Uptake kinetics of $^{99}$Tc in common duckweed

Jasper Hattink *, Jeroen J.M. de Goeij, Hubert Th. Wolterbeek

Interfaculty Reactor Institute, Delft University of Technology, Mekelweg 15, 2629 JB Delft, The Netherlands

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Abstract

The uptake of the nuclear waste product technetium-99 was studied in common duckweed ($Lemna minor$). In addition to measurements, a model involving two compartments in duckweed with different chemical forms of technetium was derived. The model was tested by chemical speciation, i.e. differentiating between reduced Tc-compounds and $\text{Tc}^{\text{VII}}\text{O}_4^-$. The $\text{TcO}_4^-$ concentrations measured were in good agreement with those predicted by the model. Two processes determine technetium uptake: (1) transport of $\text{Tc}^{\text{VII}}\text{O}_4^-$ across the cell membrane, and (2) reduction of $\text{Tc}^{\text{VII}}$. The $\text{TcO}_4^-$ concentration in duckweed reaches a steady state within 2 h while reduced Tc-compounds are stored, as a result of absence of release or re-oxidation processes. Bioaccumulation kinetic properties were derived by varying $^{99}$Tc concentration, temperature, nutrient concentrations, and light intensity. The reduction of technetium in duckweed was highly correlated with light intensity and temperature. At $25^\circ \text{C}$ the maximum reduction rate was observed at light intensities above $200 \text{ mmol m}^{-2} \text{s}^{-1}$ while half of the maximum transformation rate was reached at $41 \text{ mmol m}^{-2} \text{s}^{-1}$. Transport of $\text{TcO}_4^-$ over the cell membrane requires about $9.4 \text{ kJ mol}^{-1}$, indicating an active transport mechanism. However, this mechanism behaved as first-order kinetics instead of Michaelis–Menten kinetics between $1 \times 10^{-14}$ and $2.5 \times 10^{-5} \text{ mol l}^{-1} \text{TcO}_4^-$. Tc uptake could not be inhibited by $10^{-3} \text{ mol l}^{-1}$ nitrate, phosphate, sulphate or chloride. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Bioaccumulation; $Lemna$; Light-dependent uptake; Radionuclides; Technetium; Uptake kinetics

1. Introduction

Radionuclides are released into the environment from nuclear activities (both civil and military) and natural processes of ore weathering and cosmic radionuclide production (Santschi and Honeyman, 1989; Lieser, 1995). Relevant to man-made global contamination are the long-lived radionuclides, such as $^{14}$C, $^{137}$Cs, $^{90}$Sr, $^{99}$Tc, $^{239}$,$^{240}$Pu, $^{241}$,$^{243}$Am, and $^{237}$Np, which are partly released as low-level radioactive waste into surface waters (Aarkrog, 1986; Macaskie, 1991). $^{99}$Tc is considered as one of the most mobile among these radionuclides. Low-level waste contains approximately 10% $^{99}$Tc, which makes it an abundant long-lived radionuclide (half-life $2.13 \times 10^5$ years) among the fission products (Lieser, 1993). In the last decade, the West European reprocessing plants released about 600 TBq into the surface water (Masson et al., 1995; Leonard et al., 1997).
while the world-wide diagnostic use of the metastable $^{99m}$Tc results in unknown diffuse sources (Baptista et al., 1982; Holm, 1993). In aerobic surface waters, technetium is present as the oxyanion pertechnetate, $\text{TcO}_4^-$ (Lieser, 1993).

Radioecological research of Tc has mainly focused on the terrestrial environment, considering accumulation in agricultural crops and soil adsorption (Yoshihara, 1996). However, little is known about the biogeochemical behaviour of Tc in surface waters, where most of the Tc is discharged (MAFF, 1997). Measured concentration factors for aquatic macrophytes range from $10^2$ to $10^3$ kg$^{-1}$ on a fresh weight basis (Blaylock and Frank, 1982; Blaylock et al., 1986; MAFF, 1997; Hattink and Wolterbeek, 1999) which is one order of magnitude higher than concentration factors measured in terrestrial vegetation (Hoffman Jr, 1981; Green and Wilkins, 1995). Since aquatic plants can reach high biomass, they might significantly influence the environmental distribution of Tc. Therefore, we studied some effects of the interaction of Tc with aquatic plants in more detail.

The uptake of Tc in terrestrial higher plants can be summarised by root uptake of $\text{TcO}_4^-$, possible reduction of Tc in the roots, xylem transport of $\text{TcO}_4^-$ to leaves, photoreduction of Tc in chloroplast, followed by complexation with proteins, cysteine and glutathione (Lembrechts and Desmet, 1989; Krijger et al., 1999a,b). These complexes are rather persistent and not redistributed over the biomass (Dehut et al., 1989; Vandecasteele et al., 1989; Krijger et al., 1999a). Accumulation of Tc is strongly reduced in fertilised soils (Baptista et al., 1982; Echevarria et al., 1998), which is assigned to the analogy of $\text{TcO}_4^-$ with nitrate (Van Loon, 1986; Krijger, 1999). $\text{TcO}_4^-$ is the only chemical form of Tc known to be taken up by higher plants (Van Loon, 1986; Sheppard and Evenden, 1991; Sekine et al., 1993).

