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

In situ experimental studies were carried out aimed at the quantitative estimation of biological processes involved in nitrogen removal such as macro-algal assimilation and bacterial denitrification and their optimisation in two experimental phytotreatment ponds colonised by the macro-algae *Ulva rigida* in central Italy. Results from an *in situ* manipulative experiment estimate that *Ulva* carrying capacity defined as the macro-algal biomass in which the uptake of dissolved inorganic carbon (DIC) equals the production of oxygen (O_2), was close to $300 g \cdot m^{-2}$ dry biomass (dw). At this carrying capacity the experimental assessment of *Ulva* growth rates and *Ulva* assimilation rates and their optimisation with use of a logistic model estimated that maximum inorganic nitrogen removal (~0.04 mol $N \cdot d^{-1} m^{-2}$) was attained when *Ulva* biomass reached 150 $g_{dw} \cdot m^{-2}$ and growth rate was $0.1 \cdot d^{-1}$. Denitrification rates accounted for a small amount of total nitrogen removal (~150 µmol $N \cdot m^{-2} \cdot h^{-1}$) although an intact core incubation experiment demonstrated that denitrification increased with increasing nitrate concentrations. Based on experimental results a series of calculations have been made by use of MATLAB algorithms to facilitate manipulation of easy-to-measure variables (infrastructural, chemical and biological) and subsequent gross estimates of their effect on biological nitrogen removal efficiency, thus providing a simple tool to help decision making for infrastructure planning and management of phytotreatment ponds.

Keywords: aquaculture wastewater, phytotreatment pond, *Ulva rigida*

Introduction

Phytotreament ponds with foliose macro-algae are widely employed for the treatment of ammonia-rich water due to their simple arrangement (not needing complex infrastructure) and low cost (Redding et al., 1997; Brix, 1999; Tanner, 2001; Vymazal et al., 2001; Lin et al., 2002; Schulz et al., 2003). In terms of Italian Government law, the construction of phytotreatment ponds is compulsory for wastewater treatment under particular circumstances and various activities, the main being land-based aquaculture. The aim of phytoremediation ponds is the removal of dissolved nitrogen from wastewater to be safely discharged into the natural environment. Foliose macro-algae are primarily utilised for this purpose and *Ulva rigida* is one of the most common due to its high net growth rates, nitrogen uptake capacity and resistance to environmental stress (Laliberté et al., 1994). Coupled to dissolved nitrogen assimilation by primary producers, removal of particulate matter must be previously achieved via sedimentation pond in order to fully exploit macro-algae assimilation (Bartoli et al., 2005) and prevent deterioration of microbiological quality of the treated water (Vezzulli et al., 2005). Despite the potential usefulness of sedimentation/phytotreatment pond system for the treatment of nitrogen rich water one of the main reasons limiting their full exploitation is the lack of suitable methods to help decision making in pond infrastructure planning and pond management. Failing to observe these rules limits the usefulness of the pond and may lead (e.g. due to macro-algal collapse in an un-managed pond) to further deterioration of the wastewater. Therefore, the functionality of phytoremediation ponds is based on structural variables to be planned before construction (pond area, depth and water flow) and biological and chemical variables (macro-algae biomass, dissolved ammonia and nitrate concentrations in inlet water) to be experimentally estimated in order to optimise biological nitrogen removal (macro-algal assimilation and denitrification) through a correct management. Both of these variables are strongly correlated and estimation of their values and optimal setting requires expensive investigation often resulting economically unaffordable for the end-users. To provide a solution to this problem we developed a user-friendly tool able to facilitate manipulation of these variables (structural, chemical and biological) and provide subsequent gross estimates of their effect on biological nitrogen removal efficiency, thus providing a tool to help decision making for planning and management of phytotreatment ponds. The tool is based on a series of experimental studies aimed at the quantitative estimation of biological processes involved in nitrogen removal such as macro-algal assimilation and bacterial denitrification and their optimisation as a function of structural and chemical pond variables. The setting-up of the tool has been carried out during late spring (May

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TABLE 1 Physico-chemical properties of wastewater inflow and outflow of the phytotreatment ponds at the beginning of each experiment				
	(FALESIA srl) Inlet-May 2003	(FALESIA srl) Outlet-May 2003	(NASSA srl) Inlet-May 2002	(NASSA srl) Outlet-May 2002
Variables	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Water flow (ℓ/s)	~250		~140	
Temperature(C°)	23.5±0.5	23.5±0.6	23.6±1.2	23.7±1.1
Salinity (ppt)	37.8±0.1	37.8±0.1	41.9±0.0	42.0±0.1
pH	6.9±0.1	6.8±0.1	7.6±0.1	7.8±0.2
Oxygen (µmol $ℓ^{-1}$)	77.5±55.7	66.7±70.6	72.4±9.1	184.3±131.2
NO_3^-(µmol $ℓ^{-1}$)	6.2±0.9	5.6±1.9	3.6±2.5	10.8±8.6
NH_4^+(µmol $ℓ^{-1}$)	179.3±30.1	180.0±35.9	61.2±6.5	35.4±16.6

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 found in most Italian aquaculture ponds being characterised by:

- A mean 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 m^2 for a total surface of ~2 000 m^2 and a mean depth of ~1 m. Pond 1 is used for sedimentation purposes whilst Ponds 2 and 3 (~1 300 m^2) 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.

