ISSN 1001-0742
CN 11-2629/X

2012
Volume 24
Number 5

JOURNAL OF

ENVIRONMENTAL SCIENCES

ELSEVIER
Science Press

Sponsored by
Research Center for Eco-Environmental Sciences
Chinese Academy of Sciences

ISSN 1001–0742 **Journal of Environmental Sciences** Vol. 24 No. 5 2012

CONTENTS

Serial parameter: CN 11-2629/X*1989*m*188*en*P*26*2012-5

Available online at www.sciencedirect.com
ScienceDirect
Journal of Environmental Sciences 2012, 24(5) 800–807
JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Accelerated biodegradation of nitrophenols in the rhizosphere of *Spirodela polyrrhiza*

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Received 23 June 2011; revised 15 August 2011; accepted 30 September 2011

Abstract

We investigated the biodegradation of 2-nitrophenol (2-NP), 4-nitrophenol (4-NP), and 2,4-dinitrophenol (2,4-DNP) in the rhizosphere of *Spirodela polyrrhiza* plants by conducting degradation experiments with three river water samples supplemented with each nitrophenol (NP). We then isolated NP-degrading bacteria both from the *S. polyrrhiza* roots and from the river water. In the river water samples, removal of the three NP was accelerated in the presence of *S. polyrrhiza* plants. The three NPs persisted in an autoclaved solution with sterile plants suggests that NP removal was accelerated largely by bacterial NP biodegradation rather than by adsorption and uptake by the plants. We isolated 8 strains of NP-degrading bacteria: 6 strains from the *S. polyrrhiza* roots and 2 strains from river water without the plants. The 2-NP- and 2,4-DNP-degrading bacteria were isolated only from the *S. polyrrhiza* roots. The 4-NP-degrading bacteria different from those isolated from the river water samples were also found on *S. polyrrhiza* roots. The 2-NP- and 4-NP-degrading strains isolated from the roots utilized the corresponding NP (0.5 mmol/L) as the sole carbon and energy source. The 2,4-DNP-degrading strains isolated from the roots showed substantial 2,4-DNP-degrading activity, but the presence of other carbon and energy sources was required for their growth. The isolated NP-degrading bacteria from the roots must have contributed to the accelerated degradation of the three NPs in the rhizosphere of *S. polyrrhiza*. Our results suggested that rhizoremediation with *S. polyrrhiza* may be effective for NP-contaminated surface water.

Key words: *Spirodela polyrrhiza*; rhizosphere; nitrophenol; biodegradation

DOI: 10.1016/S1001-0742(11)60839-5

Introduction

Nitrophenols (NPs) are industrially important chemicals used mainly for the production of pharmaceuticals, dyes, pesticides, plasticizers, and explosives (Hartter, 1985; World Health Organization, 2000). NPs are formed by the partial degradation of parathion by sunlight and microorganisms in soil and water (Munnecke and Hsieh, 1976). Because of their widespread use, large quantities of NPs are commonly found as contaminants in aquatic environments, including river water, wastewater, and industrial effluents (World Health Organization, 2000). NPs are highly toxic to aquatic organisms and humans (World Health Organization, 2000). The U.S. Environmental Protection Agency (2011) has listed 2-nitrophenol (2-NP), 4-nitrophenol (4-NP), and 2,4-dinitrophenol (2,4-DNP) as priority pollutants. Thus, the study of NPs biodegradation is necessary for establishing technologies to treat water polluted by NPs and to remove NPs from contaminated environments.

Despite the recalcitrance of NPs to biodegradation, some bacteria that degrade NPs have been isolated from various environments (Kulkarni and Chaudhari, 2007; Marvin-Sikkema and de Bont, 1994; Spain, 1995). In addition, NP-contaminated soils (Ghosh et al., 2010; Pandey et al., 2006) and NP-contaminated wastewater (Heitkamp et al., 1990; Hu et al., 2008; Ray et al., 1999) have been treated by bioremediation or in bioreactors with NP-degrading bacteria. However, the potential of bioremediation for accelerated NP removal from contaminated surface water in natural aquatic environments has not been studied. Because pollutants in natural surface water are more diffusive and are present at lower concentrations than in soils and wastewater treatment plants, the immobilization and stimulation of bacteria during long-term bioremediation is important for complete cleanup of contaminated sites.

Rhizoremediation, the degradation and removal of pollutants by microbial activity in the rhizosphere of plants, is a cost-effective and environmentally friendly remediation technology. Previous studies have revealed that

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rhizosphere of terrestrial plants can accelerate biodegradation of organic pollutants, including pesticides, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls, in the soil (Chaudhry et al., 2005; Shaw and Burn, 2003). The floating aquatic plant *Spirodela polyrrhiza* (giant duckweed) and the emerged aquatic plant *Phragmites australis* (common reed) have been shown to accelerate the biodegradation of synthetic surfactants (Mori et al., 2005), single-ring aromatic compounds (Toyama et al., 2006; Yamaga et al., 2010), pyrene (Jouanneau et al., 2005; Toyama et al., 2011), and bisphenol A (Toyama et al., 2009b) in rhizosphere water and sediment. The accelerated mineralization of 4-NP in rice rhizosphere sediment has been reported (Rajasekhar and Sethunathan, 1994), but the mechanism of the accelerated mineralization and 4-NP-degrading bacteria in the rhizosphere are unclear. Furthermore, the potential of floating aquatic plants such as *S. polyrrhiza* to remove NPs from contaminated surface water has not been studied.

