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The removal of uranium from mining waste water using algal/microbial biomass

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Abstract

We describe a three step process for the removal of uranium (U) from dilute waste waters. Step one involves the sequestration of U on, in, and around aquatic plants such as algae. Cell wall ligands efficiently remove U(VI) from waste water. Growing algae continuously renew the cellular surface area. Step 2 is the removal of U-algal particulates from the water column to the sediments. Step 3 involves reducing U(VI) to U(IV) and transforming the ions into stable precipitates in the sediments. The algal cells provide organic carbon and other nutrients to heterotrophic microbial consortia to maintain the low E_H, within which the U is transformed.

Among the microorganisms, algae are of predominant interest for the ecological engineer because of their ability to sequester U and because some algae can live under many extreme environments, often in abundance. Algae grow in a wide spectrum of water qualities, from alkaline environments (Chara, Nitella) to acidic mine drainage waste waters (Mougeotia, Ulothrix). If they could be induced to grow in waste waters, they would provide a simple, long-term means to remove U and other radionuclides from U mining effluents.

This paper reviews the literature on algal and microbial adsorption, reduction, and transformation of U in waste streams, wetlands, lakes and oceans.

Keywords: Uranium; Remediation; Bioremediation; Ecological engineering; Environmental chemistry

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1. Introduction

Uranium is a ubiquitous element. It is the heaviest element occurring in nature in weighable amounts. Despite its high atomic number, it is by no means a rare element. Its relative abundance in the earth crust compares to silver, gold and the light rare earth elements and it is more abundant than tin, mercury and lead. Its naturally-occurring isotopes have masses of 238, 235 and 234. All isotopes are radioactive. The average U concentration in the earth's crust is about 3 mg kg^{-1}. Concentrations of U in geological materials are highest in continental-type rocks. Uranium forms more than 160 mineral species and accounts for 5% of all known minerals.

Dissolved U is found in most natural waters at very low concentrations, but is of some concern in waters adjacent to U mining operations when concentrations increase to levels above 1 μg dm^{-3}. ^{238}U, the most common radionuclide, has a half-life of 4.5×10^9 years, which makes clean-up environmentally important (Meinrath et al., 1996). Currently, U removal from waste streams may be tackled in four ways: (i) direct chemical methods; (ii) electrochemical treatments; (iii) ion exchange and biosorption methods; or (iv) intracellular sequestration by growing plant, algal and microbial cells. Remediation by chemical and electrochemical treatment is expensive. For U leaching from tailings and underground workings, installations may have to be in place for thousands of years. Biosorption and sequestration by living plants provide an environmentally and economically favourable method for removing U from natural waters. With proper engineering and development, an ecological self-regulating low-maintenance solution can be implemented. To provide the scientific basis to the proposed bioremediation approach, this review concentrates on what is known about U and aquatic plants, especially the algae, which inhabit U waste waters.

A cost efficient means of U removal is through the enhancement of a natural, ecological, three step process. The three steps are: (i) association of the U with organic particles (plants/algae/microbes) in the water column; (ii) provision of conditions whereby organic particles sink onto and into organic sediments; and (iii) provision of low E_H (anaerobic) conditions in the sediments with metal-reducing microbial populations. The end result of this process is that oxidized U associates with particulates, settles, becomes reduced and is bio-mineralized in the sediments – effectively creating biogenic ores (Brierley and Brierley, 1981; Anderson et al., 1989a,b; Barton et al., 1996). The advantage of using living material in the initial step is that it continually grows, providing new organic material for sequestration. This is especially useful in flow-through systems, where the supply of U to be removed is continuous. The ecological engineer must provide the proper conditions, nutrients, and organisms to optimize this three step process.

Examples from the lichens, algae, fungi, and bacteria are given, as these organisms contribute particulate and living matter to the aquatic habitat. The most common and diverse group in the aquatic environment are the algae. These organisms come in a variety of sizes and shapes, and occur in some of the most extreme environments on earth (Brock et al., 1984). Algae can be divided into a number of groups, functionally, taxonomically, and ecologically. For example, phytoplankton are algae that float in the water column; periphyton are mixed

communities which are composed of algae with moss, fungi, and bacteria that grow on rocks, on macrophytic vascular plants and on sediment surfaces (Wetzel, 1983).

Some algae are more sensitive to pollutants than others. The most sensitive ones have been used in toxicity tests (Rojickova-Padrtova and Marsalek, 1999; Franklin et al., 2000). However, there are a number of algae that are widely tolerant to metals and U, and can sequester very high concentrations of metals. Some of the algae-microbial systems can sequester metals and U in concentrations far above the level in the surrounding water (e.g. Heide et al., 1973).

Several mechanisms have been described whereby U(VI) can be associated with algae: (i) adsorption to cell surfaces; (ii) adsorption to extracellular polysaccharides; (iii) uptake into cells and incorporation into vacuoles; (iv) incorporation into $CaCO_3$ (aragonite) structures associated with some marine and freshwater algae; and (v) precipitation of U on the cell surface or internally.

2. Geochemistry

U in nature does not occur in elemental form. In humid air, U corrodes rapidly forming yellow uranyl(VI) compounds, where the linear $[O = U = O]^{2+}$ unit forms the characteristic structural unit. In the presence of phosphate(V), silicate, arsenate(V) and vanadate(V) highly insoluble orthophosphate, orthosilicate, orthoarsenate and orthovanadate compounds are formed, respectively. If these compounds are not present in significant quantities in water, the chemistry of hexavalent U is governed by its interactions with water and carbonate.

A larger group of uranyl hydroxides, oxyhydrates and uranates are known. The affinity of hexavalent U to oxygen is extreme. There are very few oxygen-free U(VI) compounds. While U(V) has been reported from laboratory experiments, it is not stable enough to allow detailed investigation and, in any case, does not play an observable role in nature. U(IV) is hydrolyzed even at very low pH. Freshly prepared U(VI) hydroxides show much higher solubility compared to uraninite minerals (Meinrath, 1998a and references given therein).

In Fig. 1, a speciation diagram of U(VI) in a dilute electrolyte solution is shown as a function of pH in equilibrium with solid phase schoepite (synthetic). Calculations are based on the IAEA (International Atomic Energy Agency) data base (IAEA, 1992). The 90% uncertainty limits (dashed lines) are calculated from the probabilistic speciation code Ljungskile (Ekberg et al., 2003; Ödegaard-Jensen et al., 2004). The calculations show the dominance of hydrolysis species in the acidic range while carbonato species prevail in the neutral to alkaline range. The 90% confidence ranges indicate the limited capability of such calculations to assess the likely species composition of a solution due to measurement uncertainties of thermodynamic data (Ödegaard-Jensen et al., 2004). A larger number of hydrolysis species, partly with an oligomeric nature, have been identified. The 90% confidence limits calculated in Fig. 1 show that a prediction of the dominating species is almost impossible.