Aquatic plants differ in nutrient uptake since they can use both foliar and root uptake (Denny, 1987). Both pathways may be used in the Tc accumulation pathway. In sediments, Tc precipitates as hydroxides, oxides, and sulfides, and complexes with humic acids as a result of the reducing circumstances (Lieser and Bauscher, 1987; Sekine et al., 1993; Aarkrog et al., 1997). Sheppard and Evenden (1991) showed that these complexes cannot be taken up by roots of aquatic macrophytes. Foliar absorption of $\text{TcO}_4^-$ should therefore be considered as the only entrance for Tc in aquatic plants. Furthermore, $\text{TcO}_4^-$ can directly diffuse to the chloroplast rather than via intermediate xylem transport. This might result in a faster uptake and reduction. In this study we focus on the accumulation processes and kinetics only. Effects of eutrophication and salinity, redistribution and release of accumulated Tc-forms, and growth will be elaborated in forthcoming articles.

The hypothesis tested is that accumulation of Tc is dominated by two processes (1) transport across the cell membrane and (2) photo reduction of Tc in the chloroplast (Fig. 1). To describe the uptake a mathematical model was derived, which was validated by additional chemical separation of $\text{TcO}_4^-$ and reduced technetium. It was anticipated that the processes are regulated by enzyme kinetics, rather than passive diffusion and chemical reduction. Therefore, $^{99}$Tc concentration, temperature, and light intensity are varied to derive kinetic properties of the underlying processes. Furthermore, uptake of Tc is studied in both presence and absence of major nutrients, to test the hypothesis of analogy with nutrients. Lemnaceae depend on their frond uptake only for nutrient requirements, and are therefore used as a model for the foliar absorption route of aquatic macrophytes. The roots of duckweed do not have a transport function as is the case for (terrestrial) plants (Landolt and Kandeler, 1987).

![Fig. 1. Schematic representation of the bioaccumulation model.](image-url)
2. The bioaccumulation model

Fig. 1 shows the (chemical) two-compartment bioaccumulation model. Three main processes are accounted for: uptake of $\text{TcO}_4^-$, release of $\text{TcO}_4^-$, and reduction of $\text{Tc}$. As a start we assume first-order rate processes, although this is not a priori correct, since many biological processes are regulated by Michaelis–Menten kinetics. By defining two phases in the uptake curve we are able to treat the uptake with (pseudo) first-order rate constants. Furthermore, dilution of the technetium concentration in duckweed as a result of increasing biomass (≈ 6% growth) and decrease of the $\text{TcO}_4^-$ concentration in the medium (< 0.1%) for the duration of the experiment (5 h) are neglected. Another important point in the model is that it only accounts for chemical species; spatial distribution of the $\text{Tc}$-species (such as a distribution over the vacuole, cytoplasm, etc.) is not incorporated. This is discussed in more detail in Section 5. The overall rates of $\text{TcO}_4^-$ transport into duckweed and formation of reduced complexes respectively, are:

$$ \frac{d[\text{TcO}_4^\text{duckweed}]}{dt} = k_1[\text{TcO}_4^\text{solution} - (k_2 + k_3)[\text{TcO}_4^\text{duckweed}] \tag{1} $$

$$ \frac{d[\text{TcX}^\text{duckweed}]}{dt} = k_3[\text{TcO}_4^\text{duckweed}] \tag{2} $$

where $k_1$ is the influx rate constant (l kg$^{-1}$ h$^{-1}$), $k_2$ the efflux rate constant (h$^{-1}$), and $k_3$ the reduction rate constant (h$^{-1}$), $t$ the time (h), $[\text{TcO}_4^\text{duckweed}]$ the $\text{TcO}_4^-$ concentration in duckweed (mol kg$^{-1}$ fresh wt.), $[\text{TcX}^\text{duckweed}]$ the concentration of reduced $\text{Tc}$ compounds in duckweed (mol kg$^{-1}$ fresh wt.), and $[\text{TcO}_4^\text{solution}]$ the $\text{TcO}_4^-$ concentration in the nutrient solution (mol l$^{-1}$).

