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Bioaugmentation involving a bacterial consortium isolated from the rhizosphere of *Spirodela polyrhiza* for treating water contaminated with a mixture of four nitrophenol isomers

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A flask-scale laboratory study was performed to assess the bioaugmentation of water contaminated with a mixture of 2-nitrophenol, 3-nitrophenol, 4-nitrophenol and 2,4-dinitrophenol by using a bacteria consortium consisting of three nitrophenol-degrading bacteria strains (*Pseudomonas* sp. strain MFR-1, *Pseudomonas* sp. strain PFR-1 and *Rhodococcus* sp. strain DFR-1), reinoculated into the roots of *Spirodela polyrhiza*. The selected strains were colonized into the root at approximately 10^4 to 10^6 colony-forming units (CFU per plant). The high populations remained stable through five sequential two-days degradation cycles and complete nitrophenol removal was achieved within five-repeated cycles. Hence, inoculation of subjected degraders into the roots of aquatic plants is an effective treatment for nitrophenol-contaminated effluents or aquatic resources.

1 Introduction

Contamination of water resources by various nitrophenol isomers poses a serious threat to human health and ecosystems. Excessive use of these nitrophenol compounds, including wood preservatives, pesticides, explosive industrial products and dyes, is evident by their presence in aquatic environments.[1] Since nitrophenols pose a wide range of risks not only to humans but also to aquatic organisms, the US Environmental Protection Agency has listed nitrophenols as priority pollutants.[2] Many methods for ensuring the safety of both humans and aquatic organisms with respect to nitrophenols have been explored, while considering the associated costs and energy consumption. Among all these treatment methods, biological treatment methods such as phytoremediation are considered economically feasible and environmental-friendly for the removal of a wide range of pollutants from aquatic environments.[3,17] However, factors such as the low speed of the process, the poor survival and low-degradation ability of microbes in the rhizosphere at contaminated sites or in polluted water sources hamper effective phytoremediation.

Bioaugmentation is a promising tool for phytoremediation, as it addresses the risks and problems associated with dissemination of microorganisms. Most remedial technologies focus on the pollutant dissipation and neglect the sustainability of pollutant removal and the ecological impact of the introduced microorganisms on the indigenous microorganisms.[3,8] Biodegradation in the rhizosphere can be stimulated by oxygen and by organic exudates released from plant roots. This process is known as the "rhizosphere effect," in which plants provide a microenvironment that is advantageous for stimulating microorganisms to be more effective decomposition of pollutants. However, the native bacterial community associated with plant roots cannot always degrade all pollutants;[10–13] therefore, phytoremediation processes are often limited or take a long time to achieve the desired goals. Moreover, the response of a single test species may differ from the responses of the same species in the entire community. Hence, inoculation of a consortium of pollutant-degrading bacteria into the rhizosphere, has greater potential than inoculation of a single species for enhancing the removal of recalcitrant pollutants.

To date, the environmental impact of remedial options has rarely been considered during decision-making processes. Accelerated degradation of synthetic surfactants and aromatic compounds in the rhizosphere of a floating aquatic plant, *Spirodela polyrhiza* (giant duckweed) have been reported by many researchers.[4–7] Moreover, our previous studies have shown that *S. polyrhiza* is efficient in promoting nitrophenols biodegradation around its roots.[8,9] However, the bioaugmentation effect on the sustainability of nitrophenol degradation in the contaminated water remains unclear. Therefore, this study aimed to

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investigate the effectiveness of bioaugmentation by using *S. polyrhiza,* in association with nitrophenol-degrading bacteria isolated from *S. polyrhiza* (3-nitrophenol-degrading *Pseudomonas* sp. strain MFR-1, 4-nitrophenol-degrading *Pseudomonas* sp. strain PFR-1, and 2,4-dinitrophenol-degrading *Rhodococcus* sp. strain DFR-1)[8,9] in flask-scale sequencing batch reactors (SBRs) for five repeated cycles to treat water contaminated by mixture of four nitrophenol isomers.