Fig. 2 summarizes the redox behaviour of U(VI) (Meinrath et al., 2003). Carbonato species are likely to govern the redox stability of the U(IV) and U(VI)

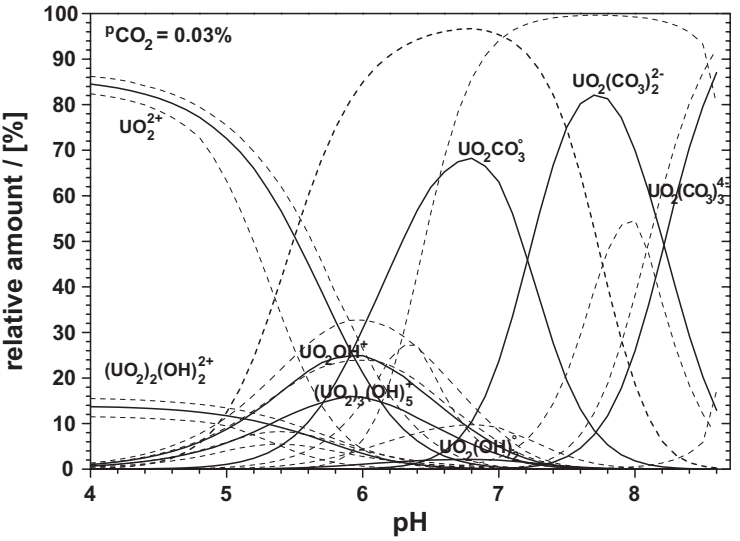

Fig. 1. Distribution of U(VI) aqueous complexes at 25 °C and ionic strength $\mu = 0.01$ M NaCl as a function of pH at atmospheric CO_2 partial pressure. Dashed lines give 90% confidence limits. The uncertainty limits have been calculated on the basis of data from IAEA (1992).

redox state. However, large uncertainties exist with regard to the nature of U(IV) species and the stability of U(V). Assuming $U(OH)_4^{\circ}$ as the prevailing U(IV) solution species (smallest stabilization of U(V)), the stability field 'a)' (cf. Fig. 2) is obtained for U(V). For this situation, the species below the stability limit of U(V) is $U(OH)_4^{\circ}$ exclusively. The formation of U(IV) carbonato species results in a strong stabilization of U(V). On the basis of the highest formation constants reported for U(IV) carbonato species, the combined fields 'a)' and 'b)' account for the stability field of U(V). Below the stability field of U(V), the respective U(IV) carbonato species are given in Fig. 2. Both situations, however, are in disagreement with experimental observations. Figs. 1 and 2 illustrate the large uncertainties that hamper the predictive power of thermodynamic simulations in U environmental chemistry. In assessing Fig. 2 it should be noted that the formation of U(IV) from U(VI) involves breaking two extremely stable U-O bonds. Hence, high overpotentials must apply, thus rendering redox state predictions on the basis of mere thermodynamic equilibrium calculations doubtful.

3. Stage 1: uranium sequestration

3.1. Adsorption

The observation that algae and aquatic macrophytes have a strong affinity for U (Scheminzky, 1959; Justyn and Stanek, 1974) led to the recognition that they could

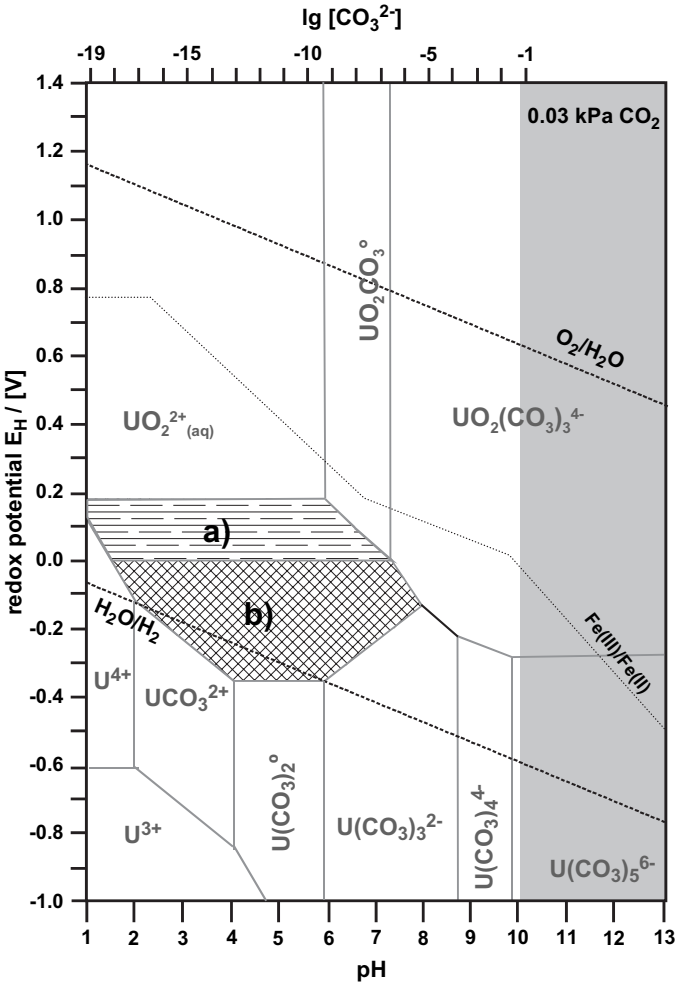

Fig. 2. E_H/pH diagram for uranium species at 25 °C and 1013 hPa in the U/H_2O/CO_2 system; $^pCO_2 = 30$ Pa. The field a) gives the calculated stability field of U(V) if the stable U(IV) species is $U(OH)_4°$, while both fields 'a)' and 'b)' give the calculated stability field of U(V) if the U(IV) carbonate species are prevailing solution species. In both cases, calculations are in disagreement with experimental observations. The stability range of water is limited by the oxidation and reduction boundaries, respectively. These limits are given as dashed lines denoted 'O_2/H_2O' and 'H_2O/H_2', respectively. These boundaries are pH-dependent. The line denoted 'Fe(III)/Fe(II)' gives the redox boundary between trivalent and divalent iron hydroxide species. It indicates that Fe(III) is capable of reoxidizing U(IV). The shaded area is physically not accessible because the carbonate content would exceed the limit of ionic strength.

provide a means of removing U from waste water. Heide et al. (1973) suggested that unicellular green algae in continuous culture could remove 95% of the dissolved U from seawater, with accumulation factors greater than 4000. Pribil and Marvan (1976) studied the U adsorption characteristics of another unicellular green alga with

similar results. Horikoshi et al. (1979, 1981) and Nakajima et al. (1979) confirmed the usefulness of algae as U adsorbents. Both cell wall structure and the U(VI) ion contribute to U adsorption by algae both in seawater and freshwater.

Biologically, metal ions are classified by their binding preferences, specifically whether they seek out O-, N-, or S-containing ligands. Pearson (1963) separated metal ions into hard acids (O-seeking) and soft acids (N- or S-seeking). Binding sites sought by hard acids on biological surfaces include carboxylate, carbonyl, alcohol, phosphate, and phosphodiester groups (ligands). Groups sought by soft acids include sulphide, disulphide, thioether, and amino groups (see Table 1).

Most plant, algal, or microbial materials found in natural waters have cell walls composed mainly of polysaccharides and carbohydrates, e.g. cellulose, xylan, and mannans (Lobban and Wynne, 1981; Myers et al., 1975; Neihof and Loeb, 1972), with a negative charge on their surfaces. The negatively charged groups attract cations such as Zn, Cu, Al and U (Barker et al., 1998; Marques et al., 1990). The polysaccharide backbone contains many other side groups, ligands such as amino, carboxyl or hydroxy, sulphide groups. These groups provide the cell wall with an overall negative charge. See Tables 1 and 2 for a list of ligands and their composition. Uranium can also be adsorbed to inorganic ligands. Zero valent iron immobilizes U(VI) (Farrell et al., 1999; Gu et al., 1998) by the formation of iron hydroxides (ferrihydrite, goethite) (Duff and Amrhein, 1996; Morrison et al., 1995; Wielinga et al., 1999, 2000; Noubactep et al., 2003).

