Manganese uptake and Mn(II) oxidation by the alga *Scenedesmus subspicatus*

Katja Knauer, Thomas Jabusch and Laura Sigg

Swiss Federal Institute for Environmental Science and Technology (EAWAG),
8600 Dübendorf, Switzerland

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ABSTRACT

To examine the relationships among manganese concentration in the culture medium, algae growth, manganese uptake and Mn(II) oxidation on the algal surface, we exposed the unicellular alga *Scenedesmus subspicatus* to a broad range of free Mn^{2+} ion concentrations. Extra- and intracellular manganese concentrations were distinguished by reducing the Mn oxides with ascorbate. A large fraction of the Mn bound by the algae occurred as reducible Mn(III/IV) oxides formed by Mn(II) oxidation, presumably on the algal surface, although these algal cells of small size are not likely to build up a large pH gradient at their surface. Limitation of growth occurred when $[\text{Mn}^{2+}]$ was lower than about 1×10^{-9} M, which corresponded to an intracellular content of 10^{-7} mol Mn (g algae dry weight) $^{-1}$. Inhibition of growth occurred at high $[\text{Mn}^{2+}]$, at which high concentrations of Mn ($> 1 \times 10^{-5}$ Mol Mn (g algae dry weight) $^{-1}$) were present both intracellularly and as extracellular Mn oxides. Phytoplankton collected in various lakes contained intracellular Mn and extracellular Mn oxides in similar concentrations as the *Scenedesmus* cultures.

Introduction

In the aquatic environment manganese occurs mainly in the three oxidation states $^{+}(\text{II})$, $^{+}(\text{III})$ and $^{+}(\text{IV})$ (Chiswell et al. 1986, Davison 1993). The redox transformations of manganese are primarily influenced by biological transformation processes, including oxidation of Mn(II), reduction of Mn(III) and Mn(IV), adsorption and uptake (Nealson et al. 1980, 1988, 1989, Diem and Stumm 1984, Tipping 1984, Richardson et al. 1988, Richardson and Stolzenbach 1995).

Manganese is an essential nutrient, and numerous enzymes utilize the redox properties of this element (Larson and Pecoraro 1992). For example, it is needed in the cell for catalyzing oxygen evolution in photosynthesis (Eyster et al. 1958, Diner et al. 1976, Sauer 1980). Hence, the growth of phytoplankton might be limited at low manganese concentrations (Sunda 1988/89). Phytoplankton cells efficiently take up and accumulate Mn(II) intracellularly (Sunda and Huntsman 1981, 1983, 1985, 1986). Phytoplankton may also catalyze the oxidation of Mn(II) to Mn(IV). Richardson et al. (1988) showed that manganese oxidation occurs in dense populations of

photosynthesizing algae that can generate microenvironments of high (>9) pH values. Mn oxidation was also shown to depend on the cell size of algae, indicating that cells larger than 20 μm can induce Mn oxidation (Richardson and Stolzenbach 1995). Price and Morel (1990) speculated that ectoenzymes may play a role in both oxidation and reduction reactions of manganese.

Relationships among Mn concentration in freshwater environments, its uptake by freshwater algae and the role of algae in forming Mn oxides are poorly known. In this study, we investigated the uptake of manganese and the formation of Mn-oxides by the green alga *Scenedesmus subspicatus*. We attempted to distinguish between intra- and extracellularly bound Mn, and especially to establish whether extracellularly bound Mn was oxidized and occurred as Mn oxide. We investigated the formation of manganese oxides on the cell surface under various conditions of pH and cell density. Growth rates of *S. subspicatus* were determined over a range of Mn concentrations to evaluate essential and toxic Mn concentration ranges. Finally, we compared the response of the algal cultures to the manganese concentrations occurring in lakes and the manganese content of phytoplankton from different lakes.

Materials and methods

Test organism and culture conditions

All experiments were conducted with axenic cultures of the unicellular green alga *Scenedesmus subspicatus* SAG 86.81 (Chlorococcales, Chlorophyta). The cultures were obtained from the Institute for Plant Physiology at the University of Göttingen, Germany. The experimental cultures were kept at 25 °C and 3000 Lux (54 $\mu\text{E m}^{-2} \text{s}^{-1}$; 12:12 h diel cycle) in 250 ml polycarbonate flasks with 100 ml medium.

