mg N-NO3- · L-1 would take between 125-175 days. We estimate the cost of the new unit at CAD $6 000, in comparison to the CAD $31 800 required in salts to achieve the same nitrate concentration through water renewal. Our system compares well with the NRR of Hignette et al. (1997) with 0.34 Kg N-NO3 · m-3 S · d-1 (at 25-30°C) and Simard et al. (2015) with 0.02 Kg N-NO3- · m-3 S· d-1 (at 12°C) both using fixed sulphur beds. A fluidised sulphur bed at the National Aquarium (Baltimore, USA) achieved NRR exceeding 1.6 Kg N-NO3- · m3 S · day-1 according to Aiken (2012). Recently, Christianson et al. (2015) also assessed fluidized-bed systems this time for treatment of aquaculture effluents and reached NRR equivalent to that of Aiken (2012). Our efforts allowed us to develop a custom-built, low complexity ASD system adapted to the conditions of our live aquatic collections. Negative effects of low temperature on bacterial conversion efficiency were successfully addressed by means of low and stable flow rates, substrate selection and stratification.

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References

Aiken D. (2012) User-friendly denitrification for sustainable life support system design. National Aquarium, Baltimore, Maryland USA (accessed on 17th May 2016 http://faculty.salisbury.edu/~kxhunter/aiken.iac.2012.pdf)

Christianson, L., C. Lépine, S. Tsukuda, Saito, K., Summerfelt, S. (2015) Nitrate removal effectiveness of fluidized sulphur-based autotrophic denitrification biofilters for recirculating aquaculture systems. Aquacultural Engineering 68: 10-18.

Dupla M., Comeau Y., Parent S., Villemur R., Jolicoeur M. (2006) Design optimization of a self-cleaning moving-bed bioreactor for seawater denitrification. Water Research 40: 249-258.

Grguric G., Wetmore S.S., Fournier R.W. (2000) Biological denitrification in a closed seawater system. Chemosphere 40: 549-55.

Hignette M., Lamort B., Langouet M., Leroy S., Martin G. (1997) Élimination des nitrates par filtration biologique autotrophe sur soufre en aquariologie marine. Congrès E.U.A.C., Munich, Salzbourg & Innsbruck 1996. Mém. Inst. Océano. P. Ricard 1997, pp: 7 – 13.

Koenig A., Liu L. (2004) Autotrophic denitrification of high-salinity wastewater using elemental sulphur: batch tests. Water Environment Research 76: 37 – 46.

Labbé N., Parent S., Villemur R. (2003) Addition of trace metals increases denitrification rate in closed marine systems. Water Research 37: 914-920.

Labelle M.-A., Juteau P., Jolicoeur M., Villemur R., Parent S., Comeau Y. (2005) Seawater denitrification in a closed mesocosm by a submerged moving bed biofilm reactor. Water Research 39: 3409-3417.

Le François N.R., Picq S., Savoie A., Boussin J.-C., Plante S., Wong E., Misserey L., Rojas S. Genet J.-P. (2015) Coupling salinity reduction to aquatic animal well-being and ecosystem representativeness at the Biodôme de Montréal. Journal of Zoo and Aquarium Research 3: 70-76.

Mahon R., Brown S.K., Zwanenburg K.C.T., Atkinson D.B., Buka K.R., Claflin L., Howell G.D., Monaco M.E., O’Boyle R.N., Sinclair M. (1998) Assemblages and biogeography of demersal fishes of the east coast of North America. Canadian Journal of Fisheries and Aquatic Sciences 55: 1704-1738.

Morris A.L., Hamlin H.J., Francis-Floyd R., Sheppard B.J., Guillette L.J. Jr. (2011) Nitrate-induced goiter in captive whitespotted bamboo sharks Chilosryllium plagiosum. Journal of Aquatic Animal Health 23: 92-99.

Parent S., Morin A. (2000) N budget as water quality management tool in closed aquatic mesocosm. Water Research 34: 1846-1856.

Pierce R.H., Weeks J.M., Prappas J.M. (1993) Nitrate toxicity to five species of marine fish. Journal of World Aquaculture Society 24: 105-107.

Sauthier N., Grasmick A., Blancheton P. (1998) Biological denitrification applied to a marine closed aquaculture system. Water Research 32: 1932-1938.

Simard M., Masson S., Mercier G., Benmoussa H., Blais J., Asce M., Coudert L. (2015) Autotrophic denitrification using elemental sulphur to remove nitrate from saline aquarium waters. Journal of Environmental Engineering 141: 04015037.

Tal Y., Nussinovitch A., Rijn, J.V. (2003) Nitrate removal in aquariums by immobilized Pseudomonas. Biotechnology Progress 19: 1019-1021.

Townsend S. (2009) Incorporating sustainable practices for zoos and aquariums: a triple bottom line approach. International Zoo Yearbook 43: 53-63.

Trouvé C., Chazal P.M., Gueroux B., Sauvaitre N. (1998) Denitrification by new strains of *Thiobacillus denitrificans* under non-standard physicochemical conditions. Effect of temperature, pH and sulphur source. Environmental Technology 19 : 601-610.

Van Bussel C.G.J., Shroeder J.P., Wuertz S., Schulz C. (2012) The chronic effect of nitrate on production performance and health status of juvenile turbot (Psetta maxima). Aquaculture 326-329 : 163-167.

Yeats P.A. (1990) Reactivity and transport of nutrients and metals in the St. Lawrence estuary. In: El-Sabh M.I., Silverberg N. (eds) Coastal and Estuarine studies, vol 39. New York, USA : Springler-Verlag, 153 – 169.