Most metals that are adsorbed or complexed to negatively charged cell walls are bound to the negatively charged ligand groups on the surface. However, some groups carry a positive charge and are thus able to adsorb and complex anionic

Table 1
List of ligand groups and their pK_s (Hunt, 1986; Segel, 1976)

Ligand group	Location	pK_s
Carboxyl	Protein c-terminal	3.5–4
Carboxyl	Beta aspartic	4–5
Carboxyl	Gamma glutamic	4–5
Carboxyl	Uronic acid	3–4.4
Carboxyl	*N*-Acetylneuraminic	2.6
Carboxyl	Lactate	3.8
Sulphonic acid	Cysteic acid	1.3
Phosphate	Serine as ester	6.8, 2.0
Phosphate	Polyol mono ester	0.9–2.1
Phosphate	Polysaccharide diester	1.5, 6.0
Amino	Cytidine (pyrimidine)	4.11
Amino	Adenosine (purine)	3.45
Imidazole	Histidine	6–7
Hydroxyl	Tyrosine-phenolic	9.5–10.5
Hydroxyl	Saccharide-alcoholic	12.0–13.0
Sulphide	Cysteine	8.3
Amino	Protein N-terminal	7.5–8.0
Amino	Lysine	8.9, 10.5
Imino	Peptide	13

Table 2
Sorption capacities of uranium(VI) for different taxa (pH is pH at which measurements were taken)

Taxon	Model	Sorption capacity (mg U gdm^{-1})	Measured pH	Reference
Scenedesmus	Measured	75		Zhang et al. (1997)
Chlorella	Measured	15.6		Horikoshi et al. (1979)
Chlorella	Measured	28.5	(pH 3.5)	McCready and Lakshmanan (1986)
Sargassum	Langmuir	560	(pH 4.0)	Yang and Volesky (1999b)
Sargassum	Langmuir	330	(pH 3.2)	Yang and Volesky (1999b)
Sargassum	Langmuir	150	(pH 2.6)	Yang and Volesky (1999b)
Sargassum	Measured	524		Fourest and Volesky (1997)
Pseudomonas	Measured	96,000		Marques et al. (1990)
Pseudomonas	Measured	541	Lyophilized	Sar and D'Souza (2001)
Pseudomonas	Measured	410	Live at pH 5	Sar and D'Souza (2001)
Saccharomyces	Measured	571		Omar et al. (1996)
Saccharomyces	Measured	360		Riordan et al. (1997)
Saccharomyces	Measured	138		Volesky and May-Phillips (1995)
Kluyveromyces	Measured	180		Bustard et al. (1996)
Talaromyces	Langmuir	323	(pH 5)	Bengtsson et al. (1995)
Aspergillus	Langmuir	423		Bhainsa and D'Souza (1999)
Aspergillus	Measured	40	(pH 4–5)	Hafez et al. (1997)
Rhizopus	Measured	>180	(pH 4)	Bhainsa and D'Souza (1999)
Rhizopus arrhizus	Measured	42.3	(pH 3.5)	McCready and Lakshmanan (1986)
Penicillium spp.	Measured	20.3	(pH 3.5)	McCready and Lakshmanan (1986)
Peltigera	Measured	42	(pH 4–5)	Haas et al. (1998)
Cladonia	Measured	29		Haas (1998)
Umbilicaria	Measured	10		Haas (1998)

metal species. The mixture of these groups on a given cell surface will determine the number of cations and anions that are complexed. Since the chemical composition and cell size are reasonably constant for a given algal species, the number of ligand groups on an alga's surface should also be fixed. More than one ligand type can be found on a given cell surface, which results in the selectivity of each cation/ligand. Various functional groups have different affinities for binding of cations and anions and thus different stability constants. Thus, a spectrum of stability constants with changing cation/surface ligand ratios can be found in natural assemblages. For example, copper (Cu^{2+}) will tend to bind first to amino acid surface groups, then to carboxylic or hydroxy-carboxylic groups (Williams, 1981).

A plausible number of high affinity surface binding sites is about 10^7–10^8 per cell for a representative phytoplankter (Morel and Hudson, 1985). Thus, some algae can have a higher affinity for some cations than others. In its cation form, U can be 'adsorbed' onto most cell surfaces. Binding anionic, complexed species of U is more difficult, as there are fewer positively charged ligands. The number of ligand sites for U(VI) on the bacterium *Shewanella putrefaciens* (Gamma Proteobacteria) was calculated to be approximately 32 μmol carboxyl sites, 9 μmol phosphoryl sites and 38 μmol amine ligand sites per gram wet weight of bacteria. These values correspond

to 2.0×10^{19} carboxyl sites, 5.5×10^{18} phosphoryl sites, and 2.3×10^{19} amine sites per gram of bacteria (Haas et al., 2001).

The cell wall structure of a bacterium, *Mycobacterium smegmatis* (Actinobacteria), has been analyzed for its carboxyl group content by Andres et al. (1994). Electron microscopic analysis of the cell surfaces and separation of the arabinogalactan-peptidoglycans (AGP) from the cell matrix indicate that about 100 mg U could potentially be adsorbed per gram cell dry weight. AGP is composed primarily of polypeptides with carboxyl groups. Approximately 200 nmol of AGP per gram dry weight of bacterium was found to be present.

At given E_H, pH and chemical conditions, several U species may prevail (Fig. 1). The speciation of U in solution regulates the U ion concentration and influences the resulting complexation reaction. The concentration of ligand sites is then proportional to the organic carbon, phosphorus, and nitrogen content of a cell suspension. If surface sites with very high affinity for certain cations exist, cell surface sites may become saturated with those cations. If the U ions were in excess of the concentration of high affinity sites, the surface of the cells could carry a constant concentration of U with respect to the cell composition. If the cells are growing, the number of available surface sites increases; in other words, the rate at which U ions are removed from solution is a function of growth rate.

Dienemann et al. (2002) characterized the U content of algae growing in wetlands in U mine effluents. They describe the algae as efficient, temporary sinks for U. Bioadsorption of up to 300 mg of U per kg dry matter (mass) led the authors to believe that algae might be useful in the removal of U from the waste stream. However, they caution that changes in E_H/pH conditions can remove the U. If the chemical conditions change, i.e. by addition of dissolved ligands (e.g. EDTA) or pH change, bound U can be released. For example, if pH drops significantly, then H^+ concentrations, by definition, increase. Dropping pH implies release of U.

Some plant/microbe populations release extracellular polysaccharides as a by-product of metabolism. These extracellular polysaccharides also contain ligands which can bind metal ions (Rai et al., 1981). Microbes such as *Pseudomonas* (Gamma Proteobacteria), and *Citrobacter* (Omnibacteria) also produce large quantities of extracellular polysaccharides (Liu and Wu, 1993; Marques et al., 1990), which can complex U. Marques et al. (1990) studied the removal of U by *Pseudomonas* exopolysaccharides. They found the complexation to be affected by pH, but not temperature. Complexation was best approximated by Freundlich isotherms, suggesting sequestration being limited to a single layer.