Medium composition

Algae were grown in a modified OECD medium (OECD test guideline 201 1984), buffered with either phosphate (5×10^{-3} M) (Kuhl 1962) or HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) (5×10^{-3} M) at a pH of 7.9 (with the exception of an experimental set in which the influence of pH on Mn oxidation in the medium was tested by working additionally with a pH of 7). The following compounds were added: NaHCO_3 (5.95×10^{-4} M), NaNO_3 (2.8×10^{-4} M), $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$ (1.22×10^{-4} M), $\text{CaCl}_2 \cdot 2 \text{H}_2\text{O}$ (1.22×10^{-4} M), KH_2PO_4 (4.5×10^{-3} M when used as the main buffer, 2.18×10^{-4} M in the HEPES medium), NaH_2PO_4 (or Na_2HPO_4 , 5×10^{-4} M, only added to the phosphate-buffered medium), H_3BO_3 (2.99×10^{-6} M), Na_2MoO_4 (2.9×10^{-8} M). Where HEPES was used as buffer, it was added to a concentration of 5×10^{-3} M. The chelator EDTA (ethylenediaminetetraacetate) was used to facilitate the control of chemical speciation in the medium and also to work at low trace metal concentrations (Sunda and Guillard 1976, Sunda and Huntsman 1983, 1985, 1986). EDTA was added as a $\text{Na}_2\text{EDTA} \cdot 2 \text{H}_2\text{O}$ stock solution to concentrations of 10^{-4} M or 10^{-5} M. Mn, Zn, Cu and Fe were added as stock solutions of

their salts $\text{MnSO}_4 \cdot 4 \text{H}_2\text{O}$, $\text{CuSO}_4 \cdot \text{H}_2\text{O}$, $\text{ZnSO}_4 \cdot 7 \text{H}_2\text{O}$ and FeEDTA (FeCl_3 and Na_2EDTA , 1:1).

Chemical speciation

Chemical speciation in the medium was calculated with the speciation program MICROQL (Westall 1979, Müller 1993), using a matrix that included 12 components and 70 species. The free manganese concentration in the cultures ranged from 10^{-4} M to 10^{-12} M. The corresponding total manganese concentrations were between 1.04×10^{-4} M and 1.78×10^{-9} M, depending on the EDTA concentration used. Free Cu^{2+} , Zn^{2+} and Fe^{3+} were controlled at $[\text{Cu}^{2+}] = 10^{-14}$ M and $[\text{Zn}^{2+}] = 10^{-9}$ M, a range comparable to reported lake concentrations (Xue and Sigg 1993, 1994); Fe^{3+} was kept at 10^{-19} M due to its low solubility (Martell and Smith 1976–1989). Total concentrations of Cu, Zn, and Fe were varied according to the calculations. Therefore, the composition of the medium was adjusted with higher total Zn concentrations at higher EDTA concentrations. The concentrations of free Ca^{2+} ions ($3.3 \pm 0.1 \times 10^{-5}$ M) and free Mg^{2+} ions ($2.78 \pm 0.01 \times 10^{-5}$ M) were constant in the different culture media; their concentrations are mostly influenced by the formation of phosphate and carbonate complexes.

Data on cell manganese content (intra- and extracellular) and growth data were compared to the initial measured total manganese and to the initial calculated free Mn^{2+} ion concentration. We measured the total metal concentrations in each flask by ICP-MS (Perkin Elmer Sciex Elan 5000) and checked the calculated values by using the program MICROQL. Furthermore, samples of the media were taken on the third and on the last day of the growth experiments, and concentrations of the trace metals and pH were measured, so that the actual concentrations and the speciation were known.

Medium preparation

The culture medium was prepared from a 10-fold concentrated solution containing macro- and micronutrients and the buffer. The pH was adjusted with NaOH after diluting this medium with $\text{Q-H}_2\text{O}$ to the final concentration. The medium was added to the culture vessels, were closed with cellulose stoppers and sterilized in a microwave oven for 7 min at 200 W (Keller et al. 1988). NaHCO_3 , EDTA and the trace metals Mn, Zn, Cu and Fe were added after heat sterilization, to prevent unwanted effects (e.g. precipitation of CaCO_3) on chemical speciation in the medium (Price et al. 1988/89). All stock solutions were filtered through sterile $0.2 \mu\text{m}$ cellulose nitrate filters from Sartorius before use. The media were left to stand for at least 24 h before inoculation, in order to achieve chemical equilibration (Price et al. 1988/89).

Chemicals of the suprapure grade were used for medium preparation. All vessels, tools and filters that were brought in contact with medium compounds or samples for analysis had been repeatedly acid-washed with 0.1 M HNO_3 suprapure and repeatedly rinsed with $\text{Q-H}_2\text{O}$.