More details on the derivation of these differential equations can be found in the article by Krijger et al. (1999a). Solving the Laplace transformations of Eqs. (1) and (2), and summing both equations, the total $\text{Tc}$ concentration in duckweed $[\text{Tc}]_{\text{duckweed}}$ as a function of time is:

$$ [\text{Tc}]_{\text{duckweed}}(t) = \frac{k_1 k_3 [\text{TcO}_4^\text{solution}]}{(k_2 + k_3)^2} (1 - e^{-(k_2 + k_3)t}) $$

$$ + \frac{k_1 k_3 [\text{TcO}_4^\text{solution}]}{k_2 + k_3} t \tag{3} $$

The first right-hand part denotes the balance between uptake and release of $\text{TcO}_4^-$, the second part the reduction of $\text{TcO}_4^-$ and accumulation. The efflux and reduction (pseudo) first-order rate constants might be strongly influenced by possible Michaelis–Menten kinetics, since the $\text{TcO}_4^-$ concentration in duckweed rises from zero to a certain steady state level. Comparing Michaelis–Menten kinetics with first-order rate processes:

$$ v = \frac{V_{\text{max}}}{K_m + [\text{TcO}_4^-]} \approx k[\text{TcO}_4^-] \tag{4} $$

in which $v$ is the reaction rate (mol kg$^{-1}$ h$^{-1}$), $V_{\text{max}}$ the maximum reaction rate (mol kg$^{-1}$ h$^{-1}$), $K_m$ the Michaelis constant (mol l$^{-1}$) and $[\text{TcO}_4^-]$ the $\text{TcO}_4^-$ concentration (mol kg$^{-1}$), $k$ the rate equation (h$^{-1}$) analogue of the (pseudo) first-order rate constants applied in Eqs. (1)–(3). This rate equation is roughly inversely proportional to the $\text{TcO}_4^-$ concentration:

$$ k = \frac{V_{\text{max}}}{K_m + [\text{TcO}_4^-]} \tag{5} $$

If the $\text{TcO}_4^-$ concentration is constant, the rate equation can be treated as first-order rate constant, and in turn, the accumulation can be described in terms of (pseudo) first-order rate constants. Therefore, two phases in the accumulation curve (Eq. (3)) can be considered. The first phase represents the uptake rate at the beginning, where efflux and reduction are negligible, while the $\text{TcO}_4^-$ concentration in the nutrient solution remains practically constant:

$$ v_{\text{uptake}} = k_1 t [\text{TcO}_4^-]_{\text{solution}} \tag{6} $$

The efflux and reduction might be treated as first-order, when the $\text{TcO}_4^-$ concentration in duckweed reaches a steady state, i.e. the second phase (Eqs. (4) and (5)):
\[
\frac{d[\text{TcO}_4^-]_{\text{duckweed}}}{dt} = 0
\]

\[\Rightarrow [\text{TcO}_4^-]_{\text{duckweed}}(t) = \frac{k_1}{k_2 + k_3} [\text{TcO}_4^-]_{\text{solution}} \quad (7)\]

From here, the accumulation of [Tc] total in duckweed follows a linear function of time:

\[ [\text{Tc}]_{\text{duckweed}}(t) = \frac{k_1 k_2}{(k_2 + k_3)^2} [\text{TcO}_4^-]_{\text{solution}} + \frac{k_1 k_3 [\text{TcO}_4^-]_{\text{solution}}}{k_2 + k_3} t \quad (8) \]

Eqs. (6) and (8) were used to fit the accumulation curves (see Section 3).

3. Material and methods

3.1. Reagents

All reagents were of p.a. quality and obtained from Sigma–Aldrich (Bornem, Belgium), Merck (Darmstadt, Germany), or Baker (Deventer, Holland). Demineralised or milli-Q water (Millipore, Milford, MA) was used throughout the experiments.

3.2. Duckweed culture

A strain of *L. minor* L. (common duckweed), which was a friendly gift of Jenner (Jenner and Janssen-Mommen, 1993) was grown in 150 ml modified Gorham solution in a climate room at 25°C and a light intensity of 120 μmol photons m⁻² s⁻¹ (Phillips PLL 83) during a 16-h light, 8-h dark period (Hughes et al., 1958; Jenner and Janssen-Mommen, 1993). Every 2 weeks, the culture was replaced by fresh nutrient solution; typical doubling time of duckweed under these conditions was 2.6 days.

3.3. Uptake experiments

Before starting the experiments, about 0.5 g duckweed (~375 fronds) was placed on 150 ml fresh nutrient solution at the experimental settings for 4–6 h. After this period, the nutrient solution was spiked with Na⁹⁹mTcO₄⁻, corresponding to 4 × 10⁻¹² mol Tc; final concentration 2.6 × 10⁻¹¹ mol l⁻¹) and, if needed, additional ⁹⁹Tc. For concentrations below the 10⁻¹² mol l⁻¹ the Mo/Tc generator was eluted twice a day, the last eluate contained a lower mass of ⁹⁹Tc, which was calculated using the decay formula for ⁹⁹Mo and ⁹⁹mTc. Uptake experiments were carried out by sampling 0.05–0.15 g fresh wt duckweed from a population (40–65% surface covering) at subsequent time points during 5 h. Two beakers were used for one uptake curve. For an accurate determination of the influx constant $k_1$, 3–4 samples were taken between 10 and 30 min. For the determination of the reduction rate, $V_{\text{red}}$, 4–8 samples were taken between 1.5–5 h. The reduction rate is defined as (see Eq. (2) and Eq. (7)):

\[ V_{\text{red}} = \frac{k_1 k_3}{k_2 + k_3} \]

Samples were spin dried for 10 min, weighed and placed in counting vials for γ ray measurements. Several accumulation curves were measured by varying [TcO₄⁻] solution, temperature, and light intensity.

