MICROBIAL ECOLOGY

Microb Ecol (2003) 46:302–311
DOI: 10.1007/s00248-002-1054-8

Microbial Processes Associated with Roots of Bulbous Rush Coated with Iron Plaques

K. Küsel,[1] A. Chabbi,[2] T. Trinkwalter[1]

[1] Department of Ecological Microbiology, BITOEK, University of Bayreuth, 95440 Bayreuth, Germany
[2] Department of Soil Protection and Recultivation, Brandenburg University of Technology, 03013 Cottbus, Germany

Received: 9 August 2002; Accepted: 20 February 2003; Online publication: 17 September 2003

ABSTRACT

Bulbous rush (*Juncus bulbosus*) is a pioneer species in acidic, iron-rich, coal mining lakes in the eastern part of Germany. *Juncus* roots are coated with iron plaques, and it has been suggested that microbial processes under the iron plaques might be supportive for *Juncus* plant growth. The objectives of this work were to enumerate the microbes involved in the turnover of iron and organic root exudates in the rhizoplane, to investigate the effect of oxygen and pH on the utilization of these exudates by the rhizobacteria, and to study the ability of the root-colonizing microbiota to reduce sulfate. Enumeration studies done at pH 3 demonstrated that 10^6 Fe(III) reducers and 10^7 Fe(II) oxidizers g (fresh wt root)$^{-1}$ were associated with *Juncus* roots. When roots were incubated in goethite-containing medium without and with supplemental glucose, Fe(II) was formed at rates approximating 1.1 mmol g (fresh wt root) $^{-1}$ d^{-1} and 3.6 mmol g (fresh wt root)$^{-1}$ d^{-1} under anoxic conditions, respectively. These results suggest that a rapid microbially mediated cycling of iron occurs in the rhizosphere of *Juncus* roots under changing redox conditions. Most-probable-number estimates of aerobes and anaerobes capable of consuming root exudates at pH 3 were similar in the rhizosphere sediment and in *Juncus* roots, but numbers of aerobes were significantly higher than those of anaerobes. At pH 3, supplemental organic exudates were primarily subject to aerobic oxidation to CO_2 and not subject to fermentation. However, at pH 4.5, root exudates were also rapidly utilized under anoxic conditions. Root-associated sulfate reduction was not observed at pH 3 to 4.5 but was observed at pH 4.9. The pH increased during all root-incubation studies both under oxic and anoxic conditions. Thus, as result of the microbial turnover of organic root exudates, pH and CO_2 levels might be elevated at the root surface and favor *Juncus* plants to colonize acidic habitats.

Correspondence to: K. Küsel; E-mail: kirsten.kuesel@bitoek.uni-bayreuth.de

Introduction

High microbial activity occurs in the rhizosphere as a result of the release of organic compounds by the roots [15]. Root exudates are thought to have a stimulatory effect on microbial growth and activity, because they are readily available for assimilation and degradation [27]. In addition, root exudates may also function as complexing ligands for immobilizing toxic metals [13, 40] or sequester nutrients such as phosphorus [2].

Bulbous rush (*Juncus bulbosus*) is a pioneer species in acidic coal mining lakes (pH 2.5–3) of the Lusatian mining district in the eastern part of Germany [6]. These lakes are characterized by a high input of sulfate, ferrous iron [Fe(II)], and protons due to the oxidation of pyrite in the surrounding mine tailings. Ferrous iron is oxidized in the oxygenated lake water and precipitates as poorly crystalline Fe(III)-oxides to the anoxic sediment, where it is utilized as electron acceptor for the oxidation of organic carbon and reduced sulfur species mainly by heterotrophic and autotrophic acidophilic bacteria [23, 33]. The reduction of sulfate appears to be restricted to deeper sediment zones with a pH of 5 and a lower availability of Fe(III) oxides [12, 33]. Because of the release of oxygen by bulbous rush roots, Fe(II) formed during the microbial reductive dissolution of Fe(III) oxides is oxidized and precipitates as iron plaque around the roots [4, 6]. These iron plaques consist of goethite and quartz, and the interstitial space between the plaque and the root surface is colonized by microorganisms. Formation of iron plaques around the roots also occurs with other macrophytes that colonize freshwater or marine sediments [11, 21, 37]. However, the extent to which the microbial oxidation of Fe(II) is involved in the formation of iron plaques is still a matter of debate.

In acidic, coal mining lakes, primary production is limited by the low solubility of inorganic carbon under acidic conditions [31]. However, the microbial oxidation of organic root exudates to CO_2 might provide an additional carbon source in the rhizosphere and be supportive for bulbous rush growth. Elevated concentrations of glucose, glycine, citrate, and malate are detected under the iron plaques compared to the surrounding porewater [4]. Microbial oxidation of these root exudates to CO_2 might also affect the pH. An increase of pH could initiate sulfate-reducing activity, because the reduction of sulfate appears to be inhibited under acidic conditions in these sediments [22].

In this study, the aerobic and anaerobic root-colonizing microorganisms were enumerated with respect to their ability to (i) utilize organic root exudates and (ii) oxidize Fe(II) or reduce Fe(III). To further evaluate the potential iron cycling in the rhizoplane, rates of root-associated reduction of Fe(III) were estimated. In addition, the effect of pH on the turnover of supplemental root exudates and the utilization of sulfate as alternative electron acceptor by the root-colonizing microorganisms was evaluated.

Materials and Methods

Field Site and Sampling

Plants of bulbous rush and vegetated sediment were collected from the littoral zone (up to a depth of 30 cm) of the acidic coal mining lake 108 in the Lusatian mining area in east central Germany during June to September 2000. Samples were placed on ice in plastic bags and transported to the laboratory. All material was processed within 24 h after collection. Sediment had an orange-brownish color and a pH of 3. In some samples obtained in late June and July, dark stripes were visible in the sediment, and the pH locally varied between 4.0 and 5.1. The pH of the lake water was approximately 3.2, the conductivity was 1400 μS, and the temperature varied between 12 and 19°C.