Total cellular, intracellular and extracellular manganese content

To measure total manganese concentration associated with algal biomass, cultures were filtered onto 0.45 μm cellulose nitrate filters (Sartorius) at the end of the exponential growth phase. Dried filters were digested with 1 ml H_2O_2 (30 %) and 4 ml HNO_3 (65 %) in the microwave oven for 14 min at 160 °C and a maximum pressure of 5 bar. The digests of the dried filtered material were analyzed for Mn, Zn, Cu, Fe, Ca and Mg with ICP-MS (Perkin Elmer Sciex Elan 5000). Total metal content was calculated on a per dry weight basis of the algae.

In order to differentiate between intra- and extracellularly bound manganese, wash experiments using the mild reducing agent ascorbate and EDTA were performed. This procedure should indicate whether the extracellular fraction of manganese occurs in the reduced Mn(II) state or as Mn(III/IV) oxides (Stauber and Florence 1985). Ascorbate reduces the Mn(III/IV) oxides, whereas high EDTA concentrations would desorb Mn(II) from the cell surfaces (Rothstein and Hayes 1956). These experiments were also conducted with batch cultures and various Mn(II) concentrations. The media were HEPES-buffered and contained either 10^{-4} M or 10^{-5} M EDTA. At the end of the exponential growth phase the cultures were filtered onto 0.45 μm filters. Before preparation and metal analysis, part of the filtered cultures were resuspended for at least 10 min in 20 ml of metal free medium containing either 3×10^{-3} M ascorbate (pH 8, Stauber and Florence 1985, Sunda and Huntsman 1987, 1988) or, in a preliminary test, 3×10^{-3} M EDTA (pH = 8). The treated filters were then digested as described above. All data points were obtained from separate cultures. Therefore no corresponding total and intracellular content data were presented at one Mn^{2+} concentration.

A further set of wash experiments was done by using different filter pore sizes to check whether the particulate Mn is bound to the algal surface, and two different pH values to study the impact of culture pH on manganese oxidation. The media for this experimental set had a pH of 7.0 or 7.9 and various manganese concentrations. At the end of exponential growth the cultures were filtered onto 3 μm nuclepore filters. Part of the cultures were resuspended in medium containing both ascorbate and EDTA. The filtered (< 3 μm) culture medium was again filtered, this time onto 0.2 μm nuclepore filters, to detect small Mn-oxide particles in the medium. Filters were either digested and analyzed for their metal contents, as described above, or treated with the dye Leukoberbelin blue (LBB), which is used for the detection of manganese oxides by a color reaction (Krumbein and Altmann 1973). The dried filters were dipped for 1 h in 2 ml of a LBB solution to develop the color reaction (Mason 1995).

The amount of other trace elements such as Cu, Zn and Fe associated with the Mn oxides on the algal cells were determined by measuring the released concentrations of these elements when the cells were washed with ascorbate. These experiments were conducted with low and high manganese encrusted algal cells.

We estimated steady state Mn(II) oxidation rates (rate_{ox}) on the surface of *S. subspicatus* with the equation: $\text{rate}_{\text{ox}} = \mu [\text{Mn}(\text{surface})]$, where μ is the specific growth rate and $[\text{Mn}(\text{surface})]$ is the surface bound manganese fraction. We calculated the uptake rate under steady state conditions for the intracellular Mn with the equation: $V_{\text{ss}} = \mu [\text{Mn}(\text{cell})]$, V_{ss} is the uptake rate under steady state conditions,

μ is the specific growth rate and $[\text{Mn}(\text{cell})]$ is the intracellular manganese content after desorption of surface bound manganese with ascorbate (Sunda and Huntsman 1985).

Preliminary tests with ascorbate and EDTA

Preliminary tests were run to determine the required ascorbate concentration as well as the resuspension time. For these tests, algal cultures were grown in a 10 l polycarbonate fermenter containing 5 l of medium (EDTA 10^{-4} M, $\text{pMn} = 6$, $\text{pCu} = 14$, $\text{pZn} = 9$, $\text{pFe} = 19$) and then harvested to obtain a high amount of algal biomass. These algae were inoculated in 300 ml sterile medium with the same free metal concentrations. For a time series 3×10^{-3} M ascorbate were added to the medium and parallel samples of 20 ml volume were taken at time intervals (0, 10, 30, 60, 120, 180 and 240 min). Samples were treated and analyzed for the cellular metal content as previously described. In the second preliminary test, algal samples were taken and were resuspended for 30 min in metal-free medium containing various ascorbate concentrations (10^{-4} M, 5×10^{-4} M, 5×10^{-3} M). Treatment and measurements were done as reported above. To compare the dissolution of manganese using ascorbate and the desorption with EDTA, two samples were resuspended for 10 min with metal-free medium containing 3×10^{-3} M EDTA.