2 Materials and methods

2.1 Plants and chemicals

In order to obtain bacteria-free *S. polyrhiza*, the plants were sterilized by a 1 min wash in 70% ethanol and a 5 min wash in sodium hypochlorite solution (5% available chlorine), rinsed twice with autoclaved deionized water, and germinated in sterile modified Hoagland nutrient medium that previously prepared by Toyama *et al.*[7] The bacteria-free plants were aseptically maintained in sterile Hoagland solution in an incubation chamber at 28 °C, 10 000 lux (16 : 8 h light–dark cycle).

A water sample of secondary effluent from a sewage treatment plant in Yamanashi (pH 7.42; NH_4^+–N, 2.26 mg L^{-1}; NO_2^-–N, 0.510 mg L^{-1}; NO_3^-–N, 9.40 mg L^{-1}; PO_4^{3-}–P, 2.09 mg L^{-1}) was used in SBR experiments.

2-NP and 3-NP were purchased from Kanto Chemical Co. Inc. (Tokyo, Japan). 4-NP and 2,4-DNP were purchased from Nacalai Tesque Inc. (Kyoto, Japan).

2.2 Microorganism strains and growth conditions

Three nitrophenol-degrading bacteria, 3-nitrophenol degrading bacteria strain MFR-1, 4-nitrophenol degrading bacteria strain PFR-1 and 2,4-dinitrophenol utilizing bacteria strain DFR-1, isolated from *S. polyrhiza* roots[8,9] were used in this study (Table 1). Strain MFR-1, PFR-1 and DFR-1 were inhabited in the roots amended with Fuefuki river water containing 3-nitrophenol, 4-nitrophenol, and 2,4-dinitrophenols for 15 days. A wide range aromatic compounds degradation has been demonstrated by these strains. Strains MFR-1 and PFR-1 were cultured in basal salts medium (BSM; 1.0 g L^{-1} $(NH_4)_2SO_4$, 1 g L^{-1} K_2HPO_4, 0.2 g L^{-1} NaH_2PO_4, 0.2 g L^{-1} $MgSO_4 \cdot 7H_2O$, 0.05 g L^{-1} NaCl, 0.05 g L^{-1} $CaCl_2$, 8.3 g L^{-1} $FeCl_3 \cdot 6H_2O$, 1.4 g L^{-1} $MnCl_2 \cdot 4H_2O$, 1.17 mg L^{-1} $Na_2MoO_4 \cdot 2H_2O$, and 1 mg L^{-1} $ZnCl_2$; pH 7.2) containing 3-NP or 4-NP as the sole carbon source (3NP–BSM or 4NP–BSM, respectively). Strain DFR-1 was cultured in BSM containing 2,4-DNP supplemented with peptone (0.5 g L^{-1}) and yeast extract (0.25 mg L^{-1}) (DNP-PY-BSM). Agar solid medium was prepared with 1.5% (w/v) agar.

2.3 Inoculation of nitrophenol-degrading bacteria on *S. polyrhiza* root surface

Each strain was cultured on rotary shaker at 28 °C and 150 rpm. Cells from late exponential phase were recovered by centrifugation (4500 × *g* at 4 °C for 20 min) and washed using BSM. Cells of each strain were then suspended into 20 mL sterile Hoagland solution at a cell density (as determined by the optical density at a wavelength of 600 nm [OD_{600}]) of 0.03. Bacteria-free plants were dipped into the above bacterial cell suspension for 10 min and washed twice with sterile Hoagland solution. The plants were identified according to their associated bacteria and used for subsequent experiments. In this investigation, we prepared single-strain PFR-1-augmented *S. polyrhiza*, single-strain DFR-1-augmented *S. polyrhiza* (plants + DFR-1), two-strain PFR-1- and DFR-1-augmented *S. polyrhiza* (plants–PFR-1/DFR-1) and three-strain MFR-1-, PFR-1- and DFR-1-augmented *S. polyrhiza* (plants–3 strains association).