Other dissolved ligands that bind U include the humic and fulvic substances found in peat bogs and acidic lakes (Titayeva, 1967; Halbach et al., 1980). Titayeva (1967) conducted both dynamic and static sorption tests on Ra and U on peat. U was sorbed better than Ra from weakly weak acid solutions (peat and water) under static conditions. Increasing the pH from 5 to 8 increased the adsorption of Ra, while the optimum pH for U was pH 6. Desorption tests using alkali extraction showed that Ra was mainly sorbed to the insoluble matrix, while most of the U desorbed with increasing alkali treatment, suggesting that it was associated with the humic substances.

The interaction of humic substances with U binding to algal cells was also studied by Szalay (1964). The adsorptive properties of humic acids probably relate to the carboxyl and hydroxyl groups associated with the breakdown of lignin. The carboxyl group, he notes, has a dissociation constant $pK_s \sim 5$. Hence the carboxyl group accepts U at about the optimum pH for U removal. Uranium is not reduced by interaction with humic acids, but rather is strongly adsorbed (Nash et al., 1981). Szalay (1964) likened humic acids to cation exchange columns, and found that Langmuir iso-pH curves described well the adsorption equilibrium of humic acids (see section on Modelling). The Langmuir equation is characterized by two constants representing a geochemical enrichment factor (GEF) and the adsorption capacity. The GEF is defined as:

$$\mathrm{GEF} = \frac{\text{conc. of U(VI) in peat}}{\text{conc. of U(VI) in water}} \tag{1}$$

GEF was measured at approximately 10,000.

Degens et al. (1979) describe the transformation of U emanating from an old underground mine into a shield lake. The U found in the sediments was associated almost completely with both phytoplankton debris and humic acids.

Living and dead cells behave in a similar fashion with respect to adsorption of U, provided the cell wall structure remains intact. Since the cell walls of dead algae/microbes and plants still contain negatively charged ligands and the overall charge of the cell wall is still negative, the dead plant material will, to some degree, adsorb positively charged U. This method has been the choice of those who would try to put adsorption into a more industrial process. Thus, several authors have reported on the use of dead biomass to adsorb cations from solution (Tsezos and Volesky, 1982; Volesky and May-Phillips, 1995; Greene et al., 1986).

The Langmuir isotherm (Langmuir, 1918) is a means to interpret hyperbolic adsorption data. It is basically the same equation used to describe Michaelis–Menten enzyme kinetics, and describes the adsorption of ions to a finite number of ligand sites in a single layer on the cell surface. Thus,

$$C_s = C_{s(sat)} K_L C_w / (1 + K_L C_w) \tag{2}$$

or in a linear form:

$$1/C_s = (1/C_w^*)(1/K_L) + (1/C_{s(sat)}) \tag{3}$$

where C_s is the concentration of uranyl on the algal surface (mg kg^{-1}), $C_{s(sat)}$ is the monolayer saturation concentration of uranyl on the algal surface (adsorption capacity: mg kg^{-1}), K_L is a constant related to the binding energy of uranyl to the algal surface (L mg^{-1}), and C_w is the concentration of uranyl in water (mg dm^{-3}).

There are certain assumptions inherent in using the Langmuir isotherm. First, the sorbent has a fixed number of sites. Second, all of the sites have equal binding enthalpies, independent of the extent of coverage. Third, maximum sorption is a monolayer on the substrate surface.

The Freundlich isotherm (Freundlich, 1926) is another common model that has no theoretical basis. The shape of the adsorption curve, however, depends on the constants K_d and N. The Freundlich equation can be linearized by plotting the ln of C_s against the ln of C_w. The slope of the ln/ln plot is $1/N$ and the ln K_d is the y intercept at ln $C_w = 1$.

In this case, K_d is proportional to the maximum adsorption constant of the Langmuir isotherm. Specifically, the Freundlich K_d represents the predicted amount of metal/actinide sorbed in milligrams per gram of sorbent at an equilibrium concentration of 1 mg dm^{-3} (Tien, 2002). It is an empirical parameter that is composed of three components, a binding constant, a term related to the number of adsorption sites, and solution composition. Solution composition is constant for any given water sample, so only the binding constant and the number of surface sites contribute to variation among K_ds. Binding energy decreases as the number of sites are filled, and so is a constant only at low sorbent concentrations. The number of surface sites will depend on the cell surface composition. The parameter N is a measure of the affinity between the sorbent and the surface. The forces between the surface layer and the sorbent are attractive if N is less than unity and repulsive if N is greater than unity (Ozer et al., 1999). Freundlich $1/N$ could be estimated based on the structure of the adsorbent cell surface and the density of carboxyl groups on that surface (Chang et al., 2000).

$$C_s = K_d C_w^{1/N} \tag{4}$$

$$\mathrm{Log}(C_s) = (1/N)\mathrm{Log}(C_w) + \mathrm{Log}(K_d) \tag{5}$$

where C_s is the concentration of uranyl on the algal surface (mg kg^{-1}), K_d is the constant related to the specific capacity of uranyl to algae surface, C_w is the concentration of uranyl in water (mg dm^{-3}) and N is a function of the intensity of adsorption.

However, speciation of hydroxo- and carboxylato species of U is highly pH-dependent. Small changes in pH may strongly affect the equilibrium (Meinrath, 1997, 1998b). Note here that competition with other cations can modify the binding of U to cell surfaces. If the chemical conditions change, such as an addition of other ligands to the water (e.g. EDTA) or a change in pH, bound U can be released.

3.2. Active intracellular uptake

Algae and other aquatic plants are known to actively pump metals across their cell membranes. This requires an energy source, and is therefore usually coupled to photosynthesis and temperature. Since large numbers of metals in the environment are essential nutrients, it is not surprising that many metals are actively pumped into algal cells. The problem arises when the external metal concentrations far exceed cell requirements. While U is not a required nutrient, it may mimic a metal that is required. Whatever the reason, U(VI) is taken up by some algal cells. Once inside the cell, U is concentrated into vacuoles and sometimes precipitated. For example, Pribil

and Marvan (1976) working with *Scenedesmus* (Chlorophyta) found that U accumulation occurred in two steps, a fast and a slow step. The second, slower step appeared to be temperature-dependent, and thus may have indicated some active uptake of U(VI). Mann and Fyfe (1984, 1985) found U(VI) crystals inside the algal cells of *Spirogyra* (Chlorophyta). Some authors looked for but could find no evidence of an active uptake in diatoms (Bacillariophyta; Goldberg et al., 1998) or *Chlorella* (Chlorophyta; Horikoshi et al., 1981).

Other organisms have also been shown to actively take up uranyl ions. For example, Golab et al. (1991) studied the biosorption of uranyl ions in *Streptomyces* (Actinobacteria) in competition with lead ions. Uranyl was found to be extracellular and intracellular, while the lead was found only extracellularly. *Peltigera* (Lichen) has been shown to take up U and, together with internal inorganic phosphate, to create uranium-phosphate crystals which are stored in the cellular mycobiont (not the alga) (Barker et al., 1998). Krueger et al. (1993) estimated that about 10% of the *Pseudomonas fluorescens* cells studied contained internal U above trace levels. They also noted that internally located U ultimately killed the cells.