Cultivation Media

The medium used for culturing heterotrophic aerobes and anaerobes contained tryptic soy broth (TSB) without dextrose (Difco Laboratories, Detroit, MI, USA) in a 1:50 dilution to achieve a final concentration of 0.55 g L^{-1}. The headspace was sterile air or N_2, respectively. After autoclaving, the pH approximated 3.1. Glucose (2 mM) was added as an additional electron donor. The basal medium used (designated Basal medium) for culturing citate-, malate-, and glycine-utilizing aerobes and anaerobes contained, in g L^{-1}: KH_2PO_4, 2.68; $K_2HPO_4{\cdot}3H_2O$, 0.073; $MgCl_2{\cdot}6H_2O$, 0.05; NaCl, 0.4; NH_4Cl, 0.125; $CaCl_2{\cdot}2H_2O$, 0.01. The headspace was sterile air or N_2, respectively. After autoclaving, the pH approximated 2.9. A mixture of citrate, malate, and glycine (2 mM, each) was added as electron donor. The medium used for culturing Fe(III)-reducing anaerobes (designated *Acidiphilium* medium) contained, in g L^{-1}: $(NH_4)_2SO_4$, 2; $K_2HPO_4{\cdot}3H_2O$, 0.5; $MgSO_4$, 0.5; KCl, 0.1; yeast extract, 0.3. The final concentration of goethite approximated 40 mM. Goethite was produced according to published protocols [9]. The gas phase was sterile N_2, and the final pH approximated 3.1. Glucose (2 mM) was added as electron donor. The medium used for culturing Fe(II)-oxidizing aerobes (designated *Thiobacillus* medium) contained, in g L^{-1}: $(NH_4)_2SO_4$, 0.14; $K_2HPO_4{\cdot}3H_2O$, 0.04; $MgSO_4$, 0.5; $CaCl_2{\cdot}2H_2O$, 0.01; KCl, 0.06; $ZnSO_4{\cdot}7H_2O$, 0.001; $CuSO_4{\cdot}5H_2O$, 0.002; $MnSO_4{\cdot}H_2O$, 0.001; $NaMoO_4{\cdot}2H_2O$, 0.0006; $CoCl_2{\cdot}6H_2O$, 0.0006; $Na_2SO_4{\cdot}10H_2O$, 0.001; $NiCl_2{\cdot}6H_2O$, 0.001; yeast extract, 0.006. The headspace was sterile air, and the final

pH approximated 3.1. Anoxic $(NH_4)_2FeSO_4 \cdot 6H_2O$ was added after autoclaving to achieve a final concentration of 40 mM Fe(II). As carbon source, CO_2 (2 mL) was added to each tube.

For the dilution series, basal medium (Basal) with an N_2 gas phase was used. All substrates were provided as sterile anoxic stock solutions or as sterile gas. MPN tubes were scored positive based on growth and/or by measuring the consumption of substrates and the formation of products. MPN tubes for Fe(III)-reducing anaerobes and Fe(II)-oxidizing aerobes were counted positive by measuring the formation or consumption of Fe(II), respectively. In MPN tubes containing *Acidiphilium* medium for culturing Fe(III)-reducing anaerobes, sulfate was apparently not consumed in inoculated tubes, because no blackening of the medium was observed that would be indicative of the formation of Fe(II) sulfides.

Enumeration and Root Incubation Studies

For enumeration studies, roots (collected in June) were carefully separated from the sediment. Healthy roots with iron plaques were excised with a clean razor blade (3 to 8 cm from the root tip), washed twice in anoxic, sterile water to remove sediment particles and loosely associated microorganisms, and weighed. Five g of roots was homogenized with a blender inside a Mecaplex H_2-free chamber (100% N_2 gas phase) and transferred to a sterile serum bottle containing 45 mL of anoxic basal medium. Similarly, 5 g of vegetated sediment was transferred to 45 mL anoxic mineral medium. Suspensions of root homogenate and sediment were serially diluted and used to inoculate various media. Numbers of cultured cells were determined by the most-probable number (MPN) technique with three replicates incubated at 15°C for 6 months; MPN values were calculated from standard MPN tables and were within 95% certainty [1].

For root incubation studies, 5 to 6 (approximately 0.11 g) washed and cut roots (collected in July) with iron plaques were transferred to sterile tubes containing 15 mL of oxic or anoxic mineral medium (Basal) with a pH of 3 or 4.5, as indicated. A mixture of malate, citrate, and glycine (1.5 to 2 mM, each) was added. To study the root-associated Fe(III)-reducing activity, approximately 0.1 g of roots was transferred to sterile tubes containing medium for Fe(III)-reducing anaerobes (*Acidiphilium*) supplemented with goethite (40 mM) and glucose (2.4 mM). To study the root-associated sulfate-reducing activity, approximately 0.1 g of roots (collected in July) was transferred to sterile tubes containing 18 mL of filter sterilized lake water at a pH of 3.0 (*in situ* pH) or adjusted to 4.5, as indicated. The gas phase was sterile N_2. Ethanol (1 mM) and 5 mL H_2 were added as electron donor. To ensure liquid sampling during long-term incubation, approximately 0.35 g of roots (collected in September) was transferred to sterile serum bottles containing 30 mL of filter sterilized lake water adjusted to pH 4.9. Either ethanol (1 mM) and 5 mL H_2 or lactate (3 mM) were added as electron donor, respectively. After increasing the pH of the lake water to 4.5 or 4.9, an orange-brownish precipitate appeared indicating the formation of Fe(III) oxides that had to be removed by centrifugation. All incubation studies were performed with three replicates. Roots without supplemental substrates served as control. The incubation temperature was 15°C. Samples were removed with sterile syringes at different time intervals, as indicated.