2.4 Nitrophenol degradation by plants–PFR-1, –DFR-1 and –PFR-1/DFR-1 associations

To assess the beneficial effect of inoculating nitrophenol-degrading bacteria on plant roots, we performed nitrophenol degradation experiments in sterile Hoagland solution using the plants–nitrophenol-degrading bacteria associations. Strains PFR-1 and DFR-1 were used as test strains. The experiments were conducted in 300 mL Erlenmeyer flasks containing 100 mL of Hoagland solution supplemented with an mixture of nitrophenols (2-, 3-, 4-nitrophenol and 2,4-dinitrophenols; 3 mg L^{-1} of each nitrophenol). We set up two different experimental groups. In the first group, 10 plants of plants–PFR-1, plants–DFR-1 or plants–PFR-1/DFR-1 association were planted in an experimental flask. The second (control) experimental group consisted of strain PFR-1, DFR-1 or a mixture of PFR-1 and DFR-1, without any plants, in sterile Hoagland solution supplemented with the mixture of nitrophenols. To obtain the bacteria for the control experiment, the roots were cut from 10 plants of each plants–bacterial strain association. The roots were transferred into tubes containing 10 mL sterile Hoagland solution, vortexed and filtered (10 μm pore size, Millipore, Tokyo, Japan) to remove root material. The bacterial cell suspensions were then inoculated into the appropriate control experimental flask. All flasks were incubated statically (28 °C; 8000 lux; 16 : 8 h

Table 1 Characteristic of nitrophenol-degrading bacteria used in this study

Strain[8,9]	Relevant characteristic(s)
Pseudomonas sp. MFR-1	3-NP degrader; Gram-negative; capable of degrading 2-nitrophenol and 3-nitrophenol, but not 4-nitrophenol or 2,4-dinitrophenols
Pseudomonas sp. PFR-1	4-NP degrader; Gram-negative; capable of degrading 2-nitrophenol, 3-nitrophenol and 4-nitrophenol but not 2,4-dinitrophenols
Rhodococcus sp. DFR-1	2,4-DNP degrader; Gram-positive; capable of degrading all four NPs (2-NP, 3-NP, 4-NP and 2,4-DNP)

light–dark). Nitrophenol concentrations were measured daily for the 4 day experimental period, and bacterial numbers were monitored at inoculation and on days 2 and 4.

2.5 SBR experiment using plants–consortium bacteria association

To assess the potential of *S. polyrhiza* and the nitrophenol-degrading rhizobacteria association for the sustainable treatment of nitrophenol-contaminated water, we conducted flask-scale SBR experiments (2 day reaction time per cycle, 5 cycles) using the plants–3 strains association. The consortium of three strains was selected due to unsustainability of nitrophenols removal over 5 cycles by two strains. Two types of nitrophenol-contaminated water, nitrophenol-contaminated sterile Hoagland solution and nitrophenol-contaminated secondary effluent water, were prepared by dissolving 3 mg L^{-1} of each of the four nitrophenols into sterile Hoagland solution or a secondary effluent sample. Two test systems were constructed in 300 mL Erlenmeyer flasks containing 100 mL of the NP-contaminated water. The experimental flask consisted of the nitrophenols-contaminated water with 10 plants of the plants–3 strains association. The flask was statically incubated at 28 ± 1 °C under fluorescent lamps at 8000 lux (16 : 8 h light–dark). After 2 days, the 10 plants were transferred to a new flask containing nitrophenols-contaminated water and incubated under the same conditions. This 2 day cycle was repeated for a total five times in triplicate. Concentrations of the four nitrophenols were monitored over the experimental period. A control experiment using 10 sterilized plants without bacterial inoculation was also conducted under the same conditions for one cycle.