3.3. Bio-precipitation

Another means of complexing metals and U with algae is through the process of bioprecipitation. This process usually occurs outside the cell, but it can occur inside as well. By setting up surface currents and changing E_H/pH conditions on the cell surface, algae can affect the geochemical conditions of the water around them, facilitating the precipitation of metals. For example, Kalin and Wheeler (1992) described a situation where algal mats altered the geochemical environment by fostering Zn carbonate precipitation in and around the algal mats.

Mann and Fyfe (1984) found a species of *Ankistrodesmus* (Chlorophyta) growing in effluent from a U mine in Elliot Lake, Canada. When electron microscope pictures of the alga were taken, cubic crystals of a U oxide were found decorating the surface of the cells. This alga, like others, probably behaves in a similar fashion to certain bacteria which are known to precipitate metals on their cell surfaces. The interaction of metal ions with bacterial surfaces is at least a two step phenomenon (Beveridge and Murray, 1980). The first event is a stoichiometric interaction between metal ion and reactive surface ligands on the cell walls. This event lowers the energy barrier for additional metal complexation and nucleates the precipitation of additional metal. As metal ions are added to the nucleation site, counter ions in solution dictate the chemical composition of the precipitate so that metal hydroxides, carbonates, sulphates, sulphides, phosphates, etc. can be formed. The eventual mineral phase is determined by the availability of metal, counter ion, and the chemical solution (pH, E_H, etc.). Over time, these hydrous precipitates dehydrate and bona fide crystalline mineral phases are developed (McLean and Beveridge, 1990). Basnakova et al. (1998) described similar uranyl phosphate crystals in *Citrobacter* (Omnibacteria).

In analyzing several species of tropical seaweeds (Chlorophyta, Phaeophyta, Rhodophyta) that incorporate calcium carbonate in their cell walls, Edington et al. (1970) noted that there was a very high correlation between the concentration of Ca

and U in red algae (Rhodophyta). The authors suggest that there are two mechanisms involved in the concentration of U. The first and primary mechanism is ion exchange or co-precipitation of the ion with the calcium carbonate matrix, and the second is a secondary mechanism that involves some form of complex formation with either the protein nitrogen or other component of the organic fraction. At given pH, pCO_2, and E_H of seawater, U is most likely to occur as the anionic species $UO_2(CO_3)_3^{4-}$ (Noubactep et al., 2003) (Fig. 2). Therefore, the U(VI) ion may not be readily available for the formation of organic complexes unless these are more stable than the $UO_2(CO_3)_3^{4-}$ complex. The structure of the U(VI) carbonato species is similar to those of analogous actinyl carbonate compounds (Meinrath, 1996). The U(VI) tricarbonato species is one of the most stable metal complexes in aqueous solution. It would readily be incorporated with calcium carbonate (or undergo anion exchange reaction) in those species of algae in which calcification occurs. In the human body, U is incorporated mainly in bone materials (Meinrath et al., 2003 and references given therein).

Phosphate can also form crystalline structures with U (Bloch, 1980). The U(VI) phosphates are very stable and have been used as a possible means to remove U from waste waters (Wright and Conca, 2002). Indirect evidence for such a relationship between U and phosphate is detailed by Abu-Hilal (1994), who noted that Red Sea corals, algae, and seagrasses collected from a phosphate-polluted site all had elevated U as compared with organisms from nearby sites with U at geogenic levels. A lichen, *Peltigera*, has been shown to take up U and together with internal inorganic phosphate to create U(VI) phosphate crystals which are stored in the cellular mycobiont (Barker et al., 1998). This formation of U(VI) phosphate crystals is widespread, although it is more likely to be found on the surface of cells (Thomas and Macaskie, 1998). *Citrobacter* (bacteria) precipitates U(VI) and other heavy metals on its cell surfaces as metal phosphates (Roig et al., 1997). Macaskie et al. (2000) describe the process whereby Citrobacter produces an overabundance of alkaline phosphatase, which in turn causes the cells to excrete phosphate, which provides the nucleus for the precipitation of U(VI) phosphate on cell surfaces.

3.4. Competition

In fresh water, algae and microbes live under conditions which can extend from pH 12 down to pH 1 (Brock et al., 1984). Most natural waters, however, show pH values between 6 and 8. Many studies with algae reported an optimum pH for U removal. Invariably, the pH was acidic to neutral. As pH climbed above neutral, the removal rate and absolute amounts removed dropped considerably. For example, for *Anacystis* (Cyanobacterium), the optimum pH range was between 3.0 and 5.0 for U removal (Liu and Wu, 1993). Yang and Volesky (1999b) used protonated *Sargassum* (Phaeophyta) to remove U. The optimum pH ranged between 2.5 and 4.0. In another example, the bioaccumulation of U by green algae was favoured in waters with higher calcium carbonate ratios, which had lower pH values than waters with higher calcium carbonate ratios (Duff et al., 1997a). *Scenedesmus* (Chlorophyta)

showed an accumulation optimum plateau between pH 5 and 8.5 (Zhang et al., 1997), or between 5.9 and 6.8 (Pribil and Marvan, 1976). Horikoshi et al. (1979) found a pH optimum for U uptake in *Chlorella regularis* between pH 6 and 7.

Not only are algae more likely to remove U under acidic conditions, but so also are lichens. Boileau et al. (1985) exposed U in the form of uranyl ions which had been pretreated to make them a cation, anion, or neutral to *Cladonia* (lichen). The maximum observable removal capacities were 49 μmol gdm^{-1} for positively charged uranyl species, 17 μmol gdm^{-1} for neutral uranyl species, and 1.6 μmol gdm^{-1} for negatively charged uranyl species. Haas et al. (1997) produced the same trends for *Peltigera* (lichen). The best pH for U accumulation is reported to be between 4 and 5. Since most plant cell walls are similar to algae and fungi, we can expect that under most conditions, U uptake/adsorption will be higher under acidic conditions. All of these acidic optima correspond to the fact that carboxyl, amino and phosphate ligands are probably the major complexing agent on/in the cell walls. These ligands have pK_s values in the pH range from 3 to 5.

Competition for sites on cell walls is strongly pH-dependent. H^+ ions behave just like any other cation. At pH values below 5, H^+ concentrations are high enough to compete with other metals in solution. At pH values below 2, H^+ outcompetes other metals and strips them from the ligands. Disregarding H^+ ions as competitors, researchers have looked at the competition of U(VI) with other metals. When in mixtures, U(VI) and other metals will compete based on molecular size and shape and the configuration of the ligand. For example, Zhang et al. (1997) grew *Scenedesmus* (Chlorophyta) in laboratory culture and then submitted it to a variety of U uptake experiments. Lithium, Na, K, and NH_4 did not affect U accumulation. However, the transition group of metals did compete with U(VI). Bhainsa and D'Souza (1999) ran a similar set of experiments with *Aspergillus* (ascomycota). Again, the optimum pH was 5, but no inhibitory effect from the addition of such ions as Fe, Ca, or Zn was observed. However, Al did interfere with U removal. Tsezos et al. (1997) also found that Al interfered with U biosorption in *Rhizopus* (Zytomycota). Liu and Wu (1993), working with *Anacystis nidulans* (Cyanobacteria), noted that of those metal ions tested, only Ca, Fe, and Zn had any appreciable effect on U accumulation. Iron was, by far, the most competitive ion, reducing U accumulation by 80%. The effect of various cations and anions on the uptake of U by *Chlorella regularis* (Chlorophyta) was examined by Nakajima et al. (1979). Uranium uptake was hindered by phosphate and carbonate ions and was not affected by cations (Na, K, Mg, Ca, Mn, Co, Ni, and Zn), nitrates, sulphates, and thiosulphates. Since the amount of U taken up decreased linearly with the amounts of carbonate added, the authors suggested that only the cation form of U(VI) was being complexed, in a manner similar to other metallic cations.