Our objectives in this study were to investigate the accelerated degradation of 2-NP, 4-NP, and 2,4-DNP in *S. polyrrhiza* rhizosphere water. We monitored NP degradation in river water supplemented with each NP in the presence and absence of *S. polyrrhiza* plants. We then isolated and characterized NP-degrading bacteria from both the *S. polyrrhiza* roots and the river water. The mechanism of the accelerated degradation of NPs in the rhizosphere of *S. polyrrhiza* was also discussed.

1 Experimental

1.1 Chemicals

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

1.2 Plant and water samples

Sterile (bacteria-free) *S. polyrrhiza* plants were prepared as described previously (Toyama et al., 2006) and aseptically maintained in Erlenmeyer flasks containing sterile modified Hoagland solution (36.1 mg/L KNO_3, 298 mg/L K_2SO_4, 3.87 mg/L NaH_2PO_4, 103 mg/L $MgSO_4{\cdot}7H_2O$, 147 mg/L $CaCl_2{\cdot}2H_2O$, 3.33 mg/L $FeSO_4{\cdot}7H_2O$, 0.95 mg/L H_3BO_3, 0.39 mg/L $MnCl_2{\cdot}4H_2O$, 0.03 mg/L $CuSO_4{\cdot}5H_2O$, 0.08 mg/L $ZuSO_4{\cdot}7H_2O$, and 0.254 mg/L $H_2MoO_4{\cdot}H_2O$; pH 7.0) until used for experiments. *Spirodela polyrrhiza* plants were grown in an incubation chamber at (28 ± 1)°C under fluorescent lamps at 8000 lux (16-hr light and 8-hr dark).

A river water sample was collected from three rivers in Yamanashi Prefecture, Japan: the Nigori River, the Fuefuki River, and the Kamanashi River (Table 1). The pH and dissolved oxygen (DO) content of the samples were measured *in situ* with a Horiba D-55 water analyzer (Horiba, Kyoto, Japan). The concentrations of NH_4^+-N, NO_2^--N, NO_3^--N, and PO_4^{3-}-P were determined with Packtest water quality analysis kits (Kyoritsu Chemical Check Lab Corp., Kyoto, Japan). The number of colony forming units in the samples was measured with 10-fold-diluted Tryptic Soy Broth agar plates. The water samples were stored at 4°C until required for experiments.

1.3 Culture media

We used a basal salts medium (BSM: 1.0 g/L $(NH_4)SO_4$, 1 g/L K_2HPO_4, 0.2 g/L NaH_2PO_4, 0.2 g/L $MgSO_4{\cdot}7H_2O$, 0.05 mg/L NaCl, 0.05 g/L $CaCl_2$, 8.3 mg/L $FeCl_3{\cdot}6H_2O$, 1.4 mg/L $MnCl_2{\cdot}4H_2O$, 1.17 mg/L $Na_2MoO_4{\cdot}2H_2O$, and 1 mg/L $ZnCl_2$; pH 7.2) containing 2-NP, 4-NP, or 2,4-DNP as the sole carbon source (2NP-BSM, 4NP-BSM, and DNP-BSM, respectively) for culturing NP-degrading bacteria. DNP-BSM containing 0.5 g/L of yeast extract and 1.0 g/L of bacto-peptone was also used for culturing 2,4-DNP-degrading bacteria. Agar solid medium was prepared with 1.5 % (*W*/*V*) agar.

1.4 NP degradation experiments in river water microcosms

To determine whether degradation of the three NPs was accelerated in *S. polyrrhiza* rhizosphere water, we conducted degradation experiments in river water microcosms as follows. Microcosm A: Twenty fronds of sterile *S. polyrrhiza* were planted in each of three 300-mL flasks containing 200-mL river water samples supplemented with one of the three NPs at 10 mg/L. Microcosm B: This control experiment consisted of three flasks containing 200-mL river water samples supplemented with one of the three NPs at 10 mg/L. A second, sterile control experiment, consisting of three flasks containing 200 mL of autoclaved (121°C, 20 min) Hoagland solution supplemented with one of the three NPs (10 mg/L) and 20 fronds of sterile *S. polyrrhiza*, was conducted to evaluate adsorption and uptake by the plants. All the microcosms were statically incubated at (28 ± 1)°C under fluorescent lamps at 8000 lux (16-hr light and 8-hr dark) for 10 days. Subsequently, the *S. polyrrhiza* plants and 10% of the water sample in each flask of microcosm A were transferred to a new river water microcosm with the same composition (i.e., 20 mL of transferred water, 180 mL of new river water, 20 fronds of transferred *S. polyrrhiza*, 10 mg/L of NP), and a second 10-day incubation was performed. In addition, 10% of the