2.6 Analytical procedures

In nitrophenols-degradation experiments, the water sampled was centrifuged (9600 × *g* at 4 °C for 10 min), and the supernatant was subjected to high performance liquid chromatography (HPLC) analysis to measure the nitrophenol concentrations. HPLC analysis was conducted in a Shimadzu system with a UV-vis detector and a Shim-pack VP-ODS column (150 mm × 4.6 mm, particle size 5 μm; Shimadzu, Kyoto, Japan). An acetonitrile–water–acetic acid mixture (500 : 498 : 2, vol/vol/vol) was used as the mobile phase at a flow rate of 1 mL min^{-1}, and detection was at a wavelength of 280 nm. For monitoring bacterial populations, bacterial cells were collected separately from bulk-water and root-surface fractions. For bulk-water samples, 5 mL bulk water was simply collected from the flask. For the root-surface fraction, three plants were collected, gently washed twice for 1 min in 20 mL sterile Hoagland solution. The roots were then cut, transferred to a tube containing 10 mL sterile Hoagland solution, subjected to ultrasonication for 60 s and vortexed for additional 60 s. The numbers of nitrophenol-degrading bacteria in the bulk-water and root-surface fractions were determined by plating serially diluted samples in triplicate using 2 nitrophenol–BSM, 3 nitrophenol–BSM, 4 nitrophenol–BSM or dinitrophenol–PY–BSM agar plates. The bacterial populations were determined as colony forming units (CFU) per milliliter for bacteria in bulk water and CFU per plant for bacteria on the root surface. The results are presented as CFU per flask, calculated as:

(CFU per mL) × (total volume of bulk water in the flask) for bacteria in the bulk water, and (CFU per plant) × (total number of plants in the flask) for bacteria on the root surface.

2.7 Statistical analysis

All the experiments were performed in triplicate and the results were indicated as the mean values with standard deviations (±95% confidence interval). Significant differences were determined by Student's t test with $p < 0.05$.

3 Results and discussion

3.1 Nitrophenols removal in the presence and absence of *S. polyrhiza*

Table 2 and Fig. 1 show the ability of strain PFR-1 and DFR-1 to colonize into the root surfaces of sterile *S. polyrhiza* and their degrading ability against four isomers of nitrophenols. Cell number of strain PFR-1 and DFR-1 readily adhered to the roots of *S. polyrhiza* after inoculation. After 1 day, the inoculated strains were released from the roots into the Hoagland solution while the populations on the roots were maintained. The population of inoculated strains on the roots increased until in the end of experiment. The *S. polyrhiza*–PFR-1 completely degraded 3-nitrophenol and 4-nitrophenol and *S. polyrhiza*–DFR-1 associations completely degraded 2-nitrophenol, 4-nitrophenol and 2,4-dinitrophenol. About 25% and 60% of 2-nitrophenol and 2,4-dinitrophenol or 54%, respectively, of 3-nitrophenol, were degraded within 4 days. In contrast, the *S. polyrhiza*–PFR-1/DFR-1 association completely degraded all four NPs within 2 days, and the degradation rates were significantly higher ($p < 0.05$) than those by the *S. polyrhiza*–PFR-1 or *S. polyrhiza*–DFR-1 associations.

In strain PFR-1 alone, 70% of 2-nitrophenol, 93% of 3-nitrophenol, 94% of 4-nitrophenol and 4% of dinitrophenol were remained over 4 days. Strain DFR-1 alone degraded 87% of 2-nitrophenol, 32% of 3-nitrophenol, 100% of 4-nitrophenol and 100% of dinitrophenol. The mixture of strains PFR-1 and DFR-1 degraded 100% of 2-nitrophenol, 30% of 3-nitrophenol, 100% of 4-nitrophenol and 100% of dinitrophenol (Fig. 1). In flasks with the single strains or the mixture of strains but without plant, the cell number after 4 days were $6.2 \pm 0.3 \times 10^6$ CFU per flask (PFR-1), $3.1 \pm 1.3 \times 10^7$ CFU per flask (DFR-1) and $9.8 \pm 0.1 \times 10^7$ CFU per flask (PFR-1/DFR-1), indicating that the population sizes were 10 to 100 times those after the initial inoculation (Table 2). There were no significant differences ($p < 0.05$) between cell numbers in flasks by the presence or absence of *S. polyrhiza*.

The results clearly show that the degradation rates of the four nitrophenol isomers by *S. polyrhiza*–nitrophenols-degrading bacteria associations were significantly higher ($p < 0.05$) than those by the strains in the absence of *S. polyrhiza*, and these abilities were also much wider than those by the strains in the absence of *S. polyrhiza*. Both strains are capable of colonizing sterilized *S. polyrhiza* roots, and *S. polyrhiza* appears to stimulate the degrading activities of both strains on the roots.