3.4. Efflux

Samples were incubated and treated as described in Section 3.3. After incubation, samples were rinsed for at least 15 min in 150 ml Tc-free nutrient solution, followed by spin drying. It was assumed that only TcO₄⁻ was released and that the remaining Tc contained mainly TcX. This was validated in additional efflux experiments in which the Tc species were monitored (data not shown). The data obtained provided information on the build up of TcX.

3.5. Chemical speciation

Chemical speciation experiments were carried out to validate the assumption of chemical compartments, and to check some of the calculated model parameters. Duplicate samples were prepared by incubating about 0.5 g fresh duckweed at the desired experimental settings. After incubation, duckweed was spin dried to remove adherent
water, weighed and homogenised. Chemical species were separated as described elsewhere by high performance liquid chromatography (HPLC) on an Alltech MF-Plus (Metal-Free) HEMA-SEC BIO 1000 size-exclusion column with $^{95m}$TcO$_4^-$ as internal standard to correct for possible artefacts. A $8.3 \times 10^{-3}$ mol l$^{-1}$ N-(2-hydroxyethyl)-piperazine-N'-ethanesulfonic acid (Hepes) buffer (pH 7.0) at a flow rate of 1 ml min$^{-1}$ was used. In this way, two species were detected: TcO$_4^-$ and reduced Tc-compounds. A further separation of the reduced Tc-compound as described in Krijger et al. (1999a) was not carried out. For more details and retention times of the different Tc-species, see Harms et al. (1996a,b, 1999).

3.6. Competition experiments

Both in excess ($10^{-3}$ mol l$^{-1}$) of nitrate, phosphate, sulphate, and chloride as well as in their absence, duckweed was incubated in a solution of $2.6 \times 10^{-11}$ mol l$^{-1}$ Tc and of $8.3 \times 10^{-3}$ mol l$^{-1}$ calcium acetate solution (pH 7.0) for 1 h. Calcium acetate was chosen to maintain equal ionic strengths in all solutions. Nitrate was supplied in the form of Ca(NO$_3$)$_2$, KNO$_3$, or Mg(NO$_3$)$_2$; phosphate as KH$_2$PO$_4$ or NaH$_2$PO$_4$; sulphate as K$_2$SO$_4$, MgSO$_4$, or Na$_2$SO$_4$; and chloride as CaCl$_2$, MgCl$_2$, or NaCl. Samples were spin dried for 10 min, weighed and placed in counting vials for $\gamma$ ray measurements.

3.7. Radionuclides and detection

$^{99}$Tc ($\beta$-emitter, $E_{\text{max}} = 292$ keV, half-life 2.1 $\times$ 10$^5$ years) was obtained from Amersham (Buckinghamshire, UK) as KTCO$_4$ in 1 M NH$_4$OH; $^{95m}$Tc ($\gamma$-emitter of mainly 204 keV (66%) and 835 keV (28%) half-life: 60 days) was obtained from Los Alamos National Laboratory (Los Alamos, NM) as NH$_4$TcO$_4$ in 1 M NH$_4$OH; $^{99m}$Tc ($\gamma$-emitter of 141 keV, half-life 6.0 h) was obtained from a $^{99}$Mo/$^{99m}$Tc generator (Malinckrodt, Petten, The Netherlands). $^{99m}$Tc and $^{95m}$Tc were measured with a Wallac (Wallay Oy, Turku, Finland) 1480 automatic 3$^\circ$ $\gamma$ counter, using a well type Na(Tl)I scintillator. Energy windows ($^{99m}$Tc 104–162 keV, $^{95m}$Tc 163–240 keV) were chosen for optimal detection and possible dual label counting, data were corrected for spill over, background and Compton radiation automatically (WALLAC, 1995). $^{99}$Tc in the nutrient solution was measured with a Packard liquid scintillation counter (LSC) in Ultima Gold™ (Packard Instruments, Groningen, The Netherlands), using appropriate correction for quenching. Energy windows were set on 5–290 keV, and the counting efficiency under these conditions was 95%.

