Chapter 3

The Potential of the Photoautotroph Synechocystis for Metal Bioremediation

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Additional information is available at the end of the chapter

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Abstract

The photoautotrophic cyanobacterium *Synechocystis* PCC6803 has received much attention as a model photosynthetic cell factory for the production of a range of important biotech products. The biomass remaining from this activity may then have further utility in processes such as metal bioremediation. In addition *Synechocystis* being an inhabitant of many natural aquatic environments is seen as an environmentally friendly alternative to using chemical precipitation methodologies for metal remediation. *Synechocystis* produces a range of extracellular polysaccharide substances (EPS) that can undergo modification as a function of culture age and growth nutrients which have been implicated in metal biosorption. Many studies have demonstrated that high levels of charged groups present in EPS are important in forming polymeric matrices with metallic ions allowing their biosorption. Genetic studies has revealed genes involved in such metal binding indicating that EPS can be modified for potential enhancement of binding or modification of the types of metals bound. The utility of metal binding to live and dead biomass of *Synechocystis* has been demonstrated for a range of metals including Cr(VI), Cd(II), Cu(II), Pb(II), Sb, Ni(II), Mn(II), Mn(IV), As(III), As(V), Cs and Hg. The potential of using *Synechocystis* as a biosorption platform is discussed.

Keywords: *Synechocystis*, EPS, metal biosorption, metal binding, metal remediation

1. Introduction

Heavy metals because of their chemical nature cannot be biodegraded by microorganisms to non-toxic species and therefore build up in the environment. Many metals undergo a change in chemical state from one form to another but ultimately they accumulate in the environment and potentially enter the human food chain through uptake by plants or animals. Removal of metals by chemical technologies has been widely used but has proven expensive or inappropriate in the case of low level metal contamination. Thus attention has focussed on newer technologies such as metal biosorption as an alternative to chemical removal.

Biosorption can simply be defined as 'the removal of substances from solution by biological material' [1]. The process is energy independent and differs from bioaccumulation which is an energy dependent transport process associated with accumulation or transport of a metal into the cell. Biomaterials and particularly biomass have a bioaffinity for metals via a number of different physico-chemical interactions with the metal. These include sorption (ad- and ab-), ion exchange and surface complexation and precipitation. There has been a large increase in published work on biosorption but so far little by way of exploitation of the process on a large scale other than by traditional sewage treatment methodologies [1]. Most biological material either living or dead can biosorb a variety of materials including metals with the vast majority of the sorption being adsorption to surface groups associated with the particular biological material. Thus far there appears to be no clear winner in terms of the best candidate as a biomass material although many bacteria and algae including cyanobacteria have been examined.

Within the domain bacteria, cyanobacteria are the only organisms to carry out oxygenic photosynthesis and are phylogenetically most closely related to gram positive microorganisms [2]. Amongst the many thousands of genera of cyanobacteria, a number of model organisms have emerged. Amongst these is *Synechocystis* which is classified within the Phyllum Cyanobacteria and is a member of the Order Chroococcales. The genus *Synechocystis* was originally described as a botanical taxon [3], with the type species being *S. aquatilis*. Members of the genera *Synechocystis* are non-nitrogen fixing, unicellular cyanobacteria which primarily inhabit fresh or marine water environments. As shown in (Table 1), more than 20 species have been characterised within the genera *Synechocystis*. These have been sub grouped on the basis of sequence and GC content into 3 groups, a high and low GC freshwater group, and a marine group [4]. *Synechocystis* PCC 6803 [5, 2] belongs to the marine group although originally isolated from a freshwater lake in the US.

Synechocystis PCC 6803 is naturally transformable [6] and can grow heterotrophically on glucose [7]. These characteristics make it of interest as a model cyanobacterium for genetic manipulation. The original PCC 6803 strain, designated the Kunisawa strain was isolated in Berkley in 1968 [8], and since then there have been a number of sub-strains derived from this original strain including Kazusa (non-transformable), China (increased settling), Amsterdam, Mu and a number of New Zealand derivatives based on individual lab publication and morphological variation. All strains (original and sub strains) demonstrate 16s rDNA sequence identity but differ phenotypically in certain traits. Phenotypic differences include increased

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settleability, motility differences, sensitivity to glucose, propagation rate and transformability [2]. Indeed, recent sequencing of a number of these 'sub'strains has revealed a small number of single nucleotide polymorphisms that may be responsible for the number of phenotypic differences observed. This phenomenon in *Synechocystis* has been termed microevolution [2].