Analytical Techniques

Growth in media lacking iron was monitored as optical density at 660 nm (OD_{660}) with a Spectronic 501 spectrophotometer (Bausch and Lomb, Rochester, NY, USA). The reduction of Fe(III) was estimated by determining the amount of Fe(II) formed [35]. To determine the formation of Fe(II), aliquots (0.2 mL) of the medium were withdrawn using sterile syringes connected to wide needles and transferred to 9.8 mL of 0.5 N HCl and incubated for 1 h at room temperature [26]. Fe(II) was measured by the phenanthroline method [39]. Headspace gases (H_2 and CO_2) were measured with Hewlett-Packard Co. (Palo Alto, CA) 5980 series II gas chromatographs [24]. Gas values included the total amounts in both the liquid and gas phases and are reported in mM (i.e., mmol L [medium or lake water]$^{-1}$). Aliphatic acids, alcohols, and sugars were determined with Hewlett Packard 1090 series II high-performance liquid chromatographs [24]. The detection limit for short-chain aliphatic acids approximated 100 μM. Glycine was determined with a Gynkotek (M480G) high-performance liquid chromatograph (Dionex, Germany) equipped with an IonPac CS12 column and a Gynkotek Detektor (UVD 160S) UV detector at 200 nm. The mobile phase was a mixture of KH_2PO_4 and K_2HPO_4 (0.05 M) at pH 5.0 with a rate of 0.5 mL min^{-1}. The detection limit for glycine approximated 50 μM. Sulfate was analyzed by ion chromatography [24]. Sediment pH was measured with an Ingold (Steinbach, Germany) U457-S7/110 combination pH electrode.

Results

Comparative Evaluation of the Microorganisms Colonizing J. bulbosus Roots and Vegetated Sediment

High numbers of Fe(II)-oxidizers cultured at pH 3 were detected in both the root and the vegetated sediment (Table 1). Compared to the sediment, roots were enriched with Fe(II)-oxidizing and Fe(III)-reducing microorganisms. The number of heterotrophic aerobes cultured at pH 3 approximated 10^4 to 10^5 g (fresh wt sediment or root)$^{-1}$, whereas the number of heterotrophic anaerobes was significantly lower ($P < 0.05$, as determined by the Mann–Whitney *U*-test) (Table 1). In general, glucose was utilized in all growth-positive MPN tubes cultured in TSB medium. Under anoxic conditions, acetate and ethanol were the main fermentation products. Compared to the low numbers of glucose-utilizing fermentors, higher numbers of glucose-utilizing anaerobes were obtained under Fe(III)-reducing conditions (Table 2). Citrate-, malate-, and gly-

Table 1. MPN values of the different metabolic types obtained from *J. bulbosus* roots and the vegetated sediment of lake 108[a]

Metabolic type	Medium	Substrate	MPN g (fresh wt)$^{-1}$	
			Roots	Vegetated sediment
Heterotrophic aerobes	TSB	Glucose	2.3×10^5 (4.9×10^4–1.1×10^6)[b]	2.3×10^4 (4.9×10^3–1.1×10^5)
Heterotrophic anaerobes	TSB	Glucose	4.0×10^2 (8.6×10^1–1.9×10^3)	9×10^2 (1.9×10^2–4.2×10^3)
Root exudate-utilizing aerobes	Basal	Citrate, malate, glycine	4.0×10^4 (8.6×10^3–9×10^5)	2.3×10^4 (4.9×10^3–1.1×10^5)
Root exudate-utilizing anaerobes	Basal	Citrate, malate, glycine	2.3×10^1 (4.9–1.1×10^2)	2.3×10^1 (4.9–1.1×10^2)
Fe(II) oxidizers	Thiobacillus	Fe(II), O_2, CO_2	2.3×10^7 (4.9×10^6–1.1×10^8)	2.3×10^5 (4.9×10^4–1.1×10^6)
Fe(III) reducers	Acidiphilium	Goethite, glucose	9×10^5 (1.9×10^5–4.2×10^6)	2.3×10^4 (4.9×10^3–1.1×10^5)

[a] MPN dilutions were incubated in three replicates at 15°C for 6 months
[b] Values in parentheses represent the range of the MPN values within 95% certainty

cine-utilizing aerobes colonizing roots or sediment were more abundant than citrate-, malate-, and glycine-utilizing anaerobes (Table 2).

Effect of pH on the Utilization of Supplemental Root Exudates under Oxic and Anoxic Conditions

Under oxic conditions at pH 3, malate, citrate, and glycine were consumed within 6 to 11 days of incubation (Fig. 1) concomitantly to the production of CO_2 (data not shown). Similar results were obtained at pH 4.5. However, under anoxic conditions, malate, citrate, and glycine were not consumed at pH 3 during an incubation of 14 days (Fig. 1). Under anoxic conditions at pH 4.5, malate and citrate were consumed within 7 days of incubation, similar to results obtained under oxic conditions, whereas glycine was not consumed during an incubation of 12 days. Acetate (3 mM) was the main organic fermentation product detected under anoxic conditions at pH 4.5 (data not shown). In controls lacking supplemental root exudates, malate, citrate, and glycine were not detected (data not shown).

The pH increased in all tubes during root incubations with and without supplemental root exudates. Under oxic conditions, the pH increased from 3.2 at the beginning of incubation to 4.7 and 3.5 at the end of incubation in tubes with and without supplemental root exudates, respectively. Under anoxic conditions, the pH increased from 3.0 at the beginning of incubation to 3.9 and 3.3 at the end of incubation in tubes with and without supplemental root exudates, respectively. Under oxic conditions, the pH increased from 4.4 at the beginning of incubation to 5.9 and 4.9 at the end of incubation in tubes with and without supplemental root exudates, respectively. Under anoxic conditions, the pH increased from 4.3 at the beginning of incubation to 5.5 and 5.1 at the end of incubation in tubes with and without supplemental root exudates, respectively.