3.8. Data analysis

The decrease of Tc concentration in the medium as a result of Tc uptake was negligible (< 0.1%). Linear regression was performed in Quattro Pro for Windows version 1.0 (Borland International) using the build in linear regression function, extended with an estimation of the S.E. for the intercept. The reduction rate was obtained directly from the fitted slope from Eq. (8) to the data sampled from 1 h on, the influx from Eq. (6), using the data sampled within the first 30 min. Flux constants were calculated using Eqs. (6) and (8), or Eqs. (7) and (8) if the TcO$_4^-$ concentration in duckweed was measured. The efflux rate $V_{\text{efflux}}$, was calculated by multiplying the efflux rate constant by the calculated or measured equilibrium level of TcO$_4^-$, (Eq.(7)):

$$V_{\text{efflux}} = \frac{k_1}{k_2 + k_3} [\text{TcO}_4^-]_{\text{solution}}$$

S.E. were calculated using the Gaussian error propagation rules.

4. Results

4.1. Test of the model

Fig. 2 shows the uptake of TcO$_4^-$ by duckweed over 5 h; the solid curve presents the results of the two-compartmental model, which is fitted to the experimental data of accumulation of total Tc (solid squares). Clearly, two compartments can be distinguished: a fast compartment representing the TcO$_4^-$, and a ‘sink’ compartment representing reduced Tc-compounds. The TcO$_4^-$ concentration
in duckweed reaches a steady state as a result of efflux and reduction. Hereafter, the formation rate of reduced Tc-compounds will become constant. Fig. 2B focuses on the formation of reduced compounds. The open symbols represent experimental values. Additional measurements of the TcO$_4^-$ concentration (open circles) and concentration of reduced Tc-forms concentration in duckweed are in good agreement with the model. These points were not used to fit the model.

4.2. Kinetics of TcO$_4^-$ accumulation

Fig. 3 shows Van’t Hoff plots for all fluxes at $10^{-14}$–$10^{-5}$ mol $l^{-1}$ TcO$_4^-$ concentrations in the nutrient solution; additional measurements of influx only were carried out till $10^{-2.6}$ mol $l^{-1}$ TcO$_4^-$. The slope of the influx graph is $1.01 \pm 0.02$, indicating a first-order process, with a rate constant ($k_1$) of $0.151 \pm 0.004$ l kg$^{-1}$ h$^{-1}$. Calculated values for the (pseudo) first-order efflux rate constant ($k_2$) and the reduction rate constant ($k_3$) are $1.58 \pm 0.09$ and $0.65 \pm 0.06$ h$^{-1}$, respectively. Data for efflux and reduction above $10^{-5}$ mol $l^{-1}$ TcO$_4^-$ were not collected.

4.3. Temperature dependence

Fig. 4 shows the temperature dependency of the rate constants between 5 and 35°C. Both influx and efflux rate constants show a linear relationship with temperature and with $Q_{10}$-values of about 1.5 and 1.3, respectively (Fig. 4A,B). The reduction rate constant shows a typical parabolic dependency, characteristic for enzymatic processes. Fig. 5 gives the Arrhenius plot for the TcO$_4^-$ equilibrium concentration in duckweed.

4.4. Light dependence

Fig. 6 shows the influence of light on the fluxes. Fig. 6A–C show influx, efflux and reduction rates, respectively. The influx is independent of light intensity. Both efflux and reduction rates show a correlation with light intensity. With low light intensities the efflux increases, while the reduction rate constant shows a strong positive dependency on light intensity. An empirical saturation model could be fitted to the reduction rates with a maximum transformation rate of $(2.1 \pm 0.2) \times 10^{-12}$ mol kg$^{-1}$ fresh wt h$^{-1}$, and $K_i'$ of $40.7 \pm 4.3$ $\mu$mol photons $s^{-1} m^{-2}$ (light intensity when half of the maximum transformation rate is reached).
4.5. Competition studies

Table 1 presents the results of the competition study. The accumulation of Tc was not inhibited by $10^{-3}$ mol l$^{-1}$ nitrate, chloride, phosphate, or sulphate. Higher concentrations of nitrate or chloride (up to $35 \times 10^{-3}$ mol l$^{-1}$) also could not inhibit the Tc accumulation (data not shown).

$$\frac{k_1}{k_2 + k_3} \times \left[ \text{TeO}_4^- \right]_{\text{nutrient solution}}$$

where $k_1$, $k_2$, and $k_3$ are influx, efflux, and reduction rate constants, respectively. Lines are drawn through the points with slope equal to one.
High calcium concentrations were applied to avoid electrostatic effects which might mask the competitive effect. Electrostatic effects were observed in studies without calcium acetate, and will be elaborated on in a forthcoming article.

5. Discussion

5.1. Bioaccumulation model

The degree of fit ($R^2 = 0.964$) and the similarity between the chemical speciation analysis and model indicate that the assumptions of negligible growth, (pseudo) first-order rate constants at $2.6 \times 10^{-11}$ mol $\text{L}^{-1} \text{TcO}_4^-$, and absence of re-oxidation or release of reduced Tc-species are valid under the given circumstances (Fig. 1). This indicates that accumulation models derived for terrestrial plants can be applied to aquatic plants (Van Loon et al., 1989), possibly with minor modifications of exclusion of root uptake and subsequent xylem transport of $\text{TcO}_4^-$ (Krijger et al., 1999a).