Synechocystis aquatilis Sauvageau 1892	*Synechocystis bourrellyi* Komárek 1976
Synechocystis buzasii Palik 1948	*Synechocystis consotia* Norris 1967
Synechocystis crassa Woronichin 1929	*Synechocystic diplococca* Pringsheim 1970
Synechocystis endobiotica Elenkin and Hollerbach 1938	*Synechocystis endophytica* Smith
Synechocystis fuscopigmentosa Kováčik 1988	*Synechocystis limnetica* Popovskaya 1968
Synechocystis major Geitler 1995	*Synechocystis minima* Woronichin 1927
Synechosystis miniscula Woronichin 1926	*Synechocystis parvula* Perfiliev 1923
Synechocystis pevalekii Ercegović 1925	*Synechocystis planctonica* Proškina-Lavrenko 1951
Synechocystis primigenia Gardner 1927	*Synechocystis sallensis* Skuja 1929
Synechocystis salina Wislouch 1924	*Synechocystis septentrionalis* Skuja 1956
Synechocystis skujae Joosten 2006	*Synechocystis thermalis* Copeland 1936
Synechocystis trididemni Lafargue 1979	*Synechocystis negrescens* Stearn 1973
Synechocystis willei Gardner	

Table 1. *Synechocystis* strains as designated in the AlgaeBase [9] with the discoverer and the year of discovery when known. In some instances these strains may be called after the discoverer such that it is not uncommon to have the 'Sauvageau strain' for *S. aquatilis*.

Cyanobacteria and in particular *Synechocystis* species, have recently received much attention as potential cell factories for the production of a wide variety of compounds of biotechnological interest. Such compounds include isobutanol, [10], 1,2-propanediol [11], isopropanol [12], 2,3-butanediol [13], ethanol [14], 3-hydroxybutyrate [15], free fatty acids [16], fatty alcohols [17], endogenously produced alka(e)nes [15], carotinoids [18], sesquiterpene β-caryophyllene [19], isoprene [20], squalene [21], poly-β-hydroxybutyrate (PHB) [22], polyhydroxyalkanoate [23], ethylene [24], cellulose [25], sucrose and glucose/fructose carbon substrates [26], mannitol [27], lactic acid [28], acetone [29] and H_2 [30]. Given the large interest in experimental production systems using *Synechocystis*, there has been an equivalent interest in utilising the biomass produced to add to the economics and increase the industrial potential of such systems. Biosorption of metals to various biomass types has emerged as one potential candidate for biomass utilisation following its use in other production processes. However, although there have been a wide range of biological materials studied [1], there is no clear best candidate as yet. Biomass with Gram negative peptidoglycan or Gram positive surface phosphate groups have clear advantages, while bacterial surface S-layers, proteins and sheaths all contribute to binding, making microbial candidates important. Attention has focussed on the potential of many cyanobacterial species for bioremediation, in particular, the model organism *Synechocystis*, given its potential availability from engineered production systems as discussed above.

The *Synechocystis* genus is of interest in this respect because of its natural freshwater or marine habitat. In addition *Synechocystis* produces EPS (Extrapolysaccharide Substances) which can be either cell attached or released exopolysaccharides (RPS) [31]. This EPS can biosorb a range of material including metals which can be produced as a by-product of other biotechnological processes at scale. Thus while there may be many candidates for biomass biosorption, the sorption efficiency, scale and economics may well be key factors in adoption of a particular technology or species for an *in situ* industrial process.

2. Characteristics of EPS produced from *Synechocystis*

The abbreviation EPS has variously been used to indicate extracellular polysaccharide, exopolysaccharides, exopolymers and extracellular polymeric substances. The material has been shown to contain nucleic acids, proteins, humic substances and lipid depending on its origin and environmental source. In addition, EPS may contain material derived from cell lysis and adsorbed materials that adhere to the natural polymers secreted from the surrounding environment. This adds to the complexity of EPS from a structural perspective. Most of this EPS is associated with the formation of aggregates or biofilms. The extracellular polysaccharide material varies in consistency, thickness and response to dye staining [32]. In laboratory culture, EPS is not an essential component, but in nature offers adaptive functionality. The characteristics of this material have been widely examined and there appears to be quite a large diversity in chemical composition and functionality. For example, in many cyanobacteria, this extra polysaccharide material plays a role in protecting cells from environmental extremes and stress. In certain strains, the release of exopolysaccharides together with sucrose and trehalose has been associated with desiccation resistance [33] and stabilization of cells when dried by air. In natural strains, dense layers may make strains less popular as food for predators relative to strains devoid of such material [34]. Attachment of benthic cyanobacteria to sediments, plant cells and other surfaces has been associated with extracellular polysaccharides and their hydrophobic nature [35]. It has also been proposed that secreted exopolysaccharides may play a role in precipitation of particles such as clay in aquatic environments clarifying the surrounding water. With precipitation more light is available for photoautotrophic metabolism [36]. The exopolysaccharides have also been proposed to disperse the cells themselves, facilitating optimum nutrient uptake [37]. In strains of *Microcystis*, the extracellular polysaccharide plays a role in metal ion accumulation, providing essential minerals for cyanobacterial metabolism. Such metals are often the few essential nutrients needed by photoautotrophic strains [38]. Protection of oxygen sensitive nitrogenase (used to fix nitrogen) has also been proposed as a function for attached extra polysaccharide material which can function by limiting oxygen transport to cells [39].