Information on experimental conditions of U 'uptake' studies, e.g. starting pH and total initial U concentration, is partly difficult to interpret. In some cases, starting conditions instead of steady-state conditions are specified in the text. In other cases, conditions must be read from figures. In Fig. 3, selected data from the references are summarized and compared to the experimental solubility curve of synthetic schoepite ($UO_3{\cdot}2H_2O$) in 0.1 M perchlorate medium (Meinrath et al., 1996;

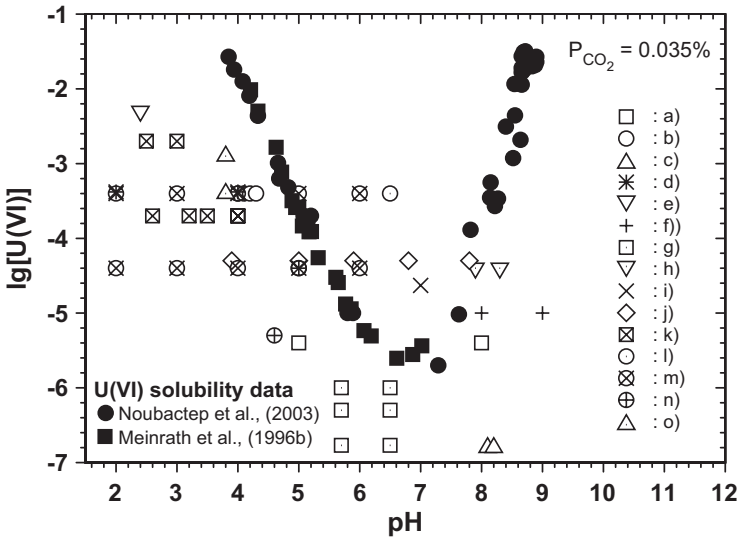

Fig. 3. Comparison literature data on experimental conditions for U uptake studies into cells with U(VI) solubilities at $I = 0.1$ M. Studies were mainly performed in low pH media with total U concentrations above 10^{-5} mol dm^{-3}. In some cases, however, near neutral media were studied where solubility limits of $UO_3{\cdot}2H_2O$ have been exceeded. (a) Nakajima et al. (1979); (b) Pilar Pons and Fuste (1993); (c) Mann and Fyfe (1984); (d) Tsezos (1983); (e) Hu et al. (1996); (f) Bender et al. (2000); (g) Franklin et al. (2000); (h) Duff et al. (1999); (i) Zhang et al. (1997); (j) Pribil and Marvan (1976); (k) Yang and Volesky (1999a,b); (l) Galun et al. (1984); (m) Guibal et al. (1992); (n) Nakajima and Sakaguchi (1986); (o) Treen-Sears et al. (1984). Liu and Wu (1993) reported trace levels of U below 10^{-10} mol dm^{-3}.

Noubactep et al., 2003). Schoepite is the most soluble U(VI) solid phase. Other solid phases will form only if they are less soluble than schoepite. It is shown that experimental solutions in most cases are below the saturation limit, while a few conditions are reported where precipitation phenomena may have spoiled the reported results. A comparison between theoretical speciation (Fig. 1) and the experimental conditions, however, indicates strongly varying solution conditions, and, hence, quite limited comparability of the reported studies. Nakajima et al. (1979), for example, added sodium carbonate to shift values between pH 4 and pH 9, whereby it remains unclear whether solutions could equilibrate with the atmosphere before the experiments or whether an excess in carbonate remained in solution. Tsezos (1983) applied high concentrations of uranyl nitrate ($UO_2(NO_3)_2{\cdot}2H_2O$) even though nitrate is complexing uranyl(VI). Franklin et al. (2000) worked with chloride solutions even though chloride may form radicals in U(VI) solutions due to electronic excitation of U(VI) by UV light.

4. Stage 2: sedimentation

The first stage in the biological removal of U is sequestration, and the previous section summarized several methods related to algae, fungi, and bacteria. The next

stage is to remove these 'biological sequestration units' from the water column. The primary means of sedimentation is simply death. As the cells die, they gradually sink to the bottom of the water column. After death, phytoplankton and bacteria can become aggregated into larger particulates, sinking faster. Algae can also be eaten by zooplankton and herbaceous fish. The resulting faecal pellets are quite dense and sink rapidly to the bottom, carrying the U with them (Fisher et al., 1987).

Sigg (1985) describes the dominant role of biogenic organic particles in binding heavy metals and transferring them into the sediments and shows these processes for fresh water lakes in Switzerland, as they are described in the ocean. This is attributed to the higher primary productivity in these lakes and the generation of more organic particulate matter which adsorbs the metals and carries them out of the water column.

Several researchers have studied U removal in nature at locations such as the Black Sea (Anderson et al., 1989b), the Pacific Ocean near Vancouver/Canada (Anderson et al., 1989a), and in a fjord off the coast of Norway (Swarzenski et al., 1999). All researchers suggest that natural U carbonates become associated with certain natural organic matter such as diatoms and that these diatoms, when dead, sink to the ocean floor, carrying U with them. All of the U found in ocean sediments appears to be bound to organic particulates in exactly the same ratios as is found in sedimenting phytoplankton. However, Sackett et al. (1973) indicate that known sinks account only for about 10% of the present-day river input of dissolved U.

Bow Lake, northwestern Ontario, contains 20 μg U kg^{-1} of water which represents a tenfold enrichment relative to other lakes from the Canadian Shield (Degens et al., 1979). Land-derived humic compounds and the plankton population (principally diatoms) jointly extracted the U from the water column; organic material collected by plankton had a U content of 200 mg kg^{-1}. The U was then transferred to the sediments where the concentration in the upper sediments was closer to 300 mg kg^{-1} sediment.

The role of phytoplankton in the accumulation and transfer of natural radionuclides (U, Th, and Ra) from the ocean surface to deep waters was assessed by Fisher et al. (1987) from analysis of a number of phytoplankton, zooplankton, sediment trap contents and deep water particles. They concluded that U and Ra concentration ratios were similar for phytoplankton, sediment trap material and deep water organic particles.

Koval'skiy and Vorotnitskaya (1965) investigated U concentrations in *Chara* (Chlorophyta) and in the mud beneath the *Chara* beds in a shallow Lake Issyk-Kul'. The U accumulated in the organic sediments via adsorption by *Chara*, then by other filamentous algae. The content of U in the lake sediments depended on the content of organic matter, as U was found mainly associated with algal debris. In the sediments of a stagnant, meromictic lake, Alberic et al. (2000) found U mostly associated with colloidal material (78%). About 40% was associated with fulvic and hydrophilic acids. Hence, as particulates break down under anoxic, anaerobic conditions, U is found to be still associated with the resultant colloidal materials and organic acids.