Growth experiments

To determine the effect of manganese on the growth of *S. subspicatus*, batch culture experiments were conducted using various manganese concentrations.

These experimental cultures were inoculated with 5 ml from a specifically designed preculture. The preculture was designed to avoid the input of significant metal amounts to the test cultures in order to achieve the low free concentrations desired in the experiments. Population density at the start of an experiment was around 10^4 cells/ml.

The specific growth rate (μ) was estimated by the linear regression of $\ln[\text{cells/ml}]$ vs. time. During 4–7 days (depending on the initial optical density (O.D.) aliquots (≤ 5 ml) were taken at time intervals to measure photometrically the increase of the optical density (O.D.) at 685 nm in the cultures. In the end of the exponential growth phase the experiments were terminated. The correlation of the O.D. with cell density was determined in a preliminary experiment. This correlation was confirmed by another preliminary test using a Galai CIS-1 laser projection particle analyzer. Manganese concentration in the culture showed no significant effect on cell size.

Phytoplankton from different lakes

Water and phytoplankton samples were taken from Lake Greifen, Sempach, and Lucerne in Switzerland and from Lake Orta in Italy in spring 1995. Go-Flow

sampling bottles (General Oceanics) were used to collect water samples from the 3 and 6 m depth at the deepest point of the lakes. The samples were transferred to acid washed polyethylene bottles. Samples were filtered in the laboratory over 0.45 μm filters to determine the total dissolved manganese concentration. Phytoplankton net tows were used to collect algae in the epilimnion of each lake from 0 to 6 m. The net mesh size was 22–95 μm to separate the zooplankton from the phytoplankton. The concentrated algae samples were placed into clean polyethylene bottles and were filtered over cellulose nitrate filter (0.45 μm) to determine the total cellular manganese content. Aliquots of the phytoplankton were washed with ascorbate as described above to determine the amount of manganese bound to the surface as Mn oxides.

Results

Relationship among growth rate, total cellular manganese content and free Mn^{2+} and total Mn concentration (Mn_T)

Limitation and inhibition of the growth of *S. subspicatus* were observed in the manganese experimental range. Limitation of growth occurred at about $[\text{Mn}^{2+}] < 10^{-9} \text{ M}$ and inhibition at $[\text{Mn}^{2+}] > 10^{-5} \text{ M}$ (Fig. 1). The optimal growth ($0.9 \pm 0.1 \text{ day}^{-1}$; replicates of each growth experiments (N), $N = 8$) occurred in the Mn^{2+} range of about 10^{-9} M to 10^{-7} M . Because large amounts of Mn were taken up by the algae

Figure 1. Relationship between growth rate (d^{-1}) of *Scenedesmus subspicatus* and calculated free Mn^{2+} (M) concentration in the culture media containing 10^{-5} and 10^{-4} M EDTA . Vertical error bars correspond to the standard deviation of the growth rate and horizontal error bars to the standard deviation of the free Mn^{2+} (M) concentration in the medium

$[\text{Mn}^{2+}]$ was not constant over the whole growth experiments, especially at low $[\text{Mn}^{2+}]$. The growth rates, as well as the cellular manganese contents are plotted in function of the initial calculated $[\text{Mn}^{2+}]$.

The total cellular manganese content increased from $1.5 \times 10^{-7} \text{ mol (g algae)}^{-1}$ (dry weight) to $2.3 \times 10^{-4} \text{ mol (g algae)}^{-1}$ with $[\text{Mn}^{2+}]$ concentration increasing from 10^{-10} M to 10^{-5} M (respectively Mn_T increasing from $2 \times 10^{-9} \text{ M}$ to $1 \times 10^{-4} \text{ M}$) (Fig. 2). The Mn content of the cells was independent of the use of either phosphate or HEPES as a buffer.

The growth limited cells in the low Mn^{2+} range contained around $10^{-7} \text{ mol M (g algae)}^{-1}$. Cells that were growth inhibited at higher Mn concentrations contained more than $10^{-5} \text{ mol Mn (g algae)}^{-1}$.

