Technical note

A simple tool to help decision making in infrastructure planning and management of phytotreatment ponds for the treatment of nitrogen-rich water

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Abstract


2002, 2003) in two experimental phytotreatment ponds for the treatment of aquaculture wastewater located in central Italy. These ponds were selected because they are representative of the average infrastructural and environmental features characterised in most Italian aquaculture ponds being characterised by:

- A depth of 1 m
- Presence of the macro-algae *Ulva rigida* growing spontaneously
- Spring/summer temperature range of 15 to 30°C
- A water pH range of between 7 and 8.

### Experimental

#### Experimental phytotreatment ponds

The ‘Falesia’ Fish Farm (Piombino, Italy) annually produces approximately 200t of gilthead sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) (Bartoli et al., 2005). Wastewater from the fish tanks is released into a lagoon system comprising three ponds connected in series. Each pond has a surface area of ~600 m² for a total surface of ~2 000 m² and a mean depth of ~1 m. Pond 1 is used for sedimentation purposes whilst Ponds 2 and 3 (~300 m²) are colonised by a natural community of the green macro-algae *Ulva rigida*. Physico-chemical properties of wastewater entering the ponds are reported in Table 1.

### Determination of *Ulva* carrying capacity

Evaluation of *Ulva* carrying capacity defined as the macro-algal biomass in which the uptake of dissolved organic carbon (DIC) equals the production of oxygen (O₂) was measured by an *in situ* manipulative experiment carried out on Pond 3 at the Falesia Fish Farm which was fully colonised by *Ulva rigida*. Three consecutive 24 h cycles of investigation were started on May 2003 in this pond. In order to investigate the effect of different macro-algal biomass approximately 20% of fresh biomass was rapidly removed (in 1 h) at the end of Cycles 1 and 2 and a 6 h interval was taken before starting a new sampling to allow for pond stabilisation. *Ulva* biomass was estimated before the beginning of each investigation cycle. Eight replicates were randomly collected in Pond 3 with a cylinder (internal diameter 700 mm, height 1 200 mm) trapping the algae along the whole water column. The macro-algal biomass within the cylinder was then collected, gently washed with pond water and weighed fresh and after oven desiccation at 70°C for 4 to 6 h. *Ulva* for fresh weight determinations was harvested from the pond with long rakes and packed in a series of perforated tanks generally used for fish harvesting. All the material was drained to remove most of the water and then weighed. Estimated macro-algal biomass was ~8 kg·m⁻² at Cycle 1, ~6 kg·m⁻² at Cycle 2 and ~4 kg·m⁻² at Cycle 3. Inlet and outlet pond water were sampled over the three cycles approximately every 3 h for a total of 48 collected samples and analysed for temperature, pH, salinity and oxygen with an YSI 556 multiple probe. Water samples (200 mℓ) were filtered with GF/F Whatman filters within 1 h of collection and immediately frozen for later analyses. Filtered water was analysed for ammonia (NH₄⁺) (Bower & Holm-Hansen, 1980), NO₃⁻ (Golterman et al., 1978), NO₂⁻ as NO₃⁻ after reduction with cadmium and dissolved inorganic carbon (DIC). Ten mℓ of unfiltered water were analysed for total dissolved inorganic carbon (DIC) by titration with 0.1 M HCl (Anderson et al., 1986).

### *Ulva* growth rates (UGR)

*Ulva* growth rates were experimentally assessed from *Ulva* samples collected from the Nassa and Falesia experimental ponds in May 2002 and May 2003 respectively. Fresh thalli were gently washed to remove epiphytic material and cut into ~300 discs (diameter = 50 mm); 20 discs were individually weighed fresh and after desiccation in an oven at 70°C whilst 4 series of 50 discs were suspended in 4 cylindrical cages (internal diameter 200 mm, height 400 mm) and positioned within the *Ulva* mats at a density close to the carrying capacity (250 gdw·m⁻²). Three days later the cages were removed and all the discs were gently washed, dried and weighed for the determination of growth rates.