The 'Nassa' Fish Farm (Orbetello,Italy) annually produces approximately 100t of gilthead sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) (Bartoli et al., 2006). Wastewater from the fish tanks is released into a lagoon system comprising four ponds connected in series. Each pond has a surface area of ~2 600 m^2 for a total surface area of ~10 000 m^2 and a mean depth of ~1 m. Pond 1 is used for sedimentation purposes whilst Ponds 2-4 (~8 000 m^2) are colonised by a natural community of the green macro-algae *Ulva rigida*. Chemical-physical 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 (O_2) 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 (NH^{4+}) (Bower & Holm-Hansen, 1980), NO_2^- (Golterman et al., 1978), NO_3^- as NO_2^- 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 g_{dw} ·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 g_{dw}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.

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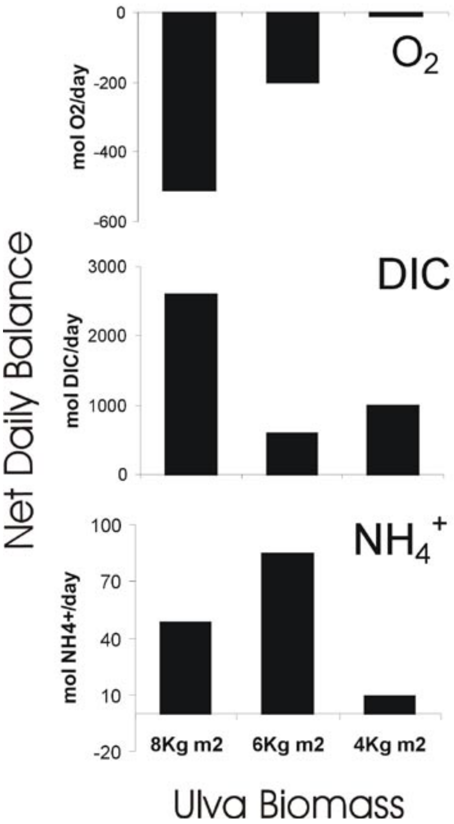

Figure 1
Average net daily balances of oxygen, dissolved inorganic carbon and ammonium during the three cycles of investigation at Falesia Fish Farm

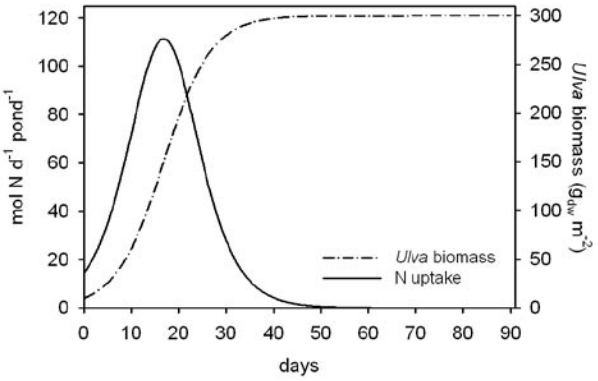

Figure 2
Theoretical N uptake by Ulva. The dashed line shows the evolution of Ulva biomass according to a logistic growth curve ($r = 0.2\ d^{-1}$, $K = 300\ g_{dw}\ m^{-2}$); the continuous line represents the associated nitrogen demand, calculated assuming an Ulva N content of 4%dw. Nitrogen demand sustaining Ulva growth equals nitrogen removed per day per pond. Maximum removal occurs when Ulva biomass reaches 150 $g_{dw}\cdot m^{-2}$ (from Bartoli et al, 2006).