Table 1 Characteristics of the three river water samples

River	pH	Dissolved oxygen (mg O_2/L)	NH_4^+-N (mg N/L)	NO_2^--N (mg N/L)	NO_3^--N (mg N/L)	PO_4^{3-}-P (mg P/L)	Colony forming units (CFU/mL)
Nigori	7.68	10.3	0.07	0.000	0.92	0.082	1.4×10^4
Fuefuki	7.17	6.20	0.00	0.000	1.16	0.100	4.6×10^4
Kamanashi	7.52	10.2	0.00	0.000	1.02	0.060	1.2×10^4

water sample in each flask of microcosm B was transferred to a new river water microcosm with the same composition (i.e., 20 mL of transferred water, 180 mL of new river water, 10 mg/L of NP), and a second 10-day incubation was performed. A separate set of river water microcosm experiments (microcosms A and B) was performed for each river water sample and each NP. The concentrations of NPs were periodically monitored during each incubation period.

1.5 Enrichment, isolation, and identification of NP-degrading bacteria

Enrichment cultures of NP-degrading bacteria were conducted separately from the river water microcosm experiments. Three fronds of *S. polyrrhiza* from each flask of microcosm A were gently washed with sterile water, and then the *S. polyrrhiza* roots were transferred to 100 mL of NP-BSM (50 mg/L). In addition, a 10-mL sample of water from each flask of microcosm B was added to 100 mL of NP-BSM (50 mg/L). The cultures were incubated at 28°C on a rotary shaker at 150 r/min for 14 days, and then 10 mL of each culture was transferred to 100 mL of fresh NP-BSM (100 mg/L) and incubated for 14 days. After a third transfer, the enrichment cultures were serially diluted and spread on NP-BSM (100 mg/L) agar plates, and the plates were incubated at 28°C.

Morphologically different colonies were screened for their ability to degrade NP. The isolated bacterial strains, designated ONR-1, PNR-1, PFR-1, PKR-1, DNR-2, DFR-1, PNW-1, and PFW-1, were characterized and identified by physiological analysis. A comparative 16S rRNA gene sequence analysis was performed as follows. Partial 16S rRNA genes were amplified by PCR using primers 10F (5′-GTTTGATCCTGGCTCA-3′) and 800R (5′-TACCAGGGTATCTAATCC-3′). The amplified 16S rRNA gene sequence was determined by Hitachi Solutions (Yokohama, Japan). The 16S rRNA sequences were compared to the reference sequence by means of a BLAST similarity search (Altschul et al., 1990), and closely related sequences were obtained from GenBank.

1.6 NP degradation tests with pure culture of isolates

Each isolate, except 2,4-DNP-degrading strains DNR-2 and DFR-1, was grown overnight in NP-BSM (0.5 mmol/L). Cells were harvested by centrifugation (10,000 $\times g$ at 4°C for 10 min), washed twice with 50 mmol/L potassium phosphate buffer (pH 7.2), and inoculated into 100 mL of NP-MSM (0.5 mmol/L) to a cell density of 0.02–0.03 (i.e., OD_{600} of 0.02–0.03). Each of the strains DNR-2 and DFR-1 was grown overnight in DNP-BSM (0.5 mmol/L) supplemented with yeast extract (0.5 g/L) and bacto-peptone (1.0 g/L). Each of the strains DNR-2 and DFR-1 was then inoculated at OD_{600} of 0.02–0.03 into 100 mL of DNP-BSM (0.1–0.5 mmol/L) either supplemented with 0.5 mmol/L glucose or unsupplemented. The cells were cultured at 28°C and 150 r/min in the dark. Cell densities and NP concentrations were monitored over the 48-hr experimental period. The experiment was conducted in triplicate.

1.7 Analytical procedures

Bacterial cell density was measured as OD_{600}. Concentrations of NPs were determined by high-performance liquid chromatography (HPLC) on 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). The river water and culture sampled at each sampling point were centrifuged (10,000 $\times g$ at 4°C for 10 min), and then the supernatant was analyzed by HPLC. For HPLC analysis, an acetonitrile-water-acetic acid mixture (500:498:2, *V*/*V*/*V*) was used as the mobile phase at a flow rate of 1 mL/min, and detection was at a wavelength of 280 nm.

1.8 Nucleotide sequence accession numbers

The 16S rRNA gene sequence data of isolated strains ONR-1, PNR-1, PFR-1, PKR-1, DNR-2, DFR-1, PNW-1, and PFW-1 have been submitted to the DDBJ/EMBL/GenBank databases under 8 accession numbers AB671547, AB671550, AB671548, AB671549, AB671546, AB671545, AB671552, and AB671551, respectively.

2 Results

2.1 Degradation of three NPs in river water microcosms in the presence and absence of *S. polyrrhiza*

We examined the degradation of 2-NP, 4-NP, and 2,4-DNP (10 mg/L each) in river water microcosms in the presence and absence of sterile *S. polyrrhiza* plants. The results are shown in Fig. 1. The 2-NP persisted in the microcosms without the plants over two 10-day incubation periods. In contrast, in the microcosms with the plants, 2-NP concentrations declined during both incubation periods; 8%–36% and 23%–100% of the 2-NP was removed from the microcosms during the first and second incubation periods, respectively (Fig. 1a).