### Denitrification rates (DR)

Denitrification rates and correlation with nitrate concentrations were measured by means of bare sediment incubations carried out only in the dark at the Nassa experimental pond with approximately 200 to 300 gdw·m⁻² macro-algal biomass. Increasing amounts of labelled nitrate (final concentration comprised between 20 and 150 µM) were added to the water phase of the cores for an evaluation of the sediment denitrification potential.

### TABLE 1

<table>
<thead>
<tr>
<th>Variables</th>
<th>(FALESIA srl) Inlet-May 2003</th>
<th>(FALESIA srl) Outlet-May 2003</th>
<th>(NASSA srl) Inlet-May 2002</th>
<th>(NASSA srl) Outlet-May 2002</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water flow (l/s)</td>
<td>~250</td>
<td>~140</td>
<td>~200</td>
<td>~120</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>23.5±0.5</td>
<td>23.5±0.6</td>
<td>23.6±1.2</td>
<td>23.7±1.1</td>
</tr>
<tr>
<td>Salinity (ppt)</td>
<td>37.8±0.1</td>
<td>37.8±0.1</td>
<td>41.9±0.0</td>
<td>42.0±0.1</td>
</tr>
<tr>
<td>pH</td>
<td>6.9±0.1</td>
<td>6.8±0.1</td>
<td>7.6±0.1</td>
<td>7.8±0.2</td>
</tr>
<tr>
<td>Oxygen (µmol ℓ⁻¹)</td>
<td>77.5±55.7</td>
<td>66.7±70.6</td>
<td>72.4±9.1</td>
<td>184.3±131.2</td>
</tr>
<tr>
<td>NO₃⁻ (µmol ℓ⁻¹)</td>
<td>6.2±0.9</td>
<td>5.6±1.9</td>
<td>3.6±2.5</td>
<td>10.8±8.6</td>
</tr>
<tr>
<td>NH₄⁺ (µmol ℓ⁻¹)</td>
<td>179.3±30.1</td>
<td>180.0±35.9</td>
<td>61.2±6.5</td>
<td>35.4±16.6</td>
</tr>
</tbody>
</table>

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The rates of denitrification (D15) were calculated according to the equations and assumptions of Nielsen (1992).

**Results**

*Ulva carrying capacity*

Results from the *in situ* manipulative experiment showed the effectiveness of the removal of macro-algal biomass on the phytotreatment pond changing from a strong heterotrophic condition to almost autotrophic (dissolved inorganic carbon [DIC] and oxygen [O2] daily balance shifted from 2 686.5 to 1 066.5 mol DIC·d^{-1} and from -518.6 to -13.0 mol O2·d^{-1} for Cycle 1 and Cycle 2 respectively) (Fig. 1). At the end of the 3rd cycle the pond became almost a sink for NH4^+ (11.4 mol NH4^+·d^{-1}) and the carrying capacity defined as the macro-algal biomass in which the uptake of DIC equals the production of O2 was identified as 300 g dw·m^{-2} of macro-algal biomass.

**Assessment and optimisation of Ulva growth rates (UGR) and Ulva assimilation rates (UAR)**

Close to the carrying capacity (250 g dw·m^{-2}) weight of dry Ulva discs augmented on average of ~2.3 mg d^{-1} in the cage compartments (0 to 40 cm depth), resulting in a specific growth rate of about 4% d^{-1}. Nitrogen assimilation by Ulva has been calculated indirectly by growth rates experiments assuming for healthy algae growing in a not limiting environment a constant nitrogen content of 4% dw. Based on experimental data, the temporal evolution of macro-algal biomass and nitrogen removal from water has been simulated with a logistic model assuming an instantaneous growth rate of 0.2·d^{-1} and the *in situ* estimated carrying capacity of 300 g dw·m^{-2}. The amount of nitrogen removed per pond per day was then calculated as a function of the *in situ* Ulva biomass. With the chosen logistic model and *in situ* parameters we calculated that the maximum inorganic nitrogen removal (~0.04 mol N·d^{-1}·m^{-2}) was attained when Ulva biomass reaches 150 g dw·m^{-2} and growth rate is 0.1·d^{-1} (Fig. 2).