Table 2 Distribution and population sizes of nitrophenol-degrading bacteria in bulk-water and root-surface fractions of microcosms in the presence and absence of *S. polyrhiza*[a]

	Bacteria population (CFU per flask)					
Time (day)	*S. polyrhiza*–PFR-1		*S. polyrhiza*–DFR-1		*S. polyrhiza*–PFR-1/DFR-1	
A test system	**Bulk water**	**Root surface**	**Bulk water**	**Root surface**	**Bulk water**	**Root surface**
0	—[b]	$(1.5 \pm 0.4) \times 10^5$	—	$(1.5 \pm 0.6) \times 10^5$	—	$(2.9 \pm 0.6) \times 10^5$
2	$(6.1 \pm 0.1) \times 10^6$	$(7.2 \pm 0.1) \times 10^6$	$(1.8 \pm 0.2) \times 10^7$	$(2.0 \pm 0.5) \times 10^7$	$(3.1 \pm 0.5) \times 10^7$	$(4.1 \pm 0.5) \times 10^7$
4	$(5.6 \pm 0.2) \times 10^6$	$(6.2 \pm 0.4) \times 10^6$	$(2.7 \pm 0.6) \times 10^7$	$(3.1 \pm 1.1) \times 10^7$	$(1.0 \pm 0.1) \times 10^8$	$(1.1 \pm 0.1) \times 10^8$
B test system	**PFR-1 alone**		**DFR-1 alone**		**PFR-1/DFR-1 alone**	
0	$(1.5 \pm 0.2) \times 10^6$	—	$(1.5 \pm 0.6) \times 10^5$	—	$(3.1 \pm 0.6) \times 10^5$	—
2	$(7.2 \pm 0.1) \times 10^6$	—	$(2.0 \pm 0.5) \times 10^7$	—	$(4.3 \pm 0.5) \times 10^7$	—
4	$(6.2 \pm 0.3) \times 10^6$	—	$(3.1 \pm 1.1) \times 10^7$	—	$(9.8 \pm 0.1) \times 10^7$	—

[a] Results are shown as mean ±95% confidence interval. [b] —, not tested.

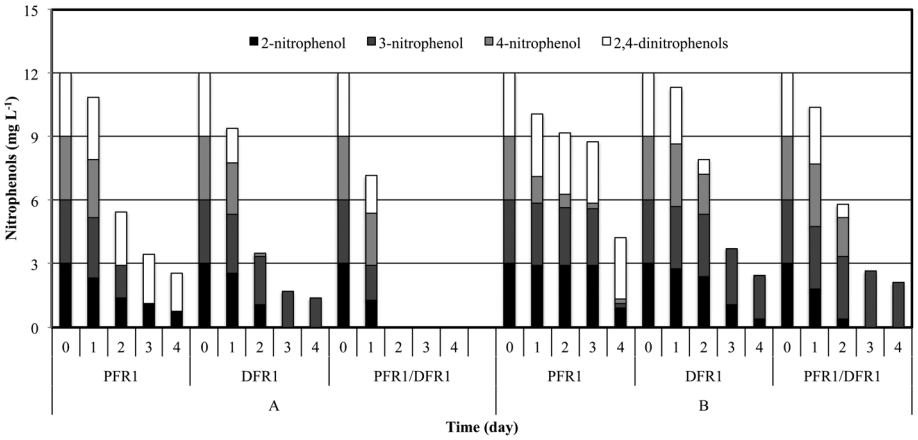

Fig. 1 Nitrophenols removal by bacteria in the presence of *S. polyrhiza*. 'A' represents nitrophenols removal by *S. polyrhiza*–strains associations, and 'B' represents nitrophenols removal by the strains alone. The initial concentration of each nitrophenols was 3 mg L^{-1}.