The 4-NP concentrations declined only slightly or not at all in the microcosms without the plants; 1%–56% and 0%–86% of the 4-NP was removed from the microcosms during the first and second incubation periods, respectively. In the microcosms with the plants, 4-NP concentrations declined during both incubation periods; 40%–89% and 25%–87% of the 4-NP was removed from the microcosms during the first and second incubation periods, respectively (Fig. 1b).

The 2,4-DNP concentrations declined only slightly or not at all in the microcosms without the plants; 0%–40% and 0%–56% of the 2,4-DNP was removed from the microcosms during the first and second incubation periods, respectively. In the microcosms with the plants, 2,4-DNP concentrations declined during both incubation periods; 52%–74% and 43%–98% of the 2,4-DNP was removed from the microcosms during the first and second incubation periods, respectively (Fig. 1c).

To evaluate the adsorption and uptake of the three NPs by the plants, we conducted sterile control experiments using autoclaved Hoagland solution supplemented with

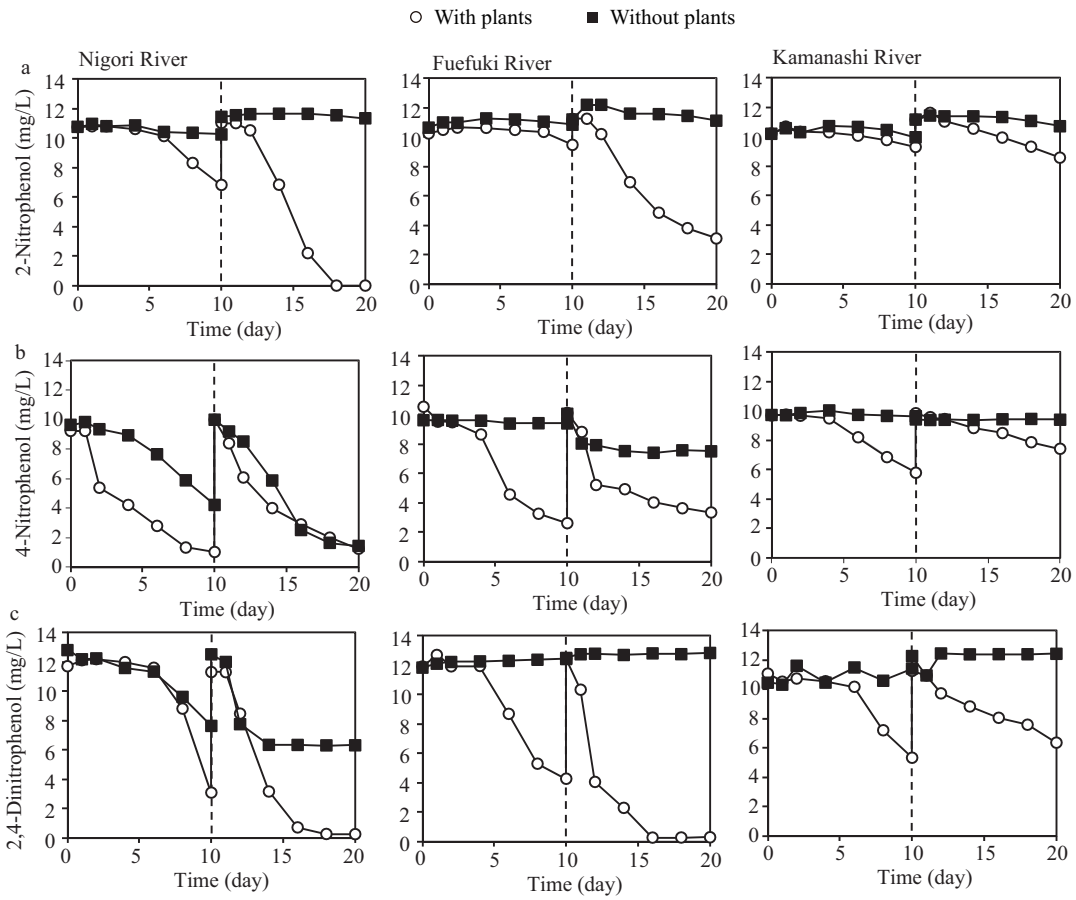

Fig. 1 Removal of 2-NP (a), 4-NP (b), and 2,4-DNP (c) from river water microcosms with *Spirodela polyrrhiza* plants and from river water microcosms without the plants. The dashed lines represent the starting points of the second incubation periods.

each NP (10 mg/L) and sterile plants. In these experiments, all three NPs declined only slightly; 18% of the 2-NP, 10% of the 4-NP, and 2% of the 2,4-DNP was removed from the microcosms over the 10-day incubation period (Fig. 2).

2.2 Isolation and identification of NP-degrading bacteria

In total, eight bacterial strains were isolated from *S. polyrrhiza* roots in microcosm A and from the river water of microcosm B after completion of the incubation experiments. We identified the isolated strains by 16S rRNA gene sequence analysis (Table 2).