These models, including the proposed model in Fig. 1, exclude surface adsorption and do not
incorporate spatial distribution of Tc over the cell organelles. In the cell wall, positively charged groups might be present due to amino-groups. However, adsorption of TcO$_4^-$, analogue to other anions, at these groups is negligible.

Fig. 2 strongly supports our hypothesis that the accumulation is ruled by processes of uptake, efflux, and reduction. However, this chemical distribution will not exclude a spatial distribution over the vacuole and other cell organelles. Efflux experiments with whole tomato plants by Krijger (1999) showed most TcO$_4^-$ to be present in the vacuole. Krijger hypothesised that this TcO$_4^-$ fraction was not available for reduction. Consequently, the reduction rates calculated in this study may be underestimated compared to the actual reduction rates. Autoradiographic studies by Woodard-Blankenship et al. (1995) showed that Tc was accumulated in the grana of the chloroplast, probably the place where Tc is reduced. Lembrechts and Desmet (1989) showed that reduction of Tc could be induced by light in chloroplast suspensions. Most likely, the reduced Tc-forms are associated with the chloroplasts and thus might match the chemical distribution.

5.2. Uptake of TcO$_4^-$

The influx, which determines the magnitude of bioaccumulation, is here defined as the amount of TcO$_4^-$ transported from the bulk solution into the cytoplasm and possible cell organelles such as the vacuole. The influx thus comprises transport across the unstirred water layer, cell wall, plasmalemma, and possible cell organelle membranes, respectively. In the following discussion only the transport over the plasmalemma is considered, assuming this as the rate determine step without any correction for cell wall effects.

The minimum energy required to transport TcO$_4^-$ across the cell membrane can be obtained using the Nernst and Ussing-Teorell equations and the cell membrane potential (Nobel, 1983):

$$\mu^i_{\text{TcO}_4^-} - \mu^o_{\text{TcO}_4^-} = zF(E_n - E_m)$$  (11)

with $\mu^i_{\text{TcO}_4^-}$ and $\mu^o_{\text{TcO}_4^-}$ the chemical potential of TcO$_4^-$ inside and outside the cell, respectively, $z$ the charge of TcO$_4^-$, $F$ the Faraday constant, and $E_n$ and $E_m$ Nernst potential for TcO$_4^-$ and cell membrane potential, respectively. $E_n$ is defined as:

$$E_n = \frac{RT}{zF} \ln \left( \frac{[\text{TcO}_4^-]_{\text{solution}}}{[\text{TcO}_4^-]_{\text{duckweed}}} \right)$$  (12)

with $T$ the temperature and $R$ the gas constant. In calculations, the chemical activity is replaced by concentration, which is justified, since low concentrations are used. It can be derived that the energy to transport TcO$_4^-$ across the membrane requires $10–18$ kJ mol$^{-1}$. The associated conditions are a cell membrane potential of between $-180$ and $-220$ mV (characteristic for Lemnaceae; Landolt and Kandeler, 1987), a temperature of 25°C and a measured equilibrium ratio of TcO$_4^-$ of the nutrient solution and duckweed of 4 kg l$^{-1}$. This energy might also be derived from Fig. 5 by combining Eqs. (11) and (12) and rewriting as:
Fig. 6. Effect of light intensity on the transformation of TcO$_4^-$ into reduced Tc-forms at a Tc nutrient solution concentration of 2.6 x 10$^{-11}$ mol l$^{-1}$. Data are expressed on a fresh weight basis. Symbols: data (□), using the influx to calculate the model parameters, (○) using the TcO$_4^-$ equilibrium concentration to calculate the model parameters, dotted lines drawn by hand (A) or fits using an empirical saturation model (B, C); error bars present (propagated) S.E. The reduction rate constant was light-dependent. Maximum reduction rate constant was calculated as 0.67 ± 0.09 h$^{-1}$, half of the maximum reduction rate constant was reached at a light intensity of 71 ± 23 μmol photons s$^{-1}$ m$^{-2}$.

\[
\ln \left( \frac{[\text{TcO}_4^-]_{\text{duckweed}}}{[\text{TcO}_4^-]_{\text{solution}}} \right) = - \frac{\left( \mu^4 - \mu^0 \right)}{R} + \frac{zF \Delta m}{R} \times \frac{1}{T} \tag{13}
\]

The slope is estimated as 1305 ± 203 K from which an energy of 6.5–10.4 kJ mol$^{-1}$ can be calculated for the transport of TcO$_4^-$ across the cell membrane.