Particularly noteworthy is the higher and wider nitrophenol-degrading ability of *S. polyrhiza*–PFR-1/DFR-1, compared with those of *S. polyrhiza*–PFR-1 and *S. polyrhiza*–DFR-1. This might result from a synergetic effect between strains PFR-1 and DFR-1. The use of bacterial consortia has been shown to be more effective in removing pollutants as compared with selected single strains in many studies.[14–16] In this study, the most effective method for simultaneously removing the four nitrophenol isomers tested was inoculation of the *S. polyrhiza* roots with a mixture of strains PFR-1 and DFR-1.

3.2 Long term performance of nitrophenols removal by *S. polyrhiza*–strains association

To achieve a complete removal of four nitrophenol isomers, the three strains of nitrophenol-degrading bacteria were inoculated onto *S. polyrhiza* roots. Sterile Hoagland solution supplemented with the mixture of nitrophenols was treated with the *S. polyrhiza* inoculated with all strains of nitrophenol-degrading bacteria (plant–strains association) in SBR experiments. This followed by monitoring the populations of three inoculated strains and the concentrations of the four nitrophenol isomers (Fig 2 and Fig. 3).

At first day of inoculation, all three strains readily adhered to the roots, MFR-1 at $1.7 \pm 0.6 \times 10^5$ CFU per plant, PFR-1 at $1.8 \pm 0.3 \times 10^5$ CFU per plant and DFR-1 at $1.9 \pm 0.1 \times 10^5$ CFU per plant. After 1 day, inoculated strains were released from the roots into the Hoagland solution while the populations on the roots were maintained. In the last days of incubation (*i.e.*, after five 2 day cycles), the populations in the flasks were $1.9 \pm 0.7 \times 10^7$ CFU per flask for MFR-1, $8.1 \pm 0.4 \times 10^5$ CFU per flask for PFR-1 and $3.5 \pm 1.6 \times 10^7$ CFU per flask for DFR-1. The notable increases by factors from about 5–100 of numbers of nitrophenols-degrading bacteria from the initial inoculation was achieved, and these higher populations persisted throughout five cycles. The increases in 3-nitrophenol-degrading MFR-1 and 2,4-dinitrophenol-degrading DFR-1 were particularly highlighted.

In the control experiment using sterile *S. polyrhiza* alone, slightly nitrophenol removal was obtained; 17% of 2-nitrophenol, 24% of 3-nitrophenol, 3% of 4-nitrophenol and 9% of 2,4-dinitrophenol were removed during the 2 day reaction cycle. In contrast, the plant–strains association consistently removed all nitrophenol isomers from the three-nitrophenols-augmented Hoagland solutions within the 2 day reaction cycle

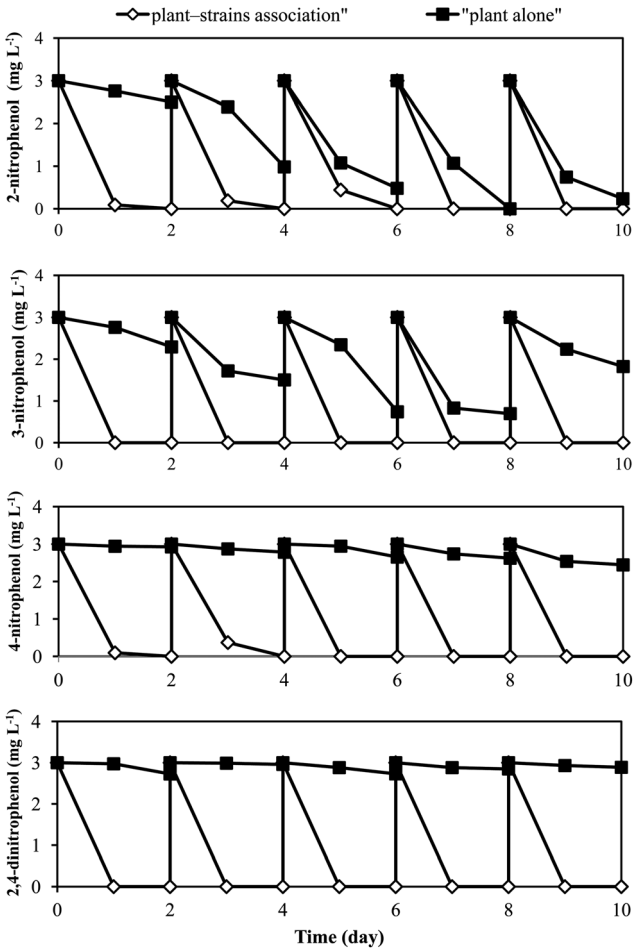

Fig. 2 Long-term performance test of nitrophenols removal by the plant–strains association.