**Denitrification rates (DR)**

Denitrification rates accounted for a small amount of total nitrogen removal at the Nassa experimental ponds with *in situ* values of ~150 µmol N·m^{-2}·h^{-1}. However, results from the concentration series experiment clearly demonstrated that denitrification increased at higher 15NO_3^- denitrification (D_{15}) concentrations and a saturation value cannot be calculated being the highest D15 value (>800 µmol N·m^{-2}·h^{-1}) still linearly correlated with the water column 15NO_3^-. The relationship occurring between 15NO_3^- concentrations (0 to 150 µM) and denitrification rates were modelled using linear regression and resulted in the following:

\[ D_{15}[\mu\text{molN} \cdot \text{m}^{-2} \cdot \text{h}^{-1}] = 5.3 \times [15\text{NO}_3^-][\mu\text{M}] + 14.8; r^2=0.94. \] (Fig. 3)

**Discussion**

Considering the optimal condition of 150 g dw·m^{-2} of macro-algal biomass, 0.04 mol N·d^{-1}·m^{-2} Ulva assimilation rates and 0.1·d^{-1}
Ulva growth rate the following calculations have been made by use of MATLAB algorithms:

a) \[ \text{MB} = (1.5^{\text{a}} \times \text{A} \times \text{UGR}) \]
where:
- \( \text{MB} \) = macro-algal biomass to be removed daily (kg·d\(^{-1}\))
- \( \text{A} \) = pond area (m\(^2\))
- \( \text{UGR} \) = \( \text{Ulva} \) growth rates (d\(^{-1}\))

b) \[ \text{AN} = (\text{UAR} \times 14^{\text{b}} \times \text{A}) \times (\text{AIW} \times 3600^{\text{c}} \times 24^{\text{d}}) \times 100^{\text{e}} \]
where:
- \( \text{AN} \) = macro-algal assimilated nitrogen (%)
- \( \text{UAR} \) = \( \text{Ulva} \) assimilation rates (µmol N·d\(^{-1}·m^{-2}\))
- \( \text{A} \) = pond area (m\(^2\))
- \( \text{AIW} \) = ammonia in inlet water (µmol)
- \( \text{F} \) = water flow (ℓ·s\(^{-1}\))

c) \[ \text{DN} = \left( (5.3^{\text{f}} + \text{NPW} + 14.8^{\text{g}}) \times \text{A} \times 24^{\text{h}} \times 14^{\text{i}} / (\text{AIW} \times 14^{\text{j}} \times \text{F} \times 3600^{\text{k}} \times 24^{\text{l}}) \right) \times 100^{\text{e}} \]
where:
- \( \text{DN} \) = denitrified nitrogen (%)
- \( \text{NPW} \) = nitrate in pond water (µmol)
- \( \text{A} \) = pond area (m\(^2\))
- \( \text{AIW} \) = ammonia in inlet water (µmol)
- \( \text{F} \) = water flow (ℓ·s\(^{-1}\))

d) \[ \text{TNR} = \text{AN} + \text{DN} \]
where:
- \( \text{TNR} \) = total nitrogen removal (%)
- \( \text{AN} \) = macro-algal assimilated nitrogen (%)
- \( \text{DN} \) = denitrified nitrogen (%)

e) \[ \text{AOW} = \text{AIW} - (\text{AIW} \times (\text{TNR} / 100^{\text{e}})) \]
where:
- \( \text{AOW} \) = ammonia in outlet water (µmol)
- \( \text{AIW} \) = ammonia in inlet water (µmol)
- \( \text{TNR} \) = total nitrogen removal (%)

and where:
- \( \text{Kg} \), m\(^2\)/µmol = conversion factor
- Conversion factor
- Coefficient of linear regression

This creates a simple tool (MATLAB function m-file) able to provide:

- Gross estimation of biological processes involved in nitrogen removal (output: macro-algal assimilated nitrogen [%], denitrified nitrogen [%], total nitrogen removal [%], ammonia outflow [µM])
- Optimal management strategies (output: macro-algal biomass to be removed daily [kg fresh biomass]).