To study U removal from a seasonally dry lake, sediment and water samples were analyzed for total organic carbon (TOC), U, and other soil parameters (Duff et al.,

1997b). Elevated levels of U enter the lake from irrigation drainage. Sediment and waters were sampled to determine which factors control U solubility and sediment U concentrations. Sediment TOC was correlated with sediment U concentrations, suggesting that U is bound to organic matter. The source of TOC was most likely from algae deposition.

In a follow-up paper, Duff et al. (1999) noted that although the U had been deposited in the sediments via dead algae, the oxidation state of the U was still mostly hexavalent. Comparing the X-ray near-edge absorption data of the pond sediments with the laboratory-produced solids, they concluded that biosorption by algae and bacteria was the dominant mechanism depositing U in the sediments. Mixed oxidation state U solids were preferentially formed in the pond sediments and in the lab except under intense SO_4-reducing conditions.

5. Stage 3: reduction/transformation/biomineralization

Once the U-algal biomass reaches the sediment interface, it continues to decompose, releasing nutrients and colloidal-bound U. If the sediments are undisturbed, the material falls into the benthic boundary layer and remains in the sediments. As the redox conditions decrease with sediment depth, the U(VI) may be reduced to U(IV). The nutrients that sustain microbial populations in the anaerobic sediments come from natural organic matter (NOM) or algal particles. Algal biomass comes in contact with sediment primarily as decomposing material. Studies on the decomposition of algae suggest that about 60% of cellular material is mineralized, with the remaining 40% persisting as particulate material (Foree and McCarty, 1970; Ulen, 1978; Fallon and Brock, 1979; Duff et al., 1999).

The decomposition of algae in the sediments provides nutrients to sustain heterotrophic microbial communities which further degrade organic matter and provide dissolved organic material for anaerobic metal-reducing microbial consortia (Fyson et al., 1998). Algal mats decomposed more slowly in the presence of heavy metals, although U was a metal that least delayed decomposition (Disnar and Trichet, 1984).

The reduction of U(VI) to U(IV), as well as the subsequent chemical reactions and transformation to U sulphide, carbonate, or other biogenic ore requires strongly reducing conditions in the sediment. U(VI) reduces to U(IV) at low E_H (<200 mV) (Fig. 2). This usually requires an anaerobic organic sediment and an active consortium of bacteria, such as sulphate-reducing bacteria (SRB), as well as other microbes to support the consortium. Redox conditions change with proximity to organic sediments. Dissolved oxygen is reduced first, followed by denitrification, followed by UO_2CO_3 reduction to uraninite and sulphate reduction to sulphide. At each step in the redox ladder, reactions may be catalyzed by different microbial enzymes. There is a succession of microbial activity from denitrifiers, to metal reducers, to sulphate reducers. Denitrification must be complete before sulphate reducers are activated. It should be noted that the formation of U(IV) requires a considerable over-potential. The often-stated claim that reducing conditions

automatically involve the formation of poorly soluble U(IV) species has never been proven experimentally. In fact, the claim that U(IV) forms in the presence of zero-valent Fe has been questioned (Noubactep et al., 2003). Freshly formed U(IV) precipitates are quite soluble. The formation of an amorphous U(IV) oxide on bacteria surfaces has been reported (Abdelouas et al., 1998) with a solubility $\geq 6\times10^{-9}$ mol dm^{-3}.

In the absence of organic reducing sediments, U bearing particles are released back into the water column when the algae decompose. Brierley and Brierley (1981) grew algae (*Chara* and *Spirogyra*; Chlorophyta) in laboratory tanks with and without an organic sediment. The U added to the water was adsorbed by the algae and sank to the bottom of the tank when they died. The U did not reappear in the water column (e.g. it was reduced and sequestered in the sediments), but was resuspended without sediments. Algal material contributed to some extent to the retention of U in sediment. While *Chara* alone was unable to significantly affect U levels in the water, the presence of decaying algae resulted in the retention of U in the sediment.

In the oceans, U(VI) carbonate and associated organic matter are sedimented in both organic, anoxic sediments and more oxic sediments (Bloch, 1980). Klinkhammer and Palmer (1991) studied the pore water of various sediment cores and determined that U diffusing into anoxic sediments remained in the sediments and underwent diagenesis. The U in more oxic sediments was released back into the water column when the organic matter was degraded. Of the U reaching the sediments, an estimated 75% remained in the sediments while 20% was redissolved. Laboratory experiments with ocean sediments carried out by Cochran et al. (1986) also support this view. Anoxic sediments tend to retain U(IV), while more oxic sediments release U after the decomposition of associated organic matter. Kalin et al. (2002) observed that when flow conditions were altered in a drainage basin receiving effluents from a waste rock pile, the shallow portion of one of the lakes in the drainage basin became oxic. The U load did not decrease as was the case in the previous 8 years of the study. This decrease in removal capacity was attributed to U release from the uppermost portion of the sediment.

Barnes and Cochran (1993) have studied U reduction in situ in coastal sediments. The U reduction and subsequent precipitation (removal from pore water) was directly related to bacterially mediated sulphide reduction. The U was released back into the pore water when the sediments were bioturbed or the sulphate reduction was halted. Uranium release also occurred as Fe and Mn were reduced. The authors attribute this to the association of U with (hydr)oxides of Fe and possibly Mn in agreement with laboratory experiments using Fe corrosion products (Noubactep et al., 2003).

The reduction/transformation process has been studied in the laboratory using microbial mats (containing algae, fungi and bacteria) (Bender et al., 2000). The mats were immobilized in silica gel and put in a column. Groundwater containing U was added and passed through the column. Over 80% of the dissolved U(VI) was removed within 15 min of treatment. X-Ray near-edge structure spectroscopy showed that the sequestered U was reduced to U(IV) within 24 h. Low redox conditions were maintained by adding nutrients to the columns.

Francis et al. (1991) studied the reduction of U in sediment samples containing U processing waste. The sediments were amended with glucose and ammonium chloride. Sediments and water were put into serum bottles and left for 50 days. Microbial activity was demonstrated by gas production and by the reduction of U in the water/sediments. They concluded that the reduction of U(VI) was due to the activity of indigenous microbes under anaerobic conditions. While U(VI) was reduced and precipitated, Fe(III) and Mn(IV) were reduced and dissolved.

Microorganisms can reduce U directly by producing H_2S or H_2 in the course of other processes (abiotic reduction) or directly with enzymes (biotic reduction, such as *Geobacter* (Delta Proteobacteria) and these bacteria used U as an electron acceptor and H_2 or acetate as an electron donor to support growth, and tolerated U(VI) concentrations as high as 8 mM.

Lovley and Phillips (1992) used SRB to reduce U(VI). They also tested the effect of toxic metals on the growth of the SRB using *Desulfovibrio desulfuricans* (Delta Proteobacteria). A pure culture was added to a closed system with lactate and U(VI). Among the anions tested, sulphate, molybdate, and nitrate, and toxic metals, Cu, Zn, Co, and Mn, only Cu in high concentrations (>100 mM) inhibited the reactions. Different bacteria require or work better with different organic substrates. Thus, Ganesh et al. (1997) found that reduction of U(VI) required different substrates by *Desulfovibrio desulfuricans* and *Shewanella alga* (Delta and Gamma Proteobacteria, respectively). The authors suggested that the best bacterium for the job depended on the organic ligands available in the waste stream.