Both calculations result in energies indicating an active uptake mechanism responsible for TcO$_4^-$ uptake, which confirms the observations that Tc uptake is inhibited if plants are treated with chemical inhibitors of the respiratory chain (Cataldo et al., 1983). However, the theoretical energies (10–18 kJ mol$^{-1}$) are comparable, but higher than the
experimentally determined ones of 6.5–10.4 kJ mol$^{-1}$ (from Fig. 5). This difference might be explained by a lower in situ Nernst energy ($E_n$) than is expected from the measured equilibrium concentration of TcO$_4^-$ in duckweed (compare Eq. (1) and the slope of Eq. (13)). A lower Nernst energy can be established by inorganic complexation of TcO$_4^-$ or storage of TcO$_4^-$ in the vacuole. Inorganic complexation of TcO$_4^-$ is most likely established by potassium, since a relatively high complexation constant ($\log K = 0.91$) is reported (Shvedov and Kotegov, 1963). In the nutrient solution (potassium concentration: 0.001 mol l$^{-1}$), almost all Tc is in the TcO$_4^-$ form. Based on the cell membrane potential, cytosolic potassium concentrations range from 1.1 to 5.3 mol l$^{-1}$. Such high cytosolic potassium concentrations are not very likely, although these concentrations are high enough to decrease the free TcO$_4^-$ concentration to such an extent as to reduce the transport energy to 0.25 and 12 kJ mol$^{-1}$, comparable with energies of 6.5–10.4 kJ mol$^{-1}$ derived from the Van’t Hoff plot.

From Eqs. (12) and (13), and the experimental energies from Fig. 5 TcO$_4^-$ equilibrium ratios can be calculated as 79–81 kg l$^{-1}$. This corresponds to 4.9–5.1% of the measured TcO$_4^-$ concentration, and the remaining fraction might be stored in the vacuole. These values are comparable to pool size ratios of cytosol for TcO$_4^-$ of 7–12% reported by Krijger (1999) for tomato roots. These calculations suggest that pertechnetate is accumulated in the vacuole. The energy needed for TcO$_4^-$ transport across the cell membrane is probably derived from exchanges with OH$^-$ or co-transport with H$^+$, and not derived from photosynthesis, since the influx rate does not change by placing duckweed in the dark (Fig. 6A) (Nobel, 1983).

In literature it is stated that the uptake of TcO$_4^-$ involves a common transport process as nitrate (Van Loon, 1986; Krijger, 1999). However, inhibition of the TcO$_4^-$ uptake could not be established by a 10$^9$-fold excess of nitrate concentration. Other anions (sulphate, phosphate, or chloride; see Table 1) also could not inhibit the Tc uptake. It is possible that Tc uptake in L. minor follows another route than competing anions or uses low-affinity carriers, characterised by high saturation values, as suggested by Krijger (1999). Some evidence may be extracted from the Van’t Hoff plot (Fig. 3A). The slope of the line equals 1, and even at 10$^{-2.6}$ mol TcO$_4^-$ l$^{-1}$ no deviation is observed. In general, enzymatic reactions are characterised by a Michaelis constant of 10$^{-2}$–

<table>
<thead>
<tr>
<th>Anion</th>
<th>Salt added</th>
<th>CF (l kg$^{-1}$)</th>
<th>Percentage of control</th>
<th>Mean (± S.D.)</th>
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<tr>
<td>Control</td>
<td>0.26</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Nitrate</td>
<td>Ca(NO$_3$)$_2$</td>
<td>0.29</td>
<td>112</td>
<td>99 (± 14) (n = 3)</td>
</tr>
<tr>
<td></td>
<td>KNO$_3$</td>
<td>0.27</td>
<td>104</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mg(NO$_3$)$_2$</td>
<td>0.21</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>Sulphate</td>
<td>MgSO$_4$</td>
<td>0.34</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td></td>
<td>K$_2$SO$_4$</td>
<td>0.29</td>
<td>112</td>
<td>110 (± 16) (n = 3)</td>
</tr>
<tr>
<td></td>
<td>Na$_2$SO$_4$</td>
<td>0.23</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td>Chloride</td>
<td>CaCl$_2$</td>
<td>0.24</td>
<td>92</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MgCl$_2$</td>
<td>0.23</td>
<td>87</td>
<td>96 (± 9) (n = 4)</td>
</tr>
<tr>
<td></td>
<td>KCl</td>
<td>0.25</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NaCl</td>
<td>0.29</td>
<td>111</td>
<td></td>
</tr>
<tr>
<td>Phosphate</td>
<td>KH$_2$PO$_4$</td>
<td>0.26</td>
<td>98</td>
<td>94 (± 5) (n = 2)</td>
</tr>
<tr>
<td></td>
<td>NaH$_2$PO$_4$</td>
<td>0.23</td>
<td>89</td>
<td></td>
</tr>
</tbody>
</table>

* The control is the uptake from the calcium acetate solution without other anions.
10⁻⁵ mol l⁻¹ (Mohr and Schopfer, 1994). Using Michaelis constants of 10⁻²–10⁻³ mol l⁻¹, Michaelis–Menten kinetics can be described theoretically by first-order kinetics within the concentration range used.