Table 2. MPN values of microorganisms obtained from *J. bulbosus* roots and the vegetated sediment of lake 108 capable of consuming supplemental root exudates[a]

Metabolic type	MPN g (fresh wt)$^{-1}$	
	Roots	Vegetated sediment
Glucose-utilizing aerobes	2.3×10^5 (4.9×10^4–1.1×10^6)[b]	2.3×10^4 (4.9×10^3–1.1×10^5)
Citrate-utilizing aerobes	4.0×10^4 (8.6×10^3–1.9×10^5)	2.3×10^4 (4.9×10^3–1.1×10^5)
Malate-utilizing aerobes	4.0×10^4 (8.6×10^3–1.9×10^5)	2.3×10^4 (4.9×10^3–1.1×10^5)
Glycine-utitizing aerobes	9×10^3 (1.9×10^3–4.2×10^4)	9×10^3 (1.9×10^3–4.2×10^4)
Glucose-utilizing anaerobes	4.0×10^2 (8.6×10^1–1.9×10^3)	9×10^2 (1.9×10^2–4.2×10^3)
Glucose-utilizing Fe(III) reducers	9×10^5 (1.9×10^5–4.2×10^6)	2.3×10^4 (4.9×10^3–1.1×10^5)
Citrate-utilizing anaerobes	2.3×10^1 (4.9–1.1×10^2)	2.3×10^1 (4.9–1.1×10^2)
Malate-utilizing anaerobes	2.3×10^1 (4.9–1.1×10^2)	2.3×10^1 (4.9–1.1×10^2)
Glycine-utilizing anaerobes	0.9×10^1 (0.19×10^1–4.2×10^1)	2.3×10^1 (4.9–1.1×10^2)

[a] MPN dilutions were incubated in three replicates at 15°C for 6 months
[b] Values in parentheses represent the range of the MPN values within 95% certainty

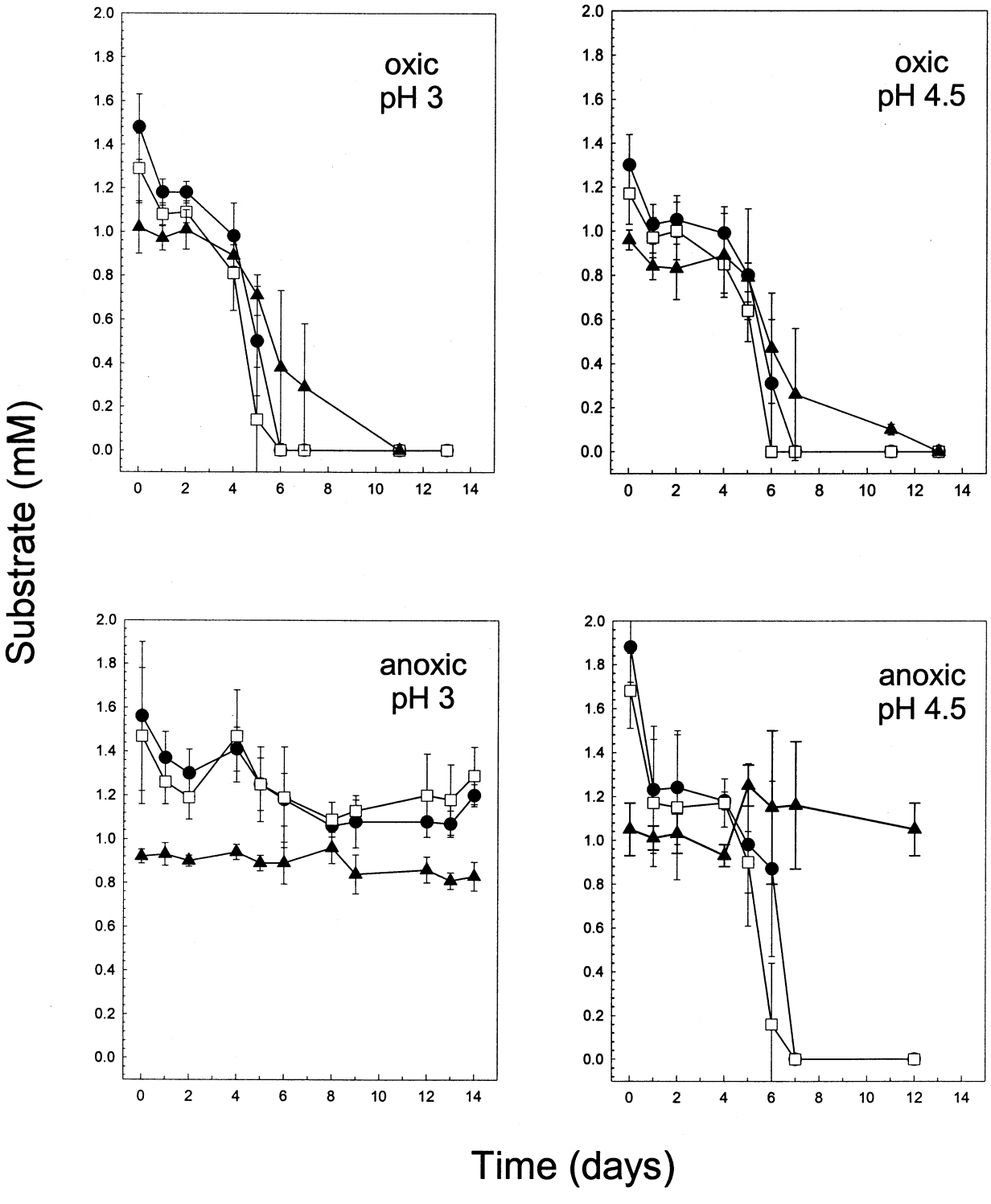

Fig. 1. Effect of pH on the consumption of supplemental root exudates by *J. bulbosus* root-associated microorganisms at 15°C under oxic and anoxic conditions. Presented are the mean values (± standard deviation) of triplicates. Symbols: (●) citrate, (□) malate, (▲) glycine.