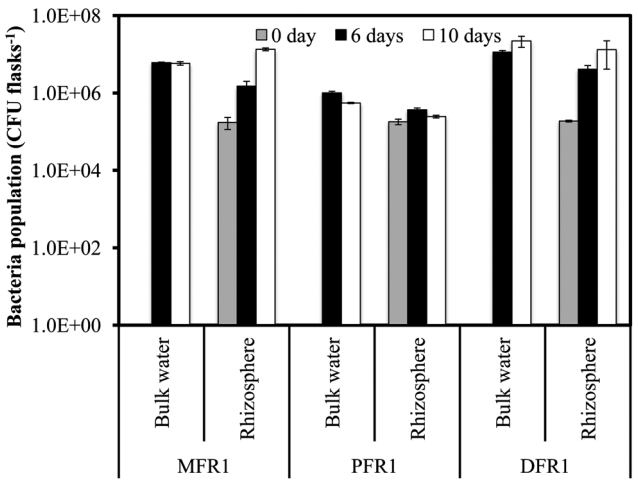

Fig. 3 Distribution and population sizes of nitrophenol-degrading bacteria in bulk-water and root-surface fractions of microcosms during long-term performance experiments.

in all five cycles. This repeated complete removal of all nitrophenols presumably indicates their biodegradation by the three inoculated strains on the *S. polyrhiza* roots.

The presence of nitrophenols in surface water, sediment and in effluents from sewage and wastewater are oftenly found in notable amount.[1] An simultaneously removal method for nitrophenols is attractive tool since nitrophenol never presence alone in the water. In this study, simultaneous and sustainable removal of four nitrophenol isomers were achieved by the inoculation of the three strains on the *S. polyrhiza* roots. The strains appear to be capable of sustainably colonizing and remaining attached to sterilized *S. polyrhiza* roots and degrading nitrophenols over the long term. These three strains are therefore suitable bacterial inocula for *S. polyrhiza* roots for NP removal.

3.3 Sustainable removal of four nitrophenol isomers from secondary effluent contaminated with nitrophenols by the *S. polyrhiza*–consortium strains association

The results presented in above sections show the ability of *S. polyrhiza*–NP-degrading rhizobacteria associations to treat Hoagland solution supplemented with NPs under sterile conditions and in artificial water. In order to assess the potential of this system for treating wastewater contaminated with nitrophenols, SBR experiments were conducted using secondary effluent water contaminated with the mixture of nitrophenols (Fig. 4). In the absence of consortium