In addition to these important roles, extracellular polysaccharide plays a key role in cell aggregation and in biofilm formation [32]. As a major structural component of biofilm, EPS plays a role in allowing microorganisms to exist in large cell densities of mixed populations. This allows extensive communication to occur and exchange of genetic material via horizontal gene transfer. Participating cyanobacteria can thus adapt and evolve through the acquisition

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of genetic material from cells present in the biofilm community. In cell suspensions, EPS is distributed between the cell surface [in the case of capsular or cell-bound EPS] and the aqueous phase containing slime or free EPS, or as a hydrated matrix in biofilm with a composition that depends on growth phase and solution chemistry [40]. This mixture mediates adhesion and binding through interfacial processes including covalent or ionic bonding, dipole interactions, steric interactions, and hydrophobic association.

Many factors associated with EPS and surface layers of the cyanobacterial biomass can affect metal biosorption. pH has a major effect, where binding is decreased as a function of low pH, while other factors include whether the biomass is free or immobilised, the growth, age and metabolic state of the biomass, surface area of the cells or biomass for binding, the presence of competing ions in the effluent, the equilibrium binding concentrations, the flow rates, the nature of the metal complex and the temperature of the binding reaction. Thus there are numerous parameters that need careful attention to ensure optimal biosorption.

2.1. Composition of the EPS and extrapolysaccharide material

Bacterial EPS can exist in many forms; as cell-bound capsular polysaccharides, unbound "slime", and as an O-antigen component of lipopolysaccharide [41]. EPS is generally observed as a sheath or capsule. This is a thin layer surrounding the cell membrane with concentric or radial fibres which vary in volume and layer composition. The material may also be observed as a slime layer, which is more loosely associated or as a soluble form which is released [42]. Much of the EPS or slime layers have limited association with the surface of the bacterial cell whereas capsular polysaccharides can be strongly connected to cell surfaces by means of a covalent attachment to phospholipid or lipid A molecules at the surface [43]. This division in nomenclature may become masked as capsular material is released and becomes free as a result of growth or leakage of the material into the growth medium.

In general, bacterial polysaccharides are composed of repeating monosaccharide units, forming homo- or hetero- polysaccharides linked via glycosidic linkages. Capsular polysaccharides are usually linear with molecular weights up to 1000 kDa. These are linked to a lipid anchor which in the classical *E. coli* system can be lipid A in the case of lipopolysaccharides. The chemical structure of lipid A is highly conserved, with the general backbone consisting of a β-1-6-linked disaccharide of 2-amino-2-deoxy-D-glucose to which fatty acids, often 3-hydroxyalkanoic acids, are linked by ester or amide linkages [41]. The inner region of the core oligosaccharide consists generally of 3-deoxy-D-manno-oct-2-ulosonic acid and L-glycero-D-manno-heptose. These together with phosphate, and less frequently hexuronic acid and other sugar acid residues, give rise to the anionic nature of both the inner core region and of lipid A. The phosphate groups present often act to link amino alcohols to 4-amino-4-deoxy-L-arabinose or other amino sugar residues present [41]. The complexity can be increased by the presence of stereoisomers, enantiomers, structural modifications of monosaccharide units and branching [44]. Table 2 illustrates the composition of EPS produced by bacteria. The exact nature of cyanobacterial EPS is largely uncharacterised but given the conservation of EPS composition in bacterial species, it can be assumed to be generally similar amongst cyanobacteria. EPS in cyanobacteria is characterized by having relatively few sugar types consisting of

the pentoses- xylose, arabinose and ribose; the hexoses -galactose, glucose (found in 90% of polymers) and mannose (found in 80% of polymers); and derived hexoses rhamnose (found in 80% of polymers), fucose, glucuronic and galacturonic acids, with occasionally methyl and amino sugars [32]. The presence of glucuronic and galacturonic acids associated with EPS further accounts for the anionic nature of many such polymers.