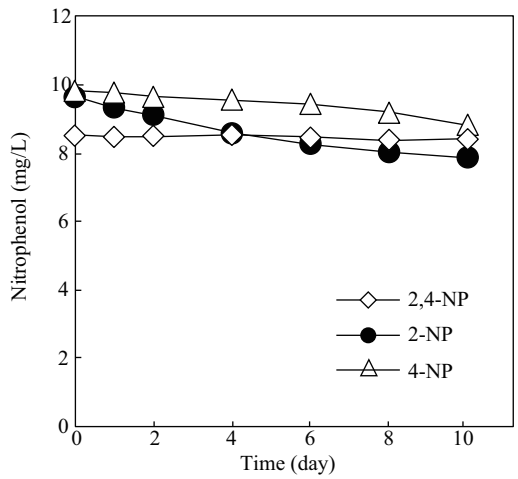

Fig. 2 Removal of the three NPs in sterile control experiments.

The 2-NP-degrading *Pseudomonas* strain ONR-1 was isolated from *S. polyrrhiza* roots in the Nigori River microcosm. The 4-NP-degrading *Pseudomonas* strains PNR-1, PFR-1, and PNW-1; 4-NP-degrading *Rhodococcus* strain PKR-1; and 4-NP-degrading *Nocardioides* strain PFW-1 were isolated both from *S. polyrrhiza* roots and from the microcosms without the plants. The 2,4-DNP-degrading *Rhodococcus* strains DNR-2 and DFR-1 were isolated from the *S. polyrrhiza* roots in the Nigori and Fuefuki River microcosms with the plants, respectively. The four isolated strains of NP-degrading bacteria were *Pseudomonas* species, three were *Rhodococcus* species, and one was a *Nocardioides* species.

2.3 Degradation of NPs by isolated strains

Figures 3–5 show the degradation of 2-NP, 4-NP, and 2,4-DNP, respectively, by isolated strains, along with cell growth. Strain ONR-1 completely degraded 0.5 mmol/L 2-NP within 9 hr, with concomitant cell growth (Fig. 3).

After a 12-hr lag period, 0.5 mmol/L 4-NP was completely degraded by strains PNR-1 and PNW-1 within 18 hr, with concomitant cell growth (Fig. 4). After a 20-hr lag period, 0.5 mmol/L 4-NP was completely degraded by strain PFR-1 within 30 hr, with concomitant cell growth (Fig. 4). And 0.5 mmol/L 4-NP was degraded by strains PFW-1 and PKR-1, within 30 and 40 hr, respectively, with concomitant cell growth (Fig. 4d, e).

Table 2 NP-degrading bacterial strains isolated from the roots of *S. polyrrhiza* plants and from bulk river water; bacterial identification using partial 16S rRNA gene sequences of the strains

Isolate	Source	Identification*			NP degraded
		Closest species (accession number)	Similarity (%)	Compared length (bp)	
ONR-1	Roots (NR)	*Pseudomonas umsongensis* strain Ps 3-10^T (AF468450)	98	686	2-NP
PNR-1	Roots (NR)	*Pseudomonas vancouverensis* strain DhA-51^T (AJ011507)	100	687	4-NP
PFR-1	Roots (FR)	*Pseudomonas umsongensis* strain Ps 3-10^T (AF468450)	99	687	4-NP
PKR-1	Roots (KR)	*Rhodococcus wratislaviensis* strain NCIMB 13082^T (Z37138)	100	644	4-NP
PNW-1	Bulk water (NR)	*Pseudomonas vancouverensis* strain DhA-51^T (AJ011507)	100	656	4-NP
PFW-1	Bulk water (FR)	*Nocardioides pyridinolyticus* strain OS4 (U61298)	99	646	4-NP
DNR-2	Roots (NR)	*Rhodococcus wratislaviensis* strain NCIMB 13082^T (Z37138)	99	672	2,4-DNP
DFR-1	Roots (FR)	*Rhodococcus wratislaviensis* strain NCIMB 13082^T (Z37138)	99	657	2,4-DNP

NR: Nigori River, FR: Fuefuki River, KR: Kamanashi River.
* Closest species and 16S rRNA gene sequence similarity between isolated strain and closest species were obtained by BLAST similarity search and GenBank.

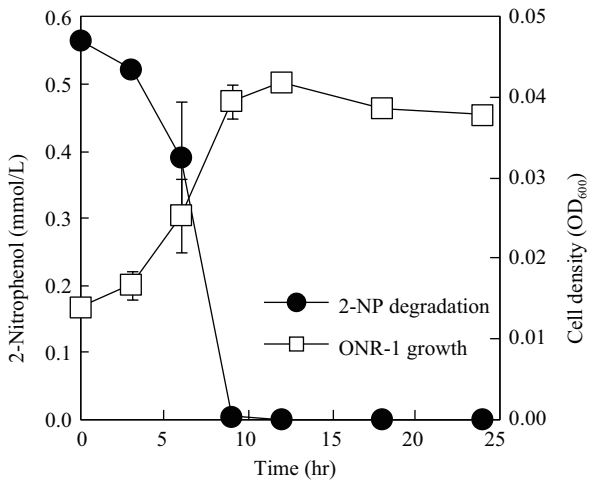

Fig. 3 Degradation of 2-NP and growth of strains ONR-1. Data are means from triplicate experiments, and the error bars indicate 95% confidence intervals.