Capacity of Root-Associated Microorganisms to Reduce Fe(III)

Fe(II) was formed by the root-associated microorganisms in tubes containing goethite as Fe(III) source both with and without supplemental glucose (Fig. 2A). Without supplemental glucose, Fe(II) was formed at a rate of 0.115 mmol Fe(II) d^{-1}. Whereas the formation of Fe(II) was not affected by the presence of supplemental glucose during

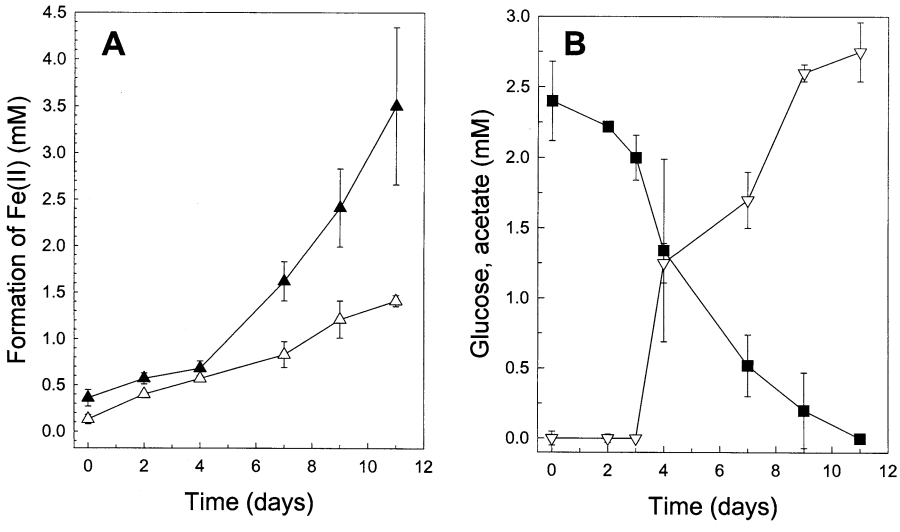

Fig. 2. Formation of Fe(II) and consumption of glucose by *J. bulbosus* root-associated microorganisms at 15°C under anoxic conditions. Presented are the mean values (± standard deviation) of triplicates. Symbols: (▲), Fe(II) with supplemental glucose; (△) Fe(II) control, (■) glucose, (▽) acetate.

the first 4 days of incubation, Fe(II) was subsequently formed at a rate of 0.397 mmol Fe(II) d^{-1}. These rates corresponded to 1.05 mmol Fe(II) g (fresh wt root)$^{-1}$ d^{-1} and 3.61 mmol Fe(II) g (fresh wt root)$^{-1}$ d^{-1} without and with supplemental glucose, respectively. Glucose was consumed within 11 days of incubation (Fig. 2B), and acetate (2.7 mM) and trace amounts of H_2 (data not shown) were detected at the end of incubation. During the first 4 days of incubation, approximately 1.1 mM of the supplemental glucose were consumed, although the formation of Fe(II) was not stimulated compared to unsupplemented controls. Thus, fermentors seemed to be also involved in glucose consumption. Approximately 12% of the reducing equivalents theoretically obtained from the oxidation of the remaining glucose were recovered in Fe(II). The pH increased from 3.3 at the beginning of incubation to 4.9 and 4.5 in the presence and absence of supplemental glucose, respectively.

Effect of pH on the Capacity of the Root-Associated Microorganisms to Reduce Sulfate

In lake water at pH 3 or adjusted to 4.5, neither endogenous sulfate (Fig. 3) nor supplemental ethanol or H_2 were consumed by the root-associated microorganisms during 21 days of incubation (data not shown). However, when lake water was adjusted to pH 4.9, sulfate was consumed after a lag phase of 18 days compared to the unsupplemented control (Fig. 3A). H_2 (3.9 mmol L^{-1} lake water) and ethanol (5.3 mM) were consumed after 4 and 15 days of incubation, respectively, concomitantly to the formation of acetate (4.3 mM). The ratio of ethanol consumed to acetate formed approximated 1.2:1, which is close to the theoretical 1:1 ratio. If the oxidation of H_2 and ethanol were completely coupled to the reduction of sulfate, 3.6 mM sulfate should theoretically be reduced [16]. However, only 1.7 mM sulfate was consumed. Thus, alternative electron acceptors present in the lake water might have also been reduced.

At the beginning of incubation, Fe(II) was not detected in lake water at pH 5. At the end of incubation, the concentration of Fe(II) approximated 1.5 mM in controls and 3.1 mM in root incubation bottles supplemented with ethanol and H_2. The source of Fe(III) is not clear. Under pH 5 conditions, the concentration of soluble Fe(III) in the lake water should be low. Thus, either Fe(III) oxides were not totally removed by centrifugation prior to root incubation or the iron plaques of the roots served as a source of Fe(III). Sorbed sulfate is released during the microbial reductive dissolution of Fe(III) oxides [33], a process that might account for increase in the concentration of sulfate during the first 18 days of incubation (Fig. 3A).

When lactate (3.5 mM) was added as electron donor to *J. bulbosus* roots incubated in lake water at pH 4.9, the concentration of sulfate decreased after 11 days of incubation (Fig. 3B). The consumption of lactate yielded acetate as a transient product and propionate. Approximately 1.5 mM sulfate was consumed. Additionally, Fe(II) increased to 3.6 mM and 1.5 mM at the end of incubation in the presence and absence of supplemental lactate, respectively.