Amongst the cyanobacteria, there is quite a wide variation in the quantity of such polymeric material produced, varying from 144 mg. L^{-1} day^{-1} in the case of *C. capsulate* ATCC 43193 to 2 mg. L^{-1} day^{-1} in the case of *Synechocystis* PCC 6803 [45], which is amongst the lowest producer of this exopolysaccharide material. There have been few studies on whether compositional change occurs in such material. In *Synechocystis*, it has been shown that strains can modify the composition of this material, particularly as a function of culture age and in response to nutrients. In many cyanobacteria, the exopolysaccharide material may be a subcomponent of the extrapolysaccharide material that remains attached to the cyanobacterial cell itself and leaks or is physically broken away due to the habitat location, flow conditions or activity of the organism. This may occur as the extrapolysaccharide layers enlarge and may become solubilized or fractured as a natural part of the growth cycle. In the case of *Synechocystis*, the small amounts released suggest that it occurs at a low level and is not a common behaviour. In *Synechocystis*, neither addition of up to 0.5M of sodium chloride, nor glyoxylate, nor altering the light intensity during growth affected release of exopolysaccharides [45] suggesting that leakage occurred rather than an active release process. The components in *Synechocystis* PCC 6803 and PCC 6714 appeared different from other cyanobacteria. Substituent group analysis showed an absence of acetate or pyruvate with some sulfate substituent detected and a rather high protein content [46]. It had been thought that sulfated exopolysaccharide material was only found in eukaryotic algae so its presence in *Synechocystis* is somewhat unusual. Such sulfated material is thought to have antiviral activity and may be a defence strategy in natural environments [32]. Acetate groups have been proposed to hinder cation binding so their absence in *Synechocystis* is of significance in its potential role in metal binding. Extrapolysaccharide material with high levels of charged groups (such as sulfated derivatives) are important in forming polymeric matrices [47] with metallic ions and indeed natural clay particles being a prerequisite for good metal binding.

Type of Microbial Compound	**Chemical Group and biosorption**
Peptidoglycan	Carboxy groups cation binding
Gram positive surface groups	Phosphate groups cation binding
EPS and related polysaccharide components	Polysaccharide groups uronic acid and sulfate
Microbial surface proteins	Charged Amino acid groups
Archael glucoproteins	Carbohydrate groups + charged amino acids
Algal cellulose	Hydroxyl groups
Fungal chitins, glucans, mannans	Amino groups of chitin

Table 2. Types of microbial surface compounds associated with biosorption.

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2.2. EPS associated genes in *Synechocystis* PCC 6803

In a study to determine key genes associated with the production of EPS in *Synechocystis*, four *Synechocystis* PCC 6803 genes, slr1875 (exoD), sll1581 (gumB), sll0923 (gumC) and sll5052 (gumD), sharing sequence homology with non-photosynthetic bacteria (in brackets), were determined [31]. The expression of these genes was shown to be dispensable for cell growth under standard laboratory conditions. In the wild type PCC 6803 strain, analysis of the EPS showed it formed a thick layer that enclosed the cell, while in the slr1875 and sll1581 deletion mutants, this layer decreased as did the tolerance of *Synechocystis* to salt and heavy metal stresses. The surface charge of *Synechocystis* PCC 6803, which plays a major role in cell interactions with other cells or surfaces, was determined by measuring the zeta potential with electrophoretic mobility. The zeta potential of the wild type strain and mutant strains were −33 mV and between −20 mV and −25 mV respectively indicating that the zeta potential can be correlated with the total amount of EPS and the resulting density of ionic surface charges produced by the cells [31].

Genome comparison of *in silico* translated genes from *Synechocystis* and *E. coli* was used to locate genes in *Synechocystis* that may modulate the cell surface moieties. The *Synechocystis* genes slr0977 and slr0982 (located in a cluster of transport genes) were shown [48] to encode homologs of the *E.coli* proteins Wzm and Wzt, the permease and oligosaccharide binding proteins functioning as an ABC-transporter. Mutation of these genes in *Synechocystis* also resulted in flocculating strains with modulated adherence properties and altered EPS.

3. Biosorption and general characteristics of absorption of metals with *Synechocystis* strains

Heavy metals are discharged from various industries, such as smelters, electroplating facilities, metal refineries, textile, mining, ceramic and glass industries. Some of the chief metals studied in terms of biosorption are those that have the potential to cause most pollution and include lead, antimony, copper, mercury, cadmium, chromium and arsenic as well as radionuclides of elements such as Cobalt, Strontium, Uranium and Thorium [1]. These all have different properties, may exist as complexes, have different oxidation states and their nature may depend on the pH of the medium. The remediation of trace amounts of metals can be carried out via electrolytic extraction, separation processes such as reverse osmosis or dialysis, chemical precipitation or solvent extraction, evaporative methods, or absorption methods such as carbon ion-exchange resin adsorption. However, because of the global problem of metal remediation and the cost of clean-up, new methodologies have been investigated and biosorption falls into this category.