Strains DNR-2 and DFR-1 completely degraded 0.1–0.5 mmol/L 2,4-DNP within 12 hr in both the presence and the absence of 0.5 mmol/L glucose as another carbon source (Fig. 5). The time required for complete degradation of 2,4-DNP by each strain in the presence of glucose was slightly shorter than that in the absence of glucose. In contrast to 2,4-DNP degradation, significant cell growth of strains DNR-2 and DFR-1 occurred in the presence of glucose but not in its absence (Fig. 5).

3 Discussion

Removal of 2-NP, 4-NP, and 2,4-DNP from river water microcosms in the presence of *S. polyrrhiza* plants was accelerated relative to removal in the absence of plants. Furthermore, all three NPs persisted in the autoclaved Hoagland solution with sterile plants. The results indicate that the accelerated removal of the three NPs resulted mainly from biodegradation by bacteria that were stimulated by the presence of *S. polyrrhiza* rather than from adsorption and uptake by the plants. The accelerated removal of the three NPs was observed for all three natural river water samples, which were collected from different freshwater sites. In addition, the accelerated removal of NPs was observed during both 10-day incubation periods (i.e., 20-day incubation). These results suggest that rhizoremediation with *S. polyrrhiza* plants can be effective for NP-contaminated surface waters.

To better understand the accelerated NP degradation in the rhizosphere of *S. polyrrhiza*, we isolated NP-degrading bacteria both from *S. polyrrhiza* roots and from river water microcosms without *S. polyrrhiza*. In addition, we compared the phylogenetic characteristics and NP-degrading activities of NP-degrading bacteria derived from the roots to the characteristics of bacteria derived from the river water microcosms without the plants. Various NP-degrading bacteria, including 2-NP-, 4-NP-, and 2,4-DNP-degrading strains, have been isolated from soil, activated sludge, sediment, and wastewater treatment plants (Kulkarni and Chaudhari, 2007; Marvin-Sikkema and de Bont, 1994; Spain, 1995). *Pseudomonas*, *Ralstonia*, *Sphingomonas*, *Burkholderia*, *Moraxella*, *Actinobacter*, *Actinomucete*, *Arthobacter*, *Bacillus*, *Rhodococcus,* and *Nocardioides* species were among the isolated NP-degrading bacteria. In this study, we also isolated a variety of NP-degrading bacteria, eight strains in total, including six strains isolated from *S. polyrrhiza* roots and two strains isolated from river water without the plants. Our isolates from *S. polyrrhiza* roots included a 2-NP-degrading *Pseudomonas* strain; 4-NP-degrading *Pseudomonas* and *Rhodococcus* strains; and 2,4-DNP-degrading *Rhodococcus* strains. We also isolated 4-NP-degrading strains belonging to *Pseudomonas* and *Nocardioides* from the water without the plants. Moreover, our isolated NP-degrading strains, except for 2,4-DNP-degrading strains DNR-2 and DFR-1, utilized the corresponding NP (0.5 mmol/L) as the sole carbon and energy source. Cells of strains DNR-2 and DFR-1 grown