Discussion

In acidic lakes of the Lusatian mining area, *J. bulbosus* is widely distributed and represents an abundant species of

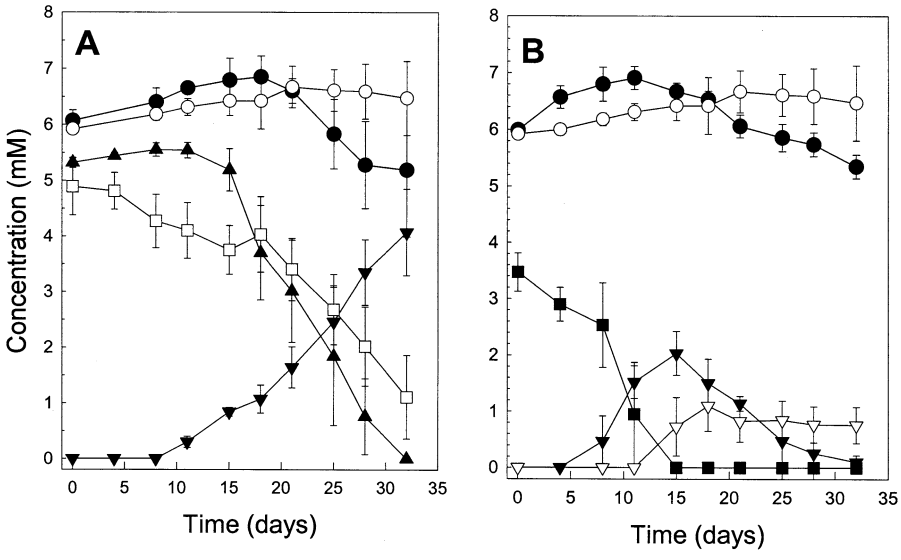

Fig. 3. Effect of supplemental ethanol and H_2 (A) and lactate (B) on the consumption of endogenous sulfate by *J. bulbosus* root-associated microorganisms at 15°C under anoxic conditions in lake water at pH 4.9. Presented are the mean values (± standard deviation) of triplicate microcosms. Symbols: (●) SO_4^{2-} with supplemental electron donor, (○) SO_4^{2-} control, (▲) ethanol, (□) H_2, (▼) acetate, (■) lactate, (△) propionate.

the area [34]. As a pioneer plant, *J. bulbosus* has various mechanisms and strategies to overcome the extreme conditions in coal mining sediments [5]. Bulbous rush can regulate the entry of transition metal ions into the cortex by maintaining amounts necessary for biological functions but avoiding excess, toxic levels [7]. The regulation of metals entering the cortex also inhibits blockages in the aeration system (aerenchyma). Thus, oxygen released by the roots can rise the redox potential in the rhizosphere [6]. Consequently, Fe(II) is oxidized, and large Fe(III)-oxide- and goethite-containing deposits are formed on the roots. These iron plaques prevent the excessive uptake of Fe(II) [7] and/or immobilize potentially toxic substances that are formed under anoxic conditions in the sediment [17, 30].

In contrast to other aquatic plant species [29, 32, 37, 38], iron plaques of bulbous rush are not directly deposited on the surface; however, a free space colonized by microorganisms exists between the root surface and the iron plaque [6]. There has been considerable debate about the potential role of microorganisms in the formation of iron plaques. Under neutral pH conditions, Fe(II) oxidation in the rhizosphere seems to result from chemical oxidation, while bacteria may act as nucleation sites for the precipitation of Fe(III) oxides [38]. However, the kinetics of chemical oxidation of Fe(II) are relatively slow at low pH. Thus, acidophilic Fe-oxidizing bacteria, such as *Acidithiobacillus ferrooxidans*, might participate in iron plaque formation under low pH conditions. The first evidence for the presence of culturable acidophilic and also neutrophilic, autotrophic Fe-oxidizing bacteria in iron plaques was presented by Emerson et al. [11]. Enumeration studies done with *J. bulbosus* roots demonstrated that Fe(II)-oxidizing microorganisms dominated the cultured, root-associated microbiota capable of growth at pH 3 (Table 1), indicating that these microorganisms might substantially contribute to the iron plaque formation in bulbous rush.

It is suggested that bacterial oxidation of Fe(II) might promote coupling between the oxidation of Fe(II) and the reduction of Fe(III) by producing poorly crystalline Fe(III) oxides which are readily available for Fe(III)-reducing bacteria [10]. Indeed, Fe(II)-oxidizing bacterial activity can lead to the formation of soluble Fe(III) ions which are subjected to diffusive transport and suboxic deposition as reactive Fe(III) oxides [36]. Under anoxic conditions, these oxides are immediately available for reduction by Fe(III)-reducing microbes. Although most of the iron present in iron plaques is Fe(III), Fe(II) compounds have been also detected on root surfaces [42]. In the present study, 10^6 Fe(III)-reducing microorganisms g (fresh wt root)$^{-1}$ were associated with *Juncus* roots, and Fe(II) was formed by *Juncus* roots at rates approximating 1.1 mmol g (fresh wt root)$^{-1}$ d^{-1} and 3.6 mmol g (fresh wt root)$^{-1}$ d^{-1} in goethite-containing medium without and with supplemental glucose under anoxic conditions, respectively. These potential Fe(III)-reduction rates exceed those observed with other aquatic macrophytes rooting in less acidic sediments with lower amounts of poorly crystalline Fe(III) oxides [21]. Thus, the present study indicates that a rapid microbially mediated cycling of iron can occur in the rhizosphere of *Juncus* roots under fluctuating redox conditions.

In the rhizoplane, the reduction of Fe(III) is thought to be one of the most active heterotrophic anaerobic proc-

esses due to the temporary high redox potential and the availability of Fe(III) oxides [21, 35]. In paddy soils, root-associated reduction of Fe(III) can inhibit methanogenesis, which is the main terminal electron-accepting process for the oxidation of organic matter in most pH neutral freshwater habitats [14]. In acidic, iron-rich sediments, the reduction of Fe(III) is the main electron accepting process for the oxidation of organic carbon, H_2, or reduced sulfur species [18]. In coal mining sediments, *Acidiphilium* species are likely involved in the reduction of Fe(III) [23]. These heterotrophic acidophiles can also reduce solid forms of Fe(III) oxides (e.g., goethite or amorphous Fe(III) hydroxide) [3]. In contrast to other neutrophilic Fe(III) reducers, *Acidiphilium* species can reduce Fe(III) under both microoxic and oxic conditions [19, 20, 25] and might thus be of ecological significance in an environment such as the rhizosphere with fluctuating redox conditions. *Acidiphilium* species can couple the reduction of Fe(III) to the complete oxidation of glucose, malate, or citrate [23]. In the present study, glucose was utilized in all dilution series that were positive for the reduction of Fe(III). Since fermentation seems to be of minor importance under low pH conditions, root exudates might be subjected to either aerobic respiration or Fe(III) reduction.