Biosorption offers the following advantages: the volume of chemical and biological sludge can be minimised, there are potentially low operating costs, the possibility of metal recovery and regeneration of the biosorbent afterwards. In recent years, there has also been a significant effort to search for new methods of metallic trace element removal that can be used *in situ* at

contaminated sites. The mechanisms by which metal ions can attach to microbial surfaces can include van der Waals forces, electrostatic interactions, precipitation extracellularly, covalent bonding, redox interactions leading to oxidation or volatilisation (as with mercury) and precipitation, or a combination of such mechanisms. The negatively charged groups (carboxyl, hydroxyl and phosphoryl) of the bacterial cell surface can adsorb metal cations. Cation exchange capacity or ability to bind metals, which can be useful in predicting microorganism-metal interactions, has been determined from pH titration curves for the cyanobacteria *Anacystis nidulans* and *Synechocystis aquatilis* and the green alga *Stichococcus bacillaris*. The results suggest that the exchange capacity is dependent on the external pH of the environment [49]. Thus the physico-chemical environment plays a major role in addition to the binding materials themselves. Many organisms capable of secreting EPS are potential candidates [41] but the ability of cyanobacterial species to grow photoautotrophically in contaminated oligotrophic marine or fresh water environments together with the potential biomass availability, their high sorption characteristics and their non-pathogenic nature makes them ideal candidates for such studies. The cyanobacterial cell surface with EPS consisting of polysaccharide, protein and lipid, together with adsorbed material make them ideal candidates. In addition, model strains such as *Synechocystis* PCC 6803 offer a tool kit of genomic techniques to explore biosorption and examine potential genetic improvements that may be possible.

3.1. Absorption of Cr(VI) and CD(II) by *Synechocystis*

Ozturk *et al* recently reported the removal of Chromium, Cr (VI) and Cadmium, Cd (II) by *Synechocystis sp*. BASO671 [50]. In their experiments, strains with a biomass density of 2.5 at OD_{665nm} were exposed to 10 ppm Cr(VI), Cd(II) and a Cr(VI) + Cd(II) mixture for 7 days in BG11 medium (the standard laboratory growth medium for *Synechocystis*), at 25^{o}C with a light (3000 lux) and a dark cycle of 12/12 h, with shaking. Metal removal was determined as metal in the medium, metal adsorbed onto the surfaces of the cells, and metal accumulated within the cells determined by atomic absorption. Consequently, around 90% of the 10 ppm Cd(II) was absorbed onto the cell surfaces and none accumulated intercellularly. With Cr(VI), some 14% of the 10 ppm Cr(VI) was found to be intercellular with none adsorbed onto the cell surface [50]. In the case of Cd(II), there was an extremely fast adsorption to the surface layers. When mixed metal solutions were added, the preference for Cd(II) binding was confirmed with less binding of Cr(VI). The results suggest that competition for functional groups on the surface of cells may favour one type of metal species over another and suggests that biosorption may be highly dependent on the initial binding kinetics. This study also highlighted a number of interesting issues relating to the production of EPS. The productivity of EPS in strains exposed to Cd(II) compared to strains exposed to Cr(VI) was superior with both strains producing less than controls without metal exposure [50]. However, higher metal exposures (beyond 10 ppm) appeared to enhance the production of EPS, further suggesting the possibility of a stress response to the metal species.

The nature of the monomer composition of *Synechocystis* EPS was monitored as a function of the addition of the single metals [Cd(II) and Cr(VI)] and the mixture of both [50]. Relative to the control, Cr(VI) decreased the uronic content (~25%) of the EPS, while Cd(II) and the mixture

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increased the uronic acid content (~25%). There was no change in glucose content, a general reduction in rhamnose content, an increase in xylose content (~100%) with Cr(VI), which reduced to zero with Cd(II). Glucuronic and galacturonic levels were increased by the presence of both metals [50]. These results suggest that not only is EPS induced in response to metals but that the nature of the EPS alters and that this alteration may be metal specific, at least in the case of chromium and cadmium in *Synechocystis*.

SEM (Scanning Electron Microscopy) and EDS (Energy Dispersive X-ray Spectroscopy) analysis of *Synechocystis* exposed to Cd(II) and Cr(VI) was also carried out and demonstrated that surface roughness was increased, with direct metal binding observed via EDS [50]. Fourier Transform Infra-Red Spectroscopy (FTIR) was also utilised to determine the nature of the functional groups involved in metal binding. Metal binding changed peaks in various parts of the FTIR spectrum, at 3400 cm^{-1} (hydroxyl and amino groups), at 2933 cm^{-1} (aromatic groups), at 1600-1725 cm^{-1} (carboxylic acid groups) and at 1034-1025 cm^{-1} (possibly carboxyl groups of polysaccharides), indicating a role for these groups in metal binding [50].