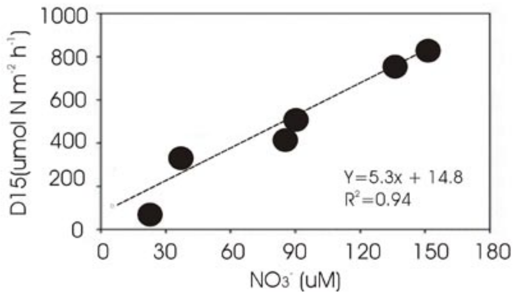

Figure 3
Rates of $^{15}NO_3^-$ denitrification (D_{15}) as function of labelled nitrate concentration in the water column (from Bartoli et al, 2006)

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 [O_2] daily balance shifted from 2 686.5 to 1 066.5 mol DIC·d^{-1} and from -518.6 to -13.0 mol O_2·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 NH_4^+ (11.4 mol NH_4^+·d^{-1}) and the carrying capacity defined as the macro-algal biomass in which the uptake of DIC equals the production of O_2 was identified as 300g_{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 $^{15}NO_3^-$ 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 $^{15}NO_3^-$. The relationship occurring between $^{15}NO_3^-$ concentrations (0 to 150 µM) and denitrification rates were modelled using linear regression and resulted in the following:

$$D_{15}\,[\mu molN\cdot m^{-2}\cdot h^{-1}] = 5.3\ ^{15}NO_3^-[\mu M]+14.8;\ r^2=0.94.\ (Fig.\ 3)$$

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}

A) File name: Ulva.xls

Phytotreatment pond average condition		
Ulva biomass (Kg fw m^2)		1,5
Water depth (m)		1,0
Temperature range (C°)		15-30
Light intensity range (uE m^{-2} s^{-1})		500-2000
pH range		7-8
Experimental measurements		
Ulva Growth rate (d^{-1})		0,1
Ulva assimilation rates (umol N d^{-1} m$^{-2)}$		40000,0
User-Friendly Tool		
INPUT		**INPUT**
Pond Area (m^2)	enter value >	1300,0
Water Flow (l s^{-1})	enter value >	250,0
Ammonia in inlet water (uM)	enter value >	179,0
Nitrate in pond water (uM)	enter value >	6,0
OUTPUT		
Biomass to be removed daily (Kg fw d^{-1})		195,0
Denitrified nitrogen(%)		0,04
Assimilated nitrogen(%)		1,3
Total nitrogen removal (%)		1,4
Ammonia in outlet water (uM)		176,5

B) File name: Ulva.xls

Phytotreatment pond average condition		
Ulva biomass (Kg fw m^2)		1,5
Water depth (m)		1,0
Temperature range (C°)		15-30
Light intensity range (uE m^{-2} s^{-1})		500-2000
pH range		7-8
Experimental measurements		
Ulva Growth rate (d^{-1})		0,1
Ulva assimilation rates (umol N d^{-1} m$^{-2)}$		40000,0
User-Friendly Tool		
INPUT		**INPUT**
Pond Area (m^2)	enter value >	8000,0
Water Flow (l s^{-1})	enter value >	140,0
Ammonia in inlet water (uM)	enter value >	61,0
Nitrate in pond water (uM)	enter value >	6,0
OUTPUT		
Biomass to be removed daily (Kg fw d^{-1})		1200,0
Denitrified nitrogen(%)		1,21
Assimilated nitrogen(%)		43,4
Total nitrogen removal (%)		44,6
Ammonia in outlet water (uM)		33,8

Figure 4
Example of management strategies and wastewater treatment prediction provided by the tool (Ulva.xls, MS Excel):
A) At the Falesia Fish Farm estimated total nitrogen removal is less than 2% even employing optimal management conditions due to structural problems of the phytotreament system such as small pond area, high water flow and high nitrogen load from wastewater
B) At the Nassa Fish Farm estimated total nitrogen removal is ~45% due to the larger pond area and reduced nitrogen load from wastewater.

Ulva growth rate the following calculations have been made by use of MATLAB algorithms:

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

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

c) $\mathbf{DN=((5.3^{(f)} * NPW + 14.8^{(f)})*A*24^{(d)}*14^{(b)})/(AIW*14^{(b)}*F*3600^{(c)}*24^{(d)})*100^{(e)}}$
where:
DN = denitrified nitrogen (%)
NPW = nitrate in pond water (µmol)
A = pond area (m^2)
AIW = ammonia in inlet water (µmol)
F = water flow (ℓ·s^{-1})

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

e) $\mathbf{AOW = AIW - (AIW*(TNR/100^{(e)}))}$
where:
AOW = ammonia in outlet water (µmol)
AIW = ammonia in inlet water (µmol)
TNR = total nitrogen removal (%)

and where:
(a) Kg_{fw} m^{-2} of macro-algal biomass
(b) grams of nitrogen per mole
(c) number of seconds per hour
(d) number of hours per day
(e) conversion factor
(f) 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 Genoa.

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

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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 m^2) 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 least ~50 m^2/pe for a load *per capita* of ~30 $gN \cdot d^{-1}$ 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 $g \cdot m^{-2}$ dry macro-algal biomass that has to be managed as well (150 $g \cdot m^{-2}$ dry macro-algal biomass corresponds to 1500 $g \cdot m^{-2}$ 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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