Roots of *J. bulbosus* were colonized by high numbers of aerobes (10^4 microorganisms g (fresh wt root)$^{-1}$) that were able to utilize root exudates. The ability of the root-colonizing microbiota to oxidize supplemental root exudates to CO_2 without a lag phase both at pH 3 and pH 4.5 indicates that these compounds can be rapidly consumed under oxic conditions. In contrast, root-colonizing microorganisms did not utilize these compounds at pH 3 under anoxic conditions. Thus, the CO_2 produced by microbial oxidation may diffuse from the roots via the internal lacunal airspace system into the leaves where it is fixed through the glycolate pathway [8]. Thus, an auxiliary source of carbon around the roots might be provided. Similar mechanisms for overcoming carbon limitation by utilization of benthic CO_2 sources are also known for other submersed macrophytes that colonize acidified lakes [41, 43].

The pH increased during all root-incubation experiments, but not in tubes containing medium or seawater lacking roots. Root-associated sulfate reduction was not observed at pH 3 to 4.5, but was observed at pH 4.9, indicating the absence of acidophilic sulfate reducers in the rhizoplane. Because of the lag phase observed prior to the onset of sulfate consumption, the root-colonizing sulfate-reducing population seemed to be small. In sediments of acidic coal mining lakes, the reduction of Fe(III) predominates because of the high availability of poorly crystalline Fe(III) oxides and the acidic conditions, whereas the reduction of sulfate is restricted to deeper sediment zones with elevated pH [33]. However, sulfate reduction can be initiated in upper sediments when the pH increases to pH 5 via reductive and fermentative microbial activities stimulated by supplemental carbon sources, such as cellobiose [22]. Apparently, the increase in pH is caused by the stimulated reduction of Fe(III) oxides through liberation of both bicarbonate and hydroxyl alkalinity, because 25% of the reducing equivalents theoretically obtained from the oxidation of cellobiose are recovered in Fe(II). However, the consumption of cellobiose in acidic sediment is slow compared to that in deeper slightly acidic sediment zones [22]. Similarly, the increased availability of carbon in the rhizosphere might lead to a pH shift during the vegetation period toward less acidic conditions and, thus, initiate sulfate reduction in the rhizosphere. Indeed, the pH of some vegetated sediment samples obtained late in July approximated 4 to 5, and dark stripes were visible in the sediment indicating sulfate-reducing activity. Thus, these results indicate that the microbially mediated turnover of carbon in the rhizosphere is supportive of bulbous rush growth by providing an additional source of CO_2 and by changing the pH of the environment.

Acknowledgements

The authors express sincere appreciation to Harold L. Drake for review of the manuscript and general support of this study. Financial support was provided by the Deutsche Forschungsgemeinschaft (DFG) (Ku 1367/1-1) and the German Ministry for Education, Science, and Technology (BMBF) (PT BEO 51-0339476 C).

References

1. Alef K (1991) Methodenhandbuch Bodenmikrobiologie: Aktivitäten, Biomasse, Differenzierung. Ecomed, Landsberg/Lech, Germany, pp 44–49
2. Bowen GD, Rovira AD (1991) The rhizosphere, the hidden half of the hidden half. In: Waisel Y, Eishel A, Kafkafi U (Eds.) Plants Roots—The Hidden Half. Marcel Dekker, New York, pp 641–649
3. Bridge TAM, Johnson DB (2000) Reductive dissolution of ferric iron minerals by *Acidiphilium* SJH. Geomicrobiol J 17:193–206