To determine the optimal biosorption process, a comparative study was carried out using dried, immobilised and live cultures of *Synechocystis sp*. with calcium alginate beads used as the immobilization substrate [51]. The removal efficiency by biosorbent was studied as a function of pH (2-8), temperature (20–40°C), initial cadmium ion concentration (50–300 mg/L), and contact time (0–120 min). The maximum biosorption capacities of the dried, immobilized dried, and immobilized live *Synechocystis sp*. and plain Ca-alginate beads were 75.7, 4.9, 4.3, and 3.9 $mg.g^{-1}$ respectively, under optimum conditions, with the biosorption equilibrium taking 15 min. These results indicated that dried *Synechocystis* biomass was superior for Cd(II) ion removal from aqueous solution by a factor of 15 fold. Interestingly, the dried material could be reused up to 5 times via adsorption and desorption cycles without significant loss in the biosorption capacity [51].

Given the large number of variables that might affect metal biosorption, an approach using response surface methodology (RSM) was employed to study the removal of Cd(II) by *Synechocystis*. RSM allows the study of the effect of several factors influencing the response to metals by varying these factors simultaneously [52]. Utilisation of this approach (RSM) has led to optimization of the critical parameters responsible for higher Cd(II) removal by *Synechocystis pevalekii*. The optimum value of pH, biomass concentration, and metal concentration were pH 6.48, 0.25 mg protein.ml^{-1} and 5 ug.ml^{-1} respectively. Modelling data predicted that 4.29 μg.ml^{-1} Cd(II) would be removed and when experimentally determined, it was found that 4.27 μg.ml^{-1} Cd(II) removal occurred [52]. This data correlated well with model data and indicated the potential utility of such models for predicting biosorption rates.

3.2. Binding of other important metals by *Synechocystis*

Binding of EPS from *Synechocystis* to Cu(II) was investigated using fluorescence spectroscopy [53]. Under different test conditions, *Synechocystis sp*. PCC 6803, grown in BG-11 media, with 72 μmol photon. m^{-2} s^{-1} of light intensity, a photoperiod of 14 hours light to 10 hours dark at 25^0C, was subjected to 0.5-4 μg.ml^{-1} of Cu (II). Three fluorescence peaks were found in the excitation-emission fluorescence spectra of EPS. Fluorescence of peak A (Ex/Em= 275/452 nm)

and peak C (Ex/Em= 350/452 nm) originated from humic-like substances and fluorescence of peak B (Ex/Em= 275/338 nm) was attributed to protein-like substances. Fluorescence of peaks A, B, and C could be quenched by Cu(II). The binding constants indicated that binding to peak A>peak B>peak C, implying that the humic-like substances in EPS have greater Cu(II) binding capacity than the protein-like substances. The binding site number in EPS-Cu(II) complexes for peaks A, B, and C was less than 1 suggesting negative co-operativity between multiple binding sites and the presence of more than one Cu binding site.

Adsorption of metals to cells can be determined through isotherms, which are defined as the amount of adsorbate (in this case metals) bound to adsorbent either as a function of concentration in liquid phase or pressure in the gas phase at constant temperature. The most common isotherms for the evaluation of adsorption kinetics are listed in Table 3. The reader is referred to [1] for a detailed examination on biosorption isotherms and equilibrium sorption studies in relation to metal biosorption. Absorption isotherms (Table 3) for Cu(II) were determined and indicated that physical adsorption followed Langmuir behaviour with the equilibrium being obtained rather slowly and possibly showing monolayer binding [54]. Absorption was shown to be a function of pH with copper hydroxides limiting absorption at alkaline pH [54]. The results suggested that not only is biomass important in metal absorption but also illustrates the importance of pH dependence with alkaline or acidic conditions promoting complexing of metallic ions rather than biomass absorption. For example, it was observed in the case of Cd(II) that complex forms were less likely to be adsorbed onto EPS of *Synechocystis aquatilis* particularly in the presence of chloride [55]. In mixed metal streams there may be competition between various metal cations for binding onto EPS. Whereas little work has been carried out on this area in *Synechocystis*, various selectivity series have been published which reflect such competition, e.g. binding of $Al^{3+} > Ag^{+} > Cu^{2+} > Cd^{2+} > Ni^{2+} > Pb^{2+} > Zn^{2+} > Co^{2+} > Cr^{3+}$ for *Chlorella vulgaris*, and binding of $Cu^{2+} > Sr^{2+} > Zn^{2+} > Mg^{2+} > Na^{+}$ for *Vaucheria* sp. [57, 1]. The presence of ions that can complex with the metal may have dramatic effects on the overall biosorption process, again indicating that variability is dependent not only on biomass factors but also on compositional aspects of the effluents being treated.