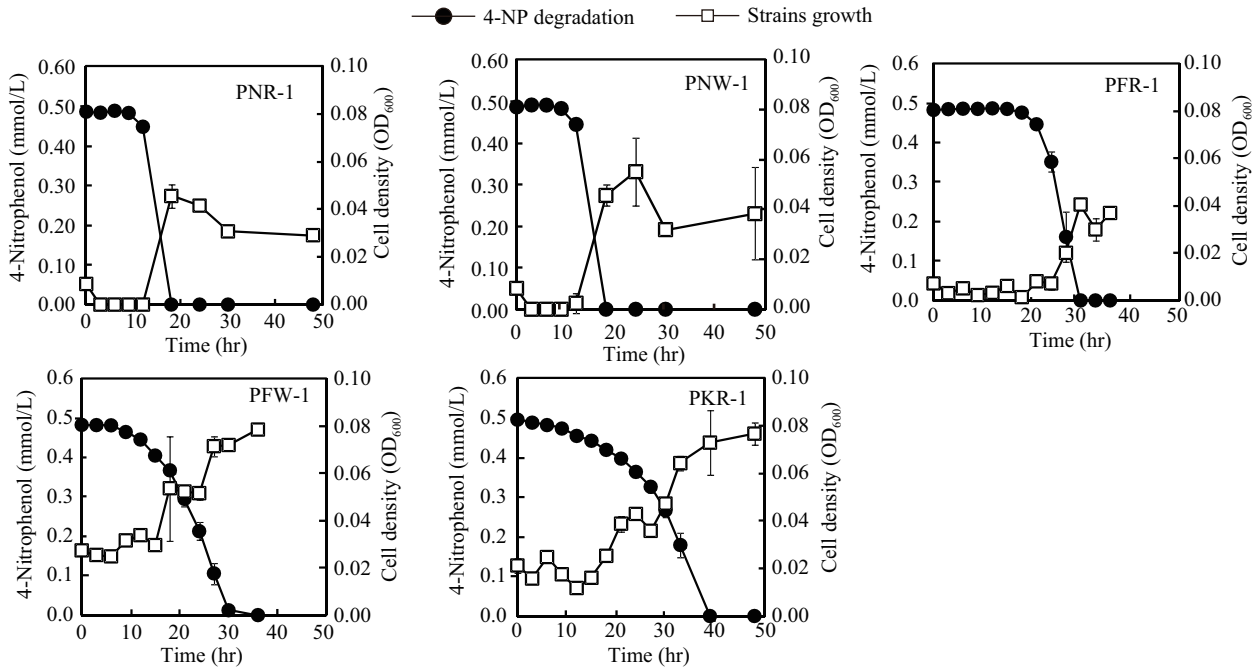

Fig. 4 Degradation of 4-NP and growth of different strains. Data are the means from triplicate experiments, and the error bars indicate 95% confidence intervals.

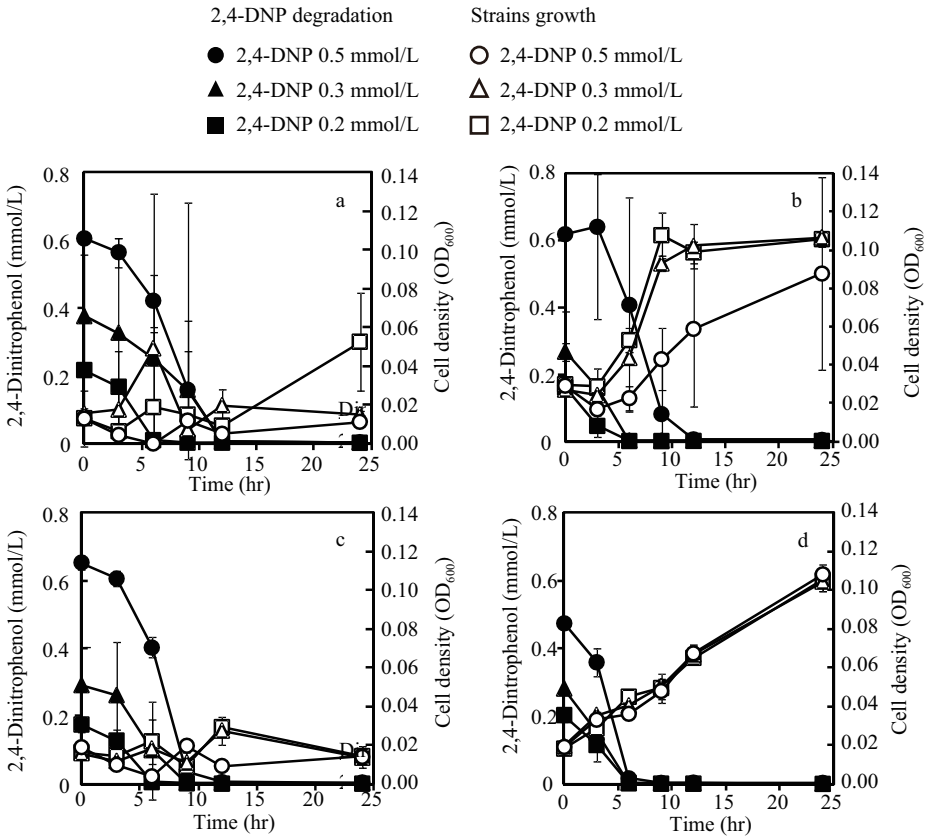

Fig. 5 Degradation of 2,4-DNP and growth of strain DNR-2 without glucose (a) and with glucose (b); degradation of 2,4-DNP and growth of strain DFR-1 without glucose (c) and with glucose (d). Data are the means from triplicate experiments, and the errors bars indicate 95% confidence intervals.

with peptone and yeast extract showed substantial 2,4-DNP-degrading activity, but the presence of other carbon and energy sources was required for their growth. To our knowledge, the bacterial strains discussed here are the first strains isolated from the aquatic rhizosphere capable of utilizing a NP as a sole and carbon energy source and of degrading 2,4-DNP. In particular, only two 2-NP-degrading bacteria have previously been reported (i.e., a *Pseudomonas* strain (Zeyer and Kearney, 1984) and an *Acinetobacter* strain (Soojhawon et al., 2005)). Thus, strain ONR-1 will be valuable for microbiological study as a new 2-NP-degrading bacterial strain.