These are calculated using structural and chemical input variables from ponds:

- Structural input: pond area [m\(^2\)], water flow [ℓ·s\(^{-1}\)]
- Chemical input: ammonia concentrations in inlet water [µM], nitrogen concentrations in pond [µM].

The current version is set up for modelling phytotreatment ponds having a depth of ~1 m, optimal macro-algal biomass of 1.5 kg·m\(^{-2}\) and environmental conditions characterised by summer temperature ranging 15 to 30°C, daylight ranging between 500 and 2 000 µE·m\(^{-2}·s^{-1}\), water pH ranging between 7.0 and 8.0 and a certain nitrification potential to allow for ammonium oxidation to nitrate (Bartoli et al., 2005). Adaptations of the tool to different environmental conditions and seasonal variations can be achieved by setting up key variables such as Ulva growth rates and Ulva assimilation rates on the basis of newly collected experimental data. Finally, to facilitate access in a user-friendly way the MATLAB version of the tool has been translated into a Windows compatible format (Ulva.xls, MS Excel) (Fig. 4) and is available upon request from the Department of Biology of the University of Genova.

Conclusions

Figure 4 shows the output of the developed tool that has been run using input variables measured at the Falesia and Nassa phytotreatment ponds respectively. At the Falesia Fish Farm even following an optimal management strategy (~195 kg fresh macro-algal biomass to be removed daily) the estimated total nitrogen removal accounts for less than ~2% of the total nitrogen inlet, with macro-algal assimilation representing the main removal processes. The low remediation potential of the Falesia system is depending upon the strong unbalance between structural and chemical pond variables such as the small area (1.300 m\(^2\)), the high water flow (250 ℓ·s\(^{-1}\)) and the high nitrogen load (179 µM) in wastewater. In contrast at the Nassa fish
farm the estimated total nitrogen removal in the managed pond (~1 200 kg fresh macro-algal biomass to be removed daily) may account for up to ~50% of total inlet due to the higher pond area (8 000 m²) and the reduced nitrogen load in wastewater (61 μM). Again macro-algal assimilation constitutes the major removal processes while denitrification only accounts for a small percentage (~1%) of nitrogen removal due to the low nitrate concentration in pond water.

By manipulating the input variables and running the tool the user can rapidly calculate the best conditions in order to obtain 100% ammonia removal from wastewater in the two ponds. As a general estimate the surface of phytotreatment ponds needed for 100% ammonia removal in wastewater using the macro-algae Ulva rigida can be calculated as being as less ~50 m²/pe for a load per capita of ~30 gN·d⁻¹ under optimal conditions. This estimate can be further improved if we consider an increase of denitrification rates, for example, by stimulating nitrification rates in pond water (e.g. oxygen supply). Of course this corresponds to the daily harvesting of ~150 g·m⁻² dry macro-algal biomass that has to be managed as well (150 g·m⁻² dry macro-algal biomass corresponds to 1500 g·m⁻² of fresh biomass). The management of harvested Ulva is a matter of debate up to now and its use for consumption by humans, by secondary cultured macro-algivores (such as abalone and sea urchins) and by other fish, has been proposed recently (Neori et al., 2004).

Successful commercial exploitation of biological systems relies on minimising development time and cost while simultaneously delivering substantial process efficiencies. The tool we have developed enables this concept by providing improved strategies for the optimisation and control of bioprocesses involved in nitrogen removal by phytotreatment pond. Recently a conceptual model for the application of bioremediation strategies in organic-rich ecosystems has been developed and is based on the mobilisation (decomposition of insoluble organic polymers) and removal of macro-elements (e.g. carbon and nitrogen) from accumulation areas (Vezzulli et al., 2004). Among systems for biological removal the use of foliose macro-algal material is considered a primary candidate; thus enhancing the potential interest of the tool we have developed within the field of applied biotechnology.

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References