Many industries, such as coatings, automotive, storage batteries, aeronautical and steel industries generate large quantities of wastewater containing various concentrations of lead. Data from storage battery producers demonstrated that the pH value of wastewater discarded by these industries ranged between pH 1.6 and 2.9, while the concentration of soluble lead was in the range of 5–15 $mg.L^{-1}$ [57]. The relationship between binding of Pb(II) and Cd(II) on the cell ultrastructure, growth and pigment content of *Synechocystis* PCC 6803 [58] was examined and a dependence on metal concentration was demonstrated. At low level absorption, few growth effects were observed, however as levels of Pb(II) increased to greater than 4 $mg.L^{-1}$, cell ultrastructure changes were observed including thylakoid deterioration suggestive of high levels of accumulation of intercellular Pb(II). Such accumulation could be useful in Pb contaminated environments. In similar studies, the optimum initial pH of biosorption was found to be pH 4.5 with the equilibrium Pb(II) uptake of 2.265 $mg.g^{-1}$ at this pH [57].

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Name of the isotherm	Equation	Principle	Reference
Henry's adsorption isotherm	$q_e = KC_e$	The amount of the adsorbate is proportional to the concentration of the adsorbent	[59]
Freundlich isotherm	$q_e = K_f C_e^{1/n}$	Describes the non-ideal and reversible adsorption not restricted a monolayer	[60]
Langmuir isotherm	$q_e = \frac{Q_{max} bC_e}{1 + bC_e}$	Assumes monolayer adsorption and can only occur at a finite number of definite localized sites, which are identical and equivalent	[60]
Brunauer-Emmett-Teller (BET) isotherm	$\frac{C_e}{q_e(C_s - C_e)} = \frac{1}{q_s C_{BET}} + \frac{(C_{BET} - 1)}{q_s C_{BET}} \frac{C_e}{C_s}$	Describes multilayer adsorption systems with relative pressure	[61]
Temkin isotherm	$q_e = B\ln A_T + B\ln C_e$ $B = \frac{RT}{b_T}$	Adsorbent–adsorbate interactions with temperature effects	[62]

Table 3. Isotherms utilized for adsorption kinetics.

Glossary of Terms: q_e, amount of adsorbate bound to the adsorbent at equilibrium ($mg.g^{-1}$); Kf, Freundlich isotherm constant ($mg.g^{-1}$) ($dm^3.g^{-1}$) related to adsorption capacity; C_e, equilibrium concentration ($mg.L^{-1}$); n, adsorption intensity; $Q_{max,}$ maximum monolayer coverage capacities ($mg.g^{-1}$); b, Langmuir isotherm constant ($dm^3.mg^{-1}$); C_{BET}, BET adsorption isotherm relating to the energy of the surface interaction ($L.mg^{-1}$); C_s, adsorbate monolayer saturation concentration ($mg.L^{-1}$); qs, theoretical isotherm saturation capacity ($mg.g^{-1}$); AT, Tempkin isotherm equilibrium binding constant ($L.g^{-1}$); B, constant related to heat of sorption ($J.\ mol^{-1}$); b_T, Temkin isotherm constant; R, Universal gas constant ($8.314\ J.mol^{-1}.{}^0K^{-2}$); T, temperature at 298^0K.

In an experimental system treating mixed metal wastes in an algal pond using *Synechocystis salina*, it was shown that 60% Cr(VI), 66% Fe(II), 70% Ni and 77% Hg was removed after 13 days of treatment. This reduction correlated with surface absorption [63], however details of the initial metal concentrations were not given.

Antimony (Sb), a non-essential element in biological systems, poses a major problem in mining areas, particularly in China. Around 80% of the world's reserves are deposited here, leaving aquatic environments in the mining areas polluted by long term leaching [64]. Conventional methodologies to remove Sb are limited to precipitation methods such as alum, lime or ferric salts precipitation. Biosorption using *Synechocystis* has been investigated as a potential economic alternative. Here, the added attraction of using *Synechocystis* lies in the fact that it is

a common inhabitant of aquatic environments in the South China region. Absorption of Sb by EPS in *Synechocystis* FASHB898 was examined. It was observed that some 50% of the Sb was absorbed in the first 30 minutes, with equilibrium being reached after 1 hour. Sorption concentrations of 2.61 $mg.L^{-1}$ of Sb per gram dry weight of biomass were determined [64]. It was shown that using initial Sb concentrations of 100 $mg.L^{-1}$ that up to 1.92 $mg.g^{-1}$ was absorbed by EPS, with some 2.64 $mg.g^{-1}$ being located intercellularly. The results of FTIR analysis confirmed that Sb binds to EPS via protein and carbohydrate group interactions as indicated for many other metals. Again it has been suggested that EPS absorption may act as a stress barrier to protect the cells from such metals [64] in natural environments.