Extracellular and intracellular manganese content

A concentration of $5 \times 10^{-3} \text{ M}$ ascorbate and a contact time of 10 min at pH 8 proved in preliminary experiments optimal to desorb the extracellular (surface-bound) Mn fraction and to obtain consistent intracellular contents.

More than 90% of the total cellular manganese could be desorbed with ascorbate from the cell surface at Mn_T of 10^{-4} M . Less than 5% of the total manganese content was removable with EDTA at Mn_T of 10^{-4} as well as at Mn_T of 10^{-8} M .

Figure 2. Relationship between total cellular manganese ($\text{mol Mn (g algae)}^{-1}$) in the green alga *Scenedesmus subspicatus* in relation to the free Mn^{2+} (M) concentration in the medium containing 10^{-5} and 10^{-4} EDTA. Experiments with phosphate buffer (squares) are distinguished from those with HEPES buffer (triangles). Vertical error bars correspond to the standard deviation of the total cellular content and horizontal error bars to the standard deviation of the free Mn^{2+} (M) concentration in the medium

These results indicate that surface-bound Mn was in the form of Mn(III/IV) oxides. Therefore, to distinguish between extracellular and intracellular manganese, we used only ascorbate for the subsequent experiments.

The intracellular Mn content increased from 1×10^{-7} to 1×10^{-5} mol/g algae with $[Mn^{2+}]$ increasing from 1×10^{-11} M to 3×10^{-5} M (respectively Mn_T increasing from 2×10^{-9} M to 1×10^{-4} M) (Fig. 3). Total and intracellular content were similar in the range of Mn^{2+} up to about 10^{-10} M (respectively Mn_T from 2×10^{-9} M to 10^{-7} M) (Fig. 3). With these intracellular Mn contents, the half-saturation constant K_M (1.39×10^{-9} M) and the maximum uptake velocity v_{max} (7.34×10^{-6} mol Mn (g algae day) $^{-1}$) of the manganese uptake system are obtained from the Michaelis-Menten equation and the reciprocal plot of Lineweaver-Burk, in relation to the free $[Mn^{2+}]$ concentration.

At higher $[Mn^{2+}]$ a substantial part of the total manganese content was present as reducible Mn(III/IV) oxides, presumably on the cell surfaces (Fig. 3 and 4). Up to 90% of the total cellular manganese content was extracellularly bound as Mn(III/IV) oxides at high Mn^{2+} (Fig. 4). Additionally, the Mn content of phytoplankton samples from the various lakes is shown in Fig. 3 (see below).

The experiments with two different filter pore sizes showed that the Mn oxides are associated with the algae. The algae collected on the 3 μ m filter (without ascorbate treatment) showed the characteristic blue color after treatment with LBB to detect Mn oxides; the intensity of the blue color increased with increasing manga-

Figure 3. Total and intracellular content of manganese (mol Mn (g algae) $^{-1}$) of *S. subspicatus* in medium containing 10^{-4} M and 10^{-5} M EDTA and of phytoplankton of Lake Greifen, Sempach, Lucerne, and Orta in relation to the free Mn^{2+} (M) concentration. Solid symbols correspond to the total cellular content, open symbols to the intracellular content

Figure 4. Surface-bound and intracellular content of manganese (mol Mn (g algae)⁻¹) of *S. subspicatus* in relation to the free Mn²⁺ (M) concentration at high (10⁶ cells/ml) and low (10⁵ cells/ml) cell density

nese concentration in the medium. The algae washed with ascorbate retained their natural green color. After removing the algae, less than 5% of the total cellular manganese content was detected on the fine filter (0.2 μm), and the filter remained white after treatment with LBB. These observations indicate that the Mn oxides were most probably bound to the surface of the cells; alternatively, the Mn oxides may become attached to the cells during filtration.

The manganese content, both the intracellular content and the surface bound Mn-oxides, was similar at low (10⁵ cells/ml) and high cell density (10⁶ cells/ml) (Fig. 4). Similar results were also obtained at both pH values (7 and 8) used (not shown).

The MnO₂ concentration per surface area was 8.92 × 10⁻⁷ g MnO₂ cm⁻² at Mn_T concentrations of 10⁻⁴ M; the thickness of the MnO₂ layer was calculated as 2.3 nm on the cell surface of *S. subspicatus* with a surface area of 8.16 × 10⁻⁷ cm² (cell)⁻¹ and corresponds thus to a few molecular layers if homogeneously distributed.