Lovley (1995) reviewed studies concerning microbial reduction of U(VI) and concluded that the process offered several advantages over currently applied technologies such as ion exchange and biosorption. The advantages included: (i) high removal rate of U per unit of biomass; (ii) reduction and removal of highly soluble U(VI) carbonate complexes; (iii) precipitation of a highly concentrated waste form (amorphous UO_2) that requires little storage space or that, alternatively, may have commercial value; (iv) the potential of treating mixed waste with organic contaminants as electron donors for the reduction of U(VI); and (v) the potential for in situ bioremediation of groundwater.

The precipitation of U requires suitable E_H/pH conditions in the sediments. It also requires a nucleus, usually one of the consortia bacteria (Beveridge et al., 1983). The precipitate formed can be uraninite (UO_2), identified by X-ray diffraction and transmission electron microscopy (Gorby and Lovley, 1992). Groudev et al. (2000) studied the transformation of U as it passed through a wetland. They identified and enumerated a number of both aerobic and anaerobic microbial consortia in the low redox sediments, and documented the presence of uraninite. They concluded that the microbial consortia and some biosorption was a viable means of U removal. The process of precipitation can be influenced by other metals such as Cu, Zn, Co, and Mn (Lovley and Phillips, 1992).

The geological record attests to the stability of U in deep anaerobic sediments. In Lake Baikal, U distribution in the sediments has been studied by Edgington et al. (1996). Based on ^{238}U and delta ^{18}O data, U has not been significantly redistributed within the Baikal sediments over at least the past 250,000 years.

Spirakis (1996) notes that U ore is often found in association with organic matter and that reduction of U from (VI) to U(IV) has long been considered the precipitation mechanism for many types of U deposits. Black shale deposits (Ranstad Shale of Sweden and Chatanooga and New Albany Shales of the USA) contain U associated with organic material and pyrite. He notes that pyrite is formed through the reduction of sulphate by organic matter and that both the adsorption and reduction of U by organic matter may have been involved in the enrichment of U in black shales.

Spirakis (1996) states that the development of roll-front U deposits is also linked to organic matter. The ore lies at the boundary between altered and unaltered rock; minerals and elements associated with uraninite in the ore include pyrite, Se, V, organic carbon, Be, Cu, As and Mo. With the exception of Be, the concentration of other elements is consistent with direct reduction or precipitation with sulphide mineral via sulphate reduction. The organic matter in association with the elements initially reduces the sulphate to sulphide and causes pyrite to form. Other U deposits could also be explained by an initial association of U with organic matter in sediments.

Landais (1996) also states that many sedimentary U deposits show close relationships between organic matter and U. According to Landais these relationships may be statistical, spatial or chemical. The chemical relationship is the result of two processes, complexation and reduction. Complexation is understood as the ionic exchange of U with carboxyl groups on humic acids, coals, and kerogens. The other process, reduction, occurs directly or indirectly under low redox conditions. Landais further states that the chemistry of the organic matter is very sensitive to the conditions of deposition and early diagenesis. Organic matter displays different characteristics depending on its origin, e.g. algal, planktonic, or geogenic.

The important final step in the described processes is the long-term immobilization of biologically reduced U. If the long-term development of a site would be considered merely from a view point of inorganic processes, the U, which is immobilized by reduction and/or mineralization, would easily become remobilized as soon as conditions change. The dashed line denoted 'Fe(III)/Fe(II)' in Fig. 2 gives the redox boundary of the Fe(III)/Fe(II) redox couple. Fe(III) can reoxidize U(IV). Several authors have outlined the relevance of biological processes for the development of stable biogenic ore deposits. In a study of 145 minerotrophic wetlands in the mountainous sub-alpine zone of the Rockies, Owen and Otton (1995) documented the vegetation type, and hydrological and topographical conditions of bogs and fens which accumulate U in sediments. Of the 145 bogs investigated, 67 showed U enrichment. In describing surficial U deposits in Canada, Culbert (1984) noted topographic similarities in Manitoba, British Columbia, the Yukon and Nova Scotia. They describe surficial 'young' deposits in semi-arid, alpine and permafrost terrain. Many more examples could be given. However, the deposit formation depends largely on site-specific conditions. The long-term treatment approach would require the creation of shallow basins where phytoplankton and periphytic algae would flourish, to create the pathway from the water to the sediment and fuel the biomineralization. A detailed discussion on the biomineralization of U is outside the

scope of this manuscript; the geological literature underscores the importance of detailed investigations of natural analogue sites.

6. Conclusions

Uranium (VI) appears in most natural waters as either a cation or complexed with carbonate (uranyl carbonate). As uranyl hydroxide, the molecule is a cation, and as uranyl carbonate, it is an anion. In cationic form, uranyl hydroxide is complexed to living plant, algal, fungal, and bacterial cell walls. It is actively taken up by some taxa but not metabolized by others. In some cells, it is precipitated on cell surfaces and in others internally. Some cells do not interact with the hydroxide at all.

The amount of uranyl ions complexed to cell walls depends on the number and density of the proper ligands in the cell walls. For uranyl, these ligands contain carboxyl groups, which have pK values between 2.6 and 5. This means that the optimum pH for uranyl complexation is between 2.6 and 5. This is borne out by numerous lab studies.

In the anionic form (as found mostly in high carbonate freshwaters and the ocean), uranyl ions are only partially complexed to plant, bacterial, algal, and fungal cell walls. The ligands present must have a positive charge. Although the ligands present usually must have a positive charge, in alkaline waters some uranyl ions are sequestered in calcium carbonate and calcium phosphate crystals common to the cell walls of some algae.

Algae do not more effectively sequester uranyl ions in their cell walls than other taxa. However, they are a diverse group of plants that can live, grow and reproduce in mining waste water and they are the most abundant and prolific taxa. Thus, understanding and control of the ecological requirements to promote and sustain growth are the underpinning of the treatment approach proposed for decommissioning U waste management areas. Algal cell walls can sequester uranyl ions whether the algae are living or dead because the cell wall structures remain relatively intact upon death. The uranyl ion can be released from these biomaterials by H^+ (e.g. high concentrations of acid (pH 2 or less)). There is, then, a potential for using algal biomass in industrial processes to remove uranyl from waste streams.

For the most part, the sequestration process can be modelled either by Freundlich or Langmuir isotherms, giving us some idea of the mechanisms involved in the complexation of uranyl ions, and in some cases, the maximum sorption capacity.

Once UO_2^{2+} ions become complexed to algal cell wall material, either whole cells, or detritus, the cells will sink to the sediments upon death. Uranyl ion can coordinate with almost any biological material, living or dead, and is transported through the water column to the sediments. This will occur more slowly in well oxygenated, turbulent waters, or faster in anoxic, still waters.

The carbon, nitrogen and phosphorus in the algal detritus are used as nutritional supplements for microbial consortia present on and in the sediments where uranyl reduction and transformation take place.

Upon reaching the sediments, particulates and bound U can either be buried into the sediments in anoxic areas, or be released and resuspended in more turbulent areas. In combination with a low E_H environment, sulphates, and minerotrophic microbial systems, the uranyl (VI) can be reduced to tetravalent U and immobilized as solid phases formed by chemical reactions with sulphates or other anions present in the sediments.

Many of the U ore bodies on the planet are associated with organic matter. Uranium ore diagenesis studied in many locations has been found to have begun with organic matter complexation, microbial reduction, and finally transformation to U and metal sulphides. The ecological approach proposed for the removal of U from waste water follows these natural steps.

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