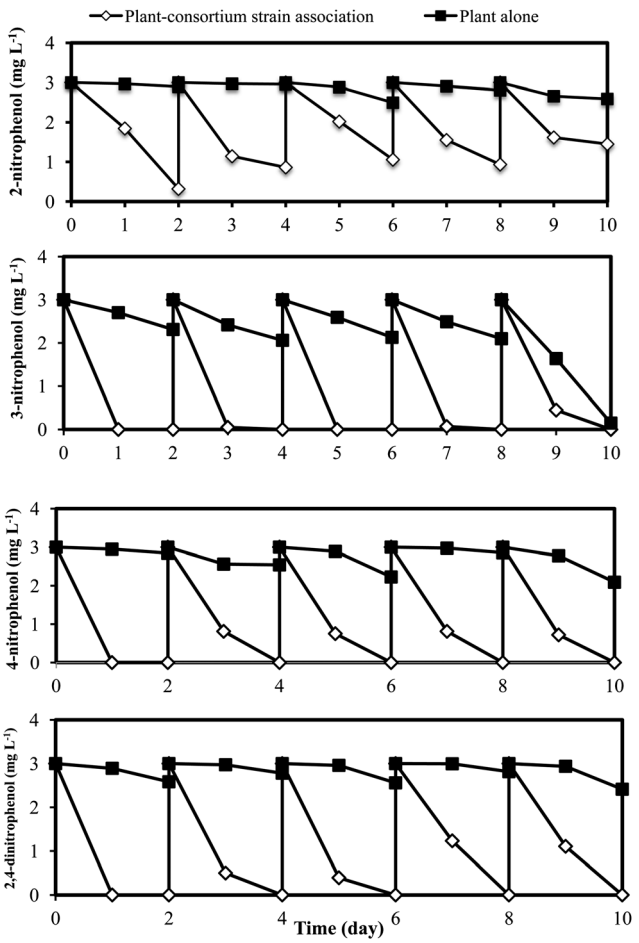

Fig. 4 Sustainable nitrophenol removal by plant–strains association in environmental contaminated water with nitrophenols.

Table 3 Growth promotion and dry weight of *S. polyrhiza* during SBR experiments using *S. polyrhiza*–strains association (test A), and *S. polyrhiza* alone (test B)

Test system	Number of fronds[a]						Biomass production rate (g dry weight per day)
	0 day	2 days	4 days	6 days	8 days	10 days	
A	10	13 ± 1.1	18 ± 0.7	23 ± 1.3	28 ± 2.3	33 ± 1.3	0.049 ± 0.001
B	10	11 ± 0.7	12 ± 1.3	13 ± 1.1	15 ± 1.7	17 ± 2.3	0.021 ± 0.003

[a] The results represent mean ±95% confidence interval.

rhizobacteria consortium, *S. polyrhiza* removed only small amounts of 2-nitrophenol (2–17%), 4-nitrophenol (5–30%) and 2,4-dinitrophenol (7–20%) from the water; the range for 3-nitrophenol removal was somewhat higher (32–95%) because of 95% removal during the last cycle. The nitrophenol removal by these uninoculated *S. polyrhiza* might have resulted from degradation by indigenous bacteria in the secondary effluent sample as well as degradation by *S. polyrhiza*. In contrast, the plant–consortium strains association repeatedly achieved complete removal of three nitrophenol isomers (3-nitrophenols, 4-nitrophenols and 2,4-dinitrophenols) and 52–81% removal for 2-NP from the contaminated water throughout all five cycles. A beneficial symbiotic interaction between nitrophenol-degrading bacteria and *S. polyrhiza* were demonstrated by a notable increase of fronds number during SBR experiment; which about 3.3 fold and 1.7 fold of test A and B increased by the end of experimental period, respectively (Table 3). Biomass production of *S. polyrhiza* in the presence of introduced bacteria was also found about 1.9 times higher than in the absence of introduced bacteria.

A variety of organic compounds, other pollutants and indigenous microorganisms could presence in the wastewaters. Under these conditions, inoculated bacteria often cannot survive and exert their full degradation ability on the target pollutants. However, in this study the introduction of three nitrophenol-degrading rhizobacteria onto *S. polyrhiza* roots resulted in the simultaneous and sustainable removal of the mixture nitrophenols tested from the contaminated secondary effluent water without any harmful to the growth of *S. polyrhiza.*

4 Conclusions

In this study, we achieved a successful bioaugmentation treatment of water contaminated with four nitrophenol isomers by inoculating the roots of giant duckweed with three nitrophenol-degrading bacterial strains originated from the Fuefuki river, namely 3-NP-degrading *Pseudomonas* sp. (MFR-1), 4-NP-degrading *Pseudomonas* sp. (PFR-1) and 2,4-DNP-degrading *Rhodococcus* sp. (DFR-1). The inoculated strains stably colonized the roots during the incubation period, resulting in simultaneous and sustainable removal of the four nitrophenols from contaminated water. This association also effectively treated the nitrophenol-contaminated secondary effluent water. Hence, rhizoaugmentation using introduced nitrophenol-degrading bacteria could be an effective and stable treatment technology for organic pollutants.

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