A 2-NP-degrading bacterium and 2,4-DNP-degrading bacteria were isolated only from *S. polyrrhiza* roots in the river water microcosms with the plants, although the *S. polyrrhiza* roots were sterilized (bacteria-free) before the start of the microcosm experiments. We did not observe substantial degradation of 2-NP, and only slight degradation of 2,4-DNP was observed in the river water without the plants. Our efforts to enrich and isolate 2-NP- and 2,4-DNP-degrading bacteria from river water without the plants were unsuccessful. These results suggest that *S. polyrrhiza* plants accumulated 2-NP- and 2,4-DNP-degrading bacteria on their roots from bulk river water during the degradation experiment, and this accumulation led to the accelerated degradation of 2-NP and 2,4-DNP and isolation of 2-NP- and 2,4-DNP-degrading bacteria. Bacteria capable of degrading 2-NP and 2,4-DNP were present in the river water samples used in this study, but in the absence of *S. polyrrhiza*, they could not fully express their degradation activities, and their cell numbers did not increase. Therefore, one of the main reasons for the accelerated degradation of 2-NP and 2,4-DNP in the rhizosphere of *S. polyrrhiza* may have been that 2-NP- and 4-NP-degrading bacteria accumulated in the rhizosphere.

We also isolated 4-NP-degrading *Pseudomonas* strains PNR-1 and PFR-1 and *Rhodococcus* strain PKR-1 from *S. polyrrhiza* roots, whereas 4-NP-degrading *Pseudomonas* strain PNW-1 and *Nocardioides* strain PFW-1 were isolated from the river water samples without the plants. Although the origins of the water samples used for the river water microcosms with and without the plants were the same, 4-NP-degrading strains derived from *S. polyrrhiza* roots in microcosms with the plants differed from those derived from river water microcosms without the plants. A *Rhodococcus* strain was found only on the roots, whereas a *Nocardioides* strain was found in bulk river water. These results suggest that *S. polyrrhiza* plants might have selectively accumulated bacteria on their roots from bulk river water and that different types of bacteria from the bulk river water must have contributed to the accelerated removal of 4-NP with support from the roots in the rhizosphere of *S. polyrrhiza*. 4-NP-degrading bacteria isolated from both the roots and the bulk river water samples had almost the same potential to effectively degrade 4-NP, as shown by the degradation tests (Fig. 4). Therefore, although 4-NP-degrading bacteria were distributed in the river water, *S. polyrrhiza* could selectively recruit and stimulate certain 4-NP-degrading bacteria in its rhizosphere, leading to the accelerated degradation of 4-NP.

Pollutant-degrading bacteria, including bacteria that degrade aromatic compounds, can accumulate at high density in the rhizosphere of terrestrial and aquatic plants (Siciliano et al., 2001; Toyama et al., 2009a, 2009b, 2011), and rhizosphere bacteria can actively degrade organic pollutants. These characteristic of the rhizosphere are attributable to the effects of oxygen and organic compounds released from the roots (Chaudhry et al., 2005; Shaw and Burn, 2003). The acceleration of NP degradation in the rhizosphere and successful isolation of various NP-degrading bacteria from the roots reported in this study might also be attributable to similar effects of *S. polyrrhiza*. Clarification of the interactions between NP-degrading bacteria and *S. polyrrhiza* underlying the accelerated NP degradation should allow development of rhizoremediation technologies.

4 Conclusions

Our study is the first to assess the potential of rhizoremediation as a practical technology for accelerated removal of 2-NP, 4-NP, and 2,4-DNP from contaminated surface water and to isolate NP-degrading bacterial strains that contribute to this accelerated NP removal. Accelerated removal of the three NPs was observed in three river water samples in the presence of *S. polyrrhiza*. The accelerated removal of the NPs resulted largely from biodegradation rather than from adsorption and uptake by the plants. From *S. polyrrhiza* roots, we isolated various NP-degrading bacteria that contributed to the accelerated biodegradation of the NPs. We conclude that rhizoremediation using *S. polyrrhiza* plants can be an effective strategy for remediation of NP-contaminated surface waters.

Acknowledgment

This research was supported by Japan Science and Technology Agency (JST), and the National Natural Science Foundation of China (NSFC) Project as the Japan-China Joint Research Program on Science and Technology (S&T) for Environmental Conservation and Construction of a Society with Less Environmental Burden with the research theme of Development of remediation technique for water environmental pollution using microorganisms and aquatic plants with purification ability-enhanced rhizosphere.

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JOURNAL OF ENVIRONMENTAL SCIENCES

Journal of Environmental Sciences (Established in 1989)
Vol. 24 No. 5 2012

Supervised by	Chinese Academy of Sciences	**Published by**	Science Press, Beijing, China
Sponsored by	Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences		Elsevier Limited, The Netherlands
		Distributed by	
Edited by	Editorial Office of Journal of Environmental Sciences (JES) P. O. Box 2871, Beijing 100085, China Tel: 86-10-62920553; http://www.jesc.ac.cn E-mail: jesc@263.net, jesc@rcees.ac.cn	Domestic	Science Press, 16 Donghuangchenggen North Street, Beijing 100717, China Local Post Offices through China
		Foreign	Elsevier Limited http://www.elsevier.com/locate/jes
Editor-in-chief	Hongxiao Tang	**Printed by**	Beijing Beilin Printing House, 100083, China

CN 11-2629/X **Domestic postcode: 2-580** **Domestic price per issue RMB ¥ 110.00**

ISSN 1001-0742