4. Chabbi A, Hines ME, Rumpel C (2001) The role of organic carbon excretion by bulbous rush roots and its turnover and utilization by bacteria under iron plaques in extremely acid sediments. Environ Exper Bot 87:237–246
5. Chabbi A, Pietsch W, Wiehe W, Hüttel RF (1998) *Juncus bulbosus:* Strategies of survival under extreme phytotoxic conditions in acid mine lakes in the Lusatian mining district, Germany. Int J Ecol Environ Sci 24:271–292
6. Chabbi A (1999) *Juncus bulbosus* as pioneer species in acidic lignite mining lakes: interactions, mechanism and survival strategies. New Phytol 144:133–142
7. Chabbi A (2003) Metal concentrations in pore water of the Lusatian lignate mining sediments and internal metal distribution in *Juncus bulbosus*. Wat Air Soil Pollut Focus 3:105–117
8. Chollet R, Ogren WL (1975) Regulation of photorespiration in C_3 and C_4 species. Bot Rev 41:137–179
9. Cornell RM, Schwertmann U (1996) The Iron Oxides. VCH Verlagsgesellschaft mbH, Weinheim, pp 491–498
10. Emerson D, Moyer C (1997) Isolation and characterization of novel iron-oxidizing bacteria that grow at circumneutral pH. Appl Environ Microbiol 63:4784–4792
11. Emerson D, Weiss JV, Megonical P (1999) Iron-oxidizing bacteria are associated with ferric hydroxide precipitates (Fe-plaque) on the roots of wetland plants. Appl Environ Microbiol 65:2758–2761
12. Fortin D, Davis B, Beveridge TJ (1996) Role of *Thiobacillus* and sulfate-reducing bacteria in iron biocycling in oxic and acidic mine tailings. FEMS Microbiol Ecol 21:11–24
13. Francis AJ, Dodge CJ, Gillow JB (1992) Biodegradation of metal citrate complexes and implications for toxic metal mobility. Nature 356:140–142
14. Frenzel P, Bosse U, Janssen PH (1999) Rice roots and methanogenesis in a paddy soil: ferric iron as an alternative electron acceptor in the rooted soil. Soil Biol Biochem 31:421–430
15. Grayston SJ, Vaughan D, Jones D (1996) Rhizosphere carbon flow in trees, in comparison with annual plants: the importance of root exudation and its impact on microbial activity and nutrient availability. Appl Soil Ecol 5:29–56
16. Hansen TA (1993) Carbon metabolism of sulfate-reducing bacteria. In: Odom JM, Singleton Jr R (Eds.) The Sulfate-Reducing Bacteria: Contemporary Perspectives. Springer Verlag, New York, pp 21–40
17. Iremonger SF, Kelly DL (1988) The responses of four Irish wetland tree species to raised soil water levels. New Phytol 109:491–497
18. Johnson DB (1995) Mineral cycling by microorganisms: iron bacteria. In: Allsopp D, Colwell RR, Hawksworth DL (Eds.) Microbial Diversity and Ecosystem Function. CAB International, University Press, Cambridge, UK, pp 137–159
19. Johnson DB, Bridge TAM (2002) Reduction of ferric iron by acidophilic heterotrophic bacteria: evidence for constitutive and inducible enzyme systems in *Acidiphilium* spp. J Appl Microbiol 92:315–321
20. Johnson DB, McGinness S (1991) Fe(III) reduction by acidophilic heterotrophic bacteria. Appl Environ Microbiol 57:207–211
21. King GM, Garey MA (1999) Ferric iron reduction by bacteria associated with the roots of freshwater and marine macrophytes. Appl Environ Microbiol 65:4393–4398
22. Küsel K, Dorsch T (2000) Effect of supplemental electron donors on the microbial reduction of Fe(II), sulfate, and CO_2 in coal mining-impacted freshwater lake sediments. Microb Ecol 40:238–249
23. Küsel K, Dorsch T, Acker G, Stackebrandt E (1999) Microbial reduction of Fe(III) in acidic sediments: Isolation of *Acidiphilium cryptum* JF-5 capable of coupling the reduction of Fe(III) to the oxidation of glucose. Appl Environ Microbiol 65:3633–3640
24. Küsel K, Drake HL (1995) Effects of environmental parameters on the formation and turnover of acetate by forest soils. Appl Environ Microbiol 61:3667–3675
25. Küsel K, Roth U, Drake HL (2002) Microbial reduction of Fe(III) in the presence of oxygen under low pH conditions. Environ Microbiol 4:414–421
26. Lovley DR, Phillips EJP (1986a) Availability of Fe(III) for microbial reduction in bottom sediments of the freshwater tidal Potomac River. Appl Environ Microbiol 52:751–757
27. Lynch JM, Whipps JM (1990) Substrate flow in the rhizosphere. Plant Soil 129:1–10
28. Marschner H, Romheld V, Cakman I (1987) Root induced-changes of nutrient availability in the rhizosphere. J Plant Nutr 10:1175–1184
29. Mendelssohn IA, Kleiss BA, Wakeley JS (1995) Factors controlling the formation of oxidized roots channels: A review. Wetlands 15:37–46
30. Mendelssohn IA, Postek MT (1982) Elemental analysis of deposits on the roots of *Spartina alterniflora loisel.* Am J Bot 69:904–912
31. Nixdorf B, Kapfer M (1998) Stimulation of phototrophic pelagic and benthic metabolism close to sediments in acidic mining lakes. Wat Air Soil Poll 108:317–330
32. Otte ML, Dekkers M, Rozema J, Broekman RA (1991) Uptake of arsenic by *Aster tripoliumi* relation to rhizosphere oxidation. Can J Bot 69:2670–2677
33. Peine A, Tritschler A, Küsel K, Peiffer S (2000) Electron flow in an iron-rich acidic sediment—evidence for an acidity-driven iron cycle. Limnol Oceanogr 45:1077–1087
34. Pietsch W (1973) Vegetationsentwicklung und Gewässergenese in den Tagebauseen des Lausitzer Braunkohlen-Revieres. Archiv Naturschutz Landschaftsforsch 13:187–217
35. Roden ER, Wetzel RG (1996) Organic carbon oxidation and suppression of methane production by microbial Fe(III) oxide reduction in vegetated and unvegetated freshwater wetland sediments. Limnol Oceanogr 41:1733–1748
36. Sobolev D, Roden EE (2001) Suboxic deposition of ferric iron by bacteria in opposing gradients of Fe(II) and oxygen at circumneutral pH. Appl Environ Microbiol 67:1328–1334

37. St-Cyr L, Crowder AA (1989) Factors affecting iron plaque on the roots of *Phragmites australis* (*Cav.*) *Trin. ex Steudel.* Plant Soil 116:85–93
38. St-Cyr L, Fortin D, Campbell PGC (1993) Microscopic observations of the iron plaque of submerged aquatic plant (*Vallisneria americana Michx*). Aquat Bot 46:155–167
39. Tamura H, Goto K, Yotsuyanagi T, Nagayama M (1974) Spectrophotometric determination of iron(II) with 1,10-phenanthroline in the presence of large amounts of iron(III). Talanta 21:314–318
40. Uren NC (2001) Types, amounts, and possible functions of compounds released into the rhizosphere by soil-grown plants. In: Pinton R, Varanini Z, Nannipieri P (Eds.) The Rhizosphere. Marcel Dekker, Inc, New York, pp 19–40
41. Waisel Y, Agami M (1996) Ecophysiology of roots of submerged aquatic plants. In: Waisel Y, Eishel A, Kafkafi U (Eds.) Plants Roots—The Hidden Half. Marcel Dekker, New York, pp 895–909
42. Wang T, Peverly JH (1999) Iron oxidation states on root surfaces of a wetland plant (*Phragmites australis*). Soil Sci Am J 63:247–252
43. Wetzel RG, Brammer ES, Linström K, Forsberg C (1985) Photosynthesis of submersed macrophytes in acidified lakes. II. Carbon limitation and utilization of benthic CO_2 sources. Aquat Bot 22:107–120