The estimated steady state Mn(II)oxidation rate increased with increasing manganese concentration, the rate was 2.25 × 10⁻⁵ ml Mn (g algae dry weight day)⁻¹ for a Mn_T concentration of 10⁻⁵ M and 7.5 × 10⁻⁵ mol Mn (g algae dry weight day)⁻¹ for Mn_T concentration of 10⁻⁴ M.

Adsorption of metal ions on the surface-bound Mn oxides

Mn oxides are surface active and tend to adsorb a wide variety of cations. The amount of copper and iron released from the algal surface after washing with ascorbate ranged between 0 and 9% and 0 and 14% of the total cellular copper and iron

Table 1. Desorption of Mn, Cu, Fe, and Zn by ascorbate treatment

MnT (M)	3.0×10^{-9}	1.6×10^{-8}	1.5×10^{-6}
Mn (% desorbed)	0	0	87.6
Cu (% desorbed)	8.5	0	0.5
Fe (% desorbed)	3.9	13.8	4.0
Zn (% desorbed)	33.8	22.8	64.7

content, respectively, and could not be related to the amount of MnO₂ associated with the cells. The amount of Zn released from the cell surface was however substantial, especially from cells with a large amount of extracellular Mn-oxide (65 % of the total cellular zinc content) (Table 1). Thus zinc seemed to adsorb on the MnO₂ associated with the cell surface.

Phytoplankton from different lakes

The total dissolved manganese concentration varied between 2×10^{-9} M and 6.5×10^{-7} M among the four lakes examined here (Fig. 3). Free Mn²⁺ is assumed to be approximately the same as the total dissolved manganese concentration because of the weak tendency of Mn to form organic complexes (Irving-Williams-order). Total cellular manganese content of the lake phytoplankton varied between 2×10^{-6} mol Mn (g algae)⁻¹ and 9.6×10^{-5} mol Mn (g algae)⁻¹. The intracellular content (defined as in the culture algae as the content after extraction with ascorbate) varied between 6×10^{-7} and 7×10^{-6} mol Mn (g algae)⁻¹ (Fig. 3). 92 % of the total cellular manganese was extracellularly bound as Mn oxides at the highest dissolved Mn concentration.

Discussion

Manganese uptake and algal growth

The results presented here indicate a limitation of the growth of *Scenedesmus subspicatus* at low Mn²⁺ concentrations, below $[Mn^{2+}] \approx 1 \times 10^{-9}$ M. Inhibition of growth at high Mn concentrations corresponded to cells with high extracellular amounts of Mn oxides, which may influence the nutrient uptake system. The Mn concentration given here for growth limiting conditions indicates an upper limit, because as stated above, it corresponds to the initial Mn²⁺ concentration in the culture medium, which decreased during growth of the algae. The discrepancy between results obtained at different EDTA concentrations may be linked to this effect. For example, we calculated the decrease of the free $[Mn^{2+}]$ during algal growth at the lowest free $[Mn^{2+}]$ of 2×10^{-10} M (EDTA = 10^{-5} M) and 2×10^{-11} M (EDTA = 10^{-4} M) (Fig. 1). In both experiments the free $[Mn^{2+}]$ was lowered by 90 %. The uptake of Mn²⁺ chang-

ed the concentration of $[Mn^{2+}]$ in the medium during the growth experiments at high algal biomass, whereas at low biomass the effect of the Mn uptake was insignificant. The calculated differences in $[Mn^{2+}]$ can however not provide a quantitative explanation of the observed differences in cellular content. The Mn content in the growth limited algae (around 1×10^{-7} mol Mn (g algae) $^{-1}$) may correspond to minimal requirements of these algae for Mn. This Mn content corresponds to a ratio of Mn:C of $2.5 \mu\text{mol (mol)}^{-1}$, using an average C content of *Scenedesmus* of $0.04 \text{ mol (g algae)}^{-1}$ (Knauer 1996). This Mn:C ratio is comparable to those observed in marine phytoplankton cells under limiting conditions, namely $6.7 \mu\text{mol (mol)}^{-1}$ for *Thalassiosira weissflogii* (Morel et al. 1991), and about $3 \mu\text{mol (mol)}^{-1}$ for *Thalassiosira pseudonana* at low competing concentrations of Zn and Cd (Sunda and Huntsman 1996).

The intracellular manganese contents measured in phytoplankton from the various lakes were higher than these minimum values, in agreement with the higher Mn concentrations available in these lakes. The intracellular Mn contents in the lake algae are comparable to those measured in *Scenedesmus*, considering the uncertainties in the dependence on $[Mn^{2+}]$ and the wide variety of algae species in the natural samples.