5.3. Efflux of TcO₄⁻

Fig. 3B shows that the efflux is proportional with the TcO₄⁻ concentration in duckweed over a wide range of TcO₄⁻ concentrations (between 10⁻¹⁴ and 10⁻⁵ mol kg⁻¹). This might imply a (pseudo) first-order mechanism responsible for the efflux of TcO₄⁻. The low Q₁₀-value of 1.3 and the linear relationship between efflux and temperature for the process till 25°C (Fig. 4B) indicates a physico-chemical rather than an enzymatic process is involved.

Fig. 6B suggests a light-dependent efflux. To understand this behaviour the definition of the efflux should be considered in more detail. The efflux is given by (see also Eq (10))

\[ v_{\text{efflux}} = k_2[TcO_4^-]_{\text{duckweed}} \]  

(14)

Both the efflux rate constant, \( k_2 \), and the TcO₄⁻ concentration in duckweed, \([TcO_4^-]_{\text{duckweed}}\), could be a function of light intensity. Direct determination of the TcO₄⁻ concentration in duckweed shows that the steady state TcO₄⁻ concentration in duckweed decreases with increasing light intensity (data not shown). The steady state TcO₄⁻ concentration in duckweed is a function of the influx, efflux and reduction rate constant; defined by (recalling Eq. (7)):

\[ [TcO_4^-]_{\text{duckweed}} = \frac{k_1}{k_2 + k_3}[TcO_4^-]_{\text{solution}} \]  

(15)

Fig. 6A clearly shows that the influx is independent of the light intensity. To obtain decreasing TcO₄⁻ concentrations in duckweed with increasing light intensities, the denominator, i.e. \( k_2 + k_3 \), should be an increasing function of the light intensity. The study of Lembrechts and Desmet (1989) suggests that the reduction process is initiated by light. The reduction rate, which is the product of the reduction rate constant \( k_3 \) and the TcO₄⁻ concentration in duckweed, increases with increasing light intensities (Fig. 6C). Taking the decreasing TcO₄⁻ concentration in duckweed into account, the reduction rate constant should be an increasing function of the light intensity, \( \phi_{\text{photon}} \).

Calculated reduction rate constants show that this function can be approached by a general saturation model:

\[ k_3(\phi_{\text{photon}}) = \frac{k_{3,\text{max}}\phi_{\text{photon}}}{K_L + \phi_{\text{photon}}} \]  

(16)

characterised by a maximum reduction rate constant \( k_{3,\text{max}} \) of 0.67 ± 0.09 h⁻¹ and a \( K_L \), the light intensity where half of the \( k_{3,\text{max}} \) is reached, of 71 ± 23 μmol photons m⁻² s⁻¹. A more detailed discussion of the reduction is given in the following paragraph. Eqs. (13) and (14) were used to describe the efflux (dotted line, Fig. 6B). An efflux rate constant, \( k_2 \), of 0.50 ± 0.03 was fitted to the efflux data. This value is not significantly different from the average of the calculated \( k_2 \)-values of 0.82 ± 0.50.

5.4. Reduction of Tc

Reduction of Tc(VII) might occur in the chloroplast following reduction routes used by nitrate and sulfate suggested by Lembrechts and Desmet (1989) or at the cell membrane level suggested by Bonotto et al. (1984), following the transmembrane reductase routes used by redox sensitive metals such as Fe and Cu (Marschner, 1995). Fig. 2B shows that the accumulation of reduced Tc compounds increases following an S-shaped curve. This indicates that the TcO₄⁻ in the cell controls the reduction. If the reduction should occur at the cell membrane, accumulation of reduced compounds should follow a linear curvature from the beginning of the experiment.

Fig. 6C and Fig. 4C show that reduction of Tc depends on light intensity and temperature, respectively. The saturation of the reduction rate is comparable to the saturation of the photosystem of Lemna, between 300 and 600 μmol m⁻² s⁻¹ (Landolt and Kandeler, 1987). The photoreduction of Tc probably occurs in chloroplasts, using electrons derived from the photosynthetic electron transport system and it is associated with some enzymatic processes (Lembrechts and Desmet, 1989). Curvature of Fig. 6C and the parabolic
shape of Fig. 4C may be explained by the correlation between photon flux and production rate of reducing agents, such as ferrodoxine (FD) or nicotinamide adenine dinucleotide phosphate (NADPH), and associated enzymatic processes. These experiments confirm the accumulation of reducing agents, such as ferrodoxine (FD) or nicotinamide adenine dinucleotide phosphate (NADPH), and associated enzymatic processes. These experiments confirm the accumulation mechanism as proposed for terrestrial higher plants, i.e. an uptake of TcO$_4^-$ followed by reduction.

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