Surface-bound Mn(III/IV) oxides

A large fraction of the total cellular manganese content in *S. subspicatus* is bound to the cell surface as Mn(III/IV) oxides, at Mn^{2+} concentrations higher than about 1×10^{-9} M (Fig. 4).

A rather rapid oxidation of Mn(II) by *S. subspicatus* takes thus place, even at relatively low pH. This oxidation process has to be examined in view of the known effects of different factors on the oxidation kinetics of manganese. The oxidation rate of Mn(II), for the autocatalytic process, is given by a rate law of the form (Morgan 1967):

$$-d \text{ Mn (II)}/dt = (k_1 + k_2[MnO_x])[OH]^{-2} [O_2] [Mn^{2+}] \quad (1)$$

The oxidation rate is thus strongly dependent on pH and on the presence of Mn oxides. Algae may influence the oxidation kinetics of Mn by elevating the pH by CO_2 uptake, either in the immediate vicinity of the cells or in the bulk solution. The initial formation of a small amount of Mn oxide may then increase the rate by autocatalysis. Furthermore, it is also possible that Mn oxidation is induced by enzymatic catalysis (Price and Morel 1990). An example of indirect Mn(II) oxidation by algal photosynthetic activity has been reported by Richardson et al. (1988), who found that pure cultures of *Chlorella* sp. could catalyze oxidation of soluble Mn(II) to particulate extracellular manganese oxides under high pH values (> 9) and in presence of dense populations that could generate microenvironments with higher pH values.

The culture alga *S. subspicatus* is an unicellular floating alga without a mucous membrane and is therefore not capable of forming aggregates. In this case, the pH effect near the cells is estimated based on the diffusion of CO_2 and the C uptake

rate, according to the calculations derived by Gavis and Ferguson (1975) and Richardson and Stolzenbach (1995). Assuming a diffusion model to a spherical cell, the difference Δc between the bulk CO_2 concentration and the concentration at the cells surface is given by:

$$\Delta c = Q/4 \pi r D \quad (2)$$

where Q = CO_2 uptake rate ($\text{mol C s}^{-1} \text{ cell}^{-1}$)

r = radius of the cell (cm)

D = diffusion coefficient ($\text{cm}^2 \text{ s}^{-1}$)

and Δc is in mol cm^{-3} .

For *S. subspicatus* Q is estimated from the average C content ($0.04 \text{ mol C (g algae)}^{-1}$), the growth rate $\mu = 1 \text{ d}^{-1}$, and with 3×10^{10} algae cells (g dry weight^{-1}) to: $Q = 1.5 \times 10^{-17} \text{ mol C s}^{-1} \text{ cell}^{-1}$. The radius of the cells is about $2\text{--}2.5 \times 10^{-4} \text{ cm}$. The diffusion coefficient for CO_2 is $D = 2 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$. The concentration difference is thus calculated to $\Delta c \approx 3 \times 10^{-7} \text{ mol C dm}^{-3}$. This concentration difference should only have a negligible effect on the pH in the immediate vicinity of the cell, as long as the total C content is not depleted in the cultures. This result is in agreement with the arguments given by Richardson and Stolzenbach (1995) for the effect of cell size on Mn oxidation. The Mn oxidation by *S. subspicatus* can therefore not be easily explained by a pH effect.

In the autocatalytic rate law given above (eq. 1), $k_2 = 1\text{--}5 \times 10^{18} \text{ M}^{-4} \text{ d}^{-1}$ (Morgan 1967, Brewer 1975, Emerson et al. 1979). At the end of a culture experiment at high Mn concentration, about $1 \times 10^{-4} \text{ mol Mn (g algae)}^{-1}$ and $0.02\text{--}0.03 \text{ g algae dm}^{-3}$ are present. It is thus estimated:

$$k_2[\text{OH}]^{-2}[\text{O}_2][\text{MnO}_x] = 6 \times 10^{-4} - 3 \times 10^{-3} (\text{d}^{-1}), \text{ pH } 8$$

$$\text{and} = 6 \times 10^{-6} - 3 \times 10^{-5} (\text{d}^{-1}), \text{ pH } 7$$

Using these values, oxidation rates at a concentration of $[\text{Mn}^{2+}] = 1 \times 10^{-5} \text{ M}$ are estimated to $3 \times 10^{-7} - 1.5 \times 10^{-6} \text{ mol (g algae day)}^{-1}$ at pH 8. These values are thus lower than those observed in the experiments. The Mn oxidation by *S. subspicatus* can not be fully accounted for by the autocatalytic effects. These results may therefore indicate that a directly catalyzed Mn(II) oxidation takes place on the surface of *S. subspicatus*. This process may either involve an enzymatic catalysis, or the oxidation may be induced by a favorable coordination of Mn(II) on the cell surface. Mn(II) bound to oxygen donor atoms, e.g. to OH-groups at the surfaces of oxides, or to oxygen functional groups of ligands is oxidized faster than Mn^{2+} in solution (Wehrli 1990). Coordination of Mn(II) to the algal surface may catalyze the oxidation. Furthermore, the initially formed Mn(IV) oxide may be more reactive than assumed above for the autocatalytic rate.

The results on the phytoplankton from different lakes also indicate that Mn oxides are associated with phytoplankton, and therefore that Mn oxidation takes place during phytoplankton growth. The Mn content of the lake algae was in agreement with the range in the culture algae (Fig. 3), within the limits given under different

conditions. In the cases of the eutrophic lakes Greifen and Sempach, high pH may occur during photosynthesis, and account for a catalysis of Mn oxidation. In the case of Lake Orta with the highest concentration of surface bound Mn oxides, pH is only 7.3, and other catalytic effects have to be important.

Our results are consistent with those of Sunda et al. (1985), who observed no surface bound Mn in marine algae, grown at low free Mn^{2+} ion concentrations of $10^{-8.6}$, by desorption with NTA or with ascorbate. At similar manganese concentrations, no oxidation on the cell surface of *S. subspicatus* occurred in our experiments. Nakajima et al. (1979) also demonstrated in their manganese uptake experiments with living and scalded cells of *Chlorella* sp. that less than 5% of the total cellular Mn was adsorbed but most of the manganese could be eluted with an EDTA solution from the scalded cells at a Mn_T concentration of 1.85×10^{-7} M. Manganese might adsorbed to algal surfaces as Mn^{2+} , and than be taken up or be oxidized by living cells only. Stauber and Florence (1985) showed in experiments with the marine diatom *Nitzschia closterium* that the main fraction of the total manganese was located on the cell surface at Mn_T concentrations in the medium of 0.76×10^{-7} M and 7.64×10^{-7} M. Algal cells were washed with HNO_3 or ascorbic acid, and they interpreted their results that most of the extracellular manganese was present as $\text{Mn}(\text{OH})_2$ and $\text{Mn}(\text{OH})_3$ and not as colloidal MnO_2 . Studies by Richardson et al. (1988) and Richardson and Stolzenbach (1995) described Mn oxidation in a rather qualitative than quantitative way; no extracellular Mn content was measured neither calculations of Mn oxidation rates were done.

Studies in natural waters have clearly shown that the oxidation rates of Mn are much faster than the rates predicted by abiotic surface catalysis of Mn(II) oxidation and are most likely bacterially mediated at oxic-anoxic boundaries (Nealson et al. 1988, Emerson et al. 1979, Tipping 1984, Diem and Stumm 1984, Mason 1995). Mn cycling in the epilimnion of lakes is however poorly known, where algae may play an important role in the Mn(II) uptake and oxidation. Our results for the Mn oxidation catalyzed by *Scenedesmus* are comparable to Mn(II) oxidation rates of Metallogenium-like morphotypes obtained in-situ in Lake Washington (Maki et al. 1987), but are 100 times lower than Mn(II) oxidation rates estimated from Tipping et al. (1984) at the oxic-anoxic boundary in freshwater lakes. These results indicate thus that Mn oxidation on algal surfaces could be a significant mechanism in lakes with relatively high Mn concentrations, for example due to mixing with water from reducing zones.

Conclusions

The results presented here indicate that the cellular content in *Scenedesmus subspicatus* is dominated by intracellular uptake at low Mn concentrations, where the Mn requirements under growth limiting conditions are similar to those of marine algae. At higher Mn concentrations, Mn oxidation takes place on the cell surfaces of *Scenedesmus subspicatus*, although the algal cells are of small size and are not likely to build up a large pH gradient at their surface.

In addition to the well studied microbial Mn oxidation at oxic/anoxic boundaries, phytoplankton may have a great impact on the manganese cycle in freshwater,

by transforming manganese from the soluble Mn(II) state to intracellularly bound Mn and to particulate Mn(III/IV) oxides.

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