In a study examining resistance to Nickel (Ni), 10 different *Synechocystis* strains were initially examined for nickel resistance. The EC_{50} values of the 10 isolates ranged from 2.56 to 17.41 $mg.L^{-1}$, while the EPS concentrations of the 10 isolates ranged from 44 to 143 $mg.L^{-1}$. *Synechocystis sp*. BASO403 and *Synechocystis sp*. BASO404 were chosen on the basis of greatest resistance and highest EPS to examine Ni(II) biosorption [65], thus illustrating the potential utility of certain *Synechocystis* strains for (Ni) removal.

Engineered nanoparticles, particularly particles containing titanium dioxide (TiO_2) are finding application in industry particularly in paints, cosmetics and as part of solar cells. Although relatively inert, TiO_2 can be activated by UV light producing reactive oxygen species which can be antibacterial [66]. Thus with the increased potential use of such nanomaterial's, biological treatment regimens could be compromised by the killing effects on bacterial communities in treatment facilities. It has been demonstrated that *Synechocystis* PCC 6803 has significant ability to biosorb TiO_2 [67]. The response of wild-type *Synechocystis,* which possesses abundant EPS surrounding the cells, to that of an EPS-depleted mutant was also examined and indicated that the EPS play a crucial role in S*ynechocystis* protection against cell killing caused by TiO_2 nanoparticles [67] indicating that it may have potential in remediation of this emerging class of compound.

Manganese (Mn) uptake to cells of *Synechocystis* was measured in cells incubated with Mn solutions. *Synechocystis* cells were shown to be able to take up 150 μM of Mn(II) or Mn(IV) in 48 hours [68]. The predominant accumulation of Mn was associated with the outer membrane for both Mn substrates. Large manganese deposits were found associated with the EPS of *Synechocystis* cells. TEM analysis demonstrated that Mn accumulation occurred on the cell surface and analysis demonstrated that the attached material was manganese phosphate. This bound material withstood multiple washes and appeared to be quite stably bound, indicative of tight binding and its potential as a biosorption material [68].

Arsenic (As) is a widely used component of batteries, a dopant in semiconductors and in optoelectronics. Additionally, it is used in some pesticides and herbicides. Toxicity to humans occurs mainly via drinking water and it is thus important to remove even trace amounts from water. Arsenic is present in two biologically active forms, As(V) and As(III), depending on the redox potential of the environment. Oxidation of As(III) to As(V) is a detoxification process, since As(V) is less toxic than As(III) [69] while arsenate methylation is also a common detoxifying mechanism in many microbial systems. Examination of the response of *Synechocystis* PCC 6803 to arsenic revealed that the organism can grow and accumulate arsenic to high levels. Biomass of *Synechocystis* could accumulate up to 0.38 $g.kg^{-1}$ dry weight when treated with 100 μM sodium arsenite over a 14 day period [70]. When treated with arsenate for six weeks,

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Synechocystis produced volatile arsenicals. An *Ars*M homolog of a known arsenic methylases from *Synechocystis* sp. PCC 6803 was purified and shown to play a role in methylating arsenite *in vitro* with trimethylarsine as the end product. This illustrated the potential utility of this organism in detoxification of arsenic compounds. Amongst a number of cyanobacteria examined, *Synechocystis* was shown to have one of the highest levels of tolerance to arsenic and to be able to accumulate arsenic at a high rate [71]. Genomic studies on tolerance to arsenic have shown that arsenic resistance in *Synechocystis* PCC 6803, in addition to *ars*M (the methylase), was mediated by the *ars*BHC operon which was regulated by *ars*R and two additional arsenate reductases encoded by the *ars*I1 and *ars*I2 genes [72]. *Ars*B encoded an arsenite transporter, *ars*H an FMN-quinone reductase and *ars*C a FMN-quinone reductase. Using a gene array study, a highly orchestrated response to arsenic was observed in *Synechocystis* with 421 genes involved, of which, 179 were induced while 242 were repressed on arsenic addition based on transcriptomic studies [72]. These arrays of genes, whose expression was modified by arsenic were shown to be associated with the repression of growth, the lowering of energy metabolism and the induction of general stress responses which form part of the core transcriptional response to stress in many organisms. The most highly induced genes were those for the *ars*BHC operon [72].

In *Synechocystis* PCC 6803 similar systems for detoxification of mercury are observed as found in many other microbial systems. The protein Grx1, annotated as Slr1562 in the *Synechocystis* genome, selectively interacts with the putative mercuric reductase protein, Slr1849, in PCC 6803. Grx1 which is designated *Mer*A- like, appears to play a major role in catalysing NADPH-driven reduction of mercuric and uranyl ions [73]. In addition to a defence role against the toxicity of such metals, the presence of this system may also have a bioremediation role in mixed effluents. However, its potential has not been realised nor have comparative studies been carried out comparing its detoxification abilities with other organisms.

Sorption of caesium (Cs) by *Synechocystis* PCC 6803 has been examined at concentrations between 1 to 100 μM Cs in the presence of three clay types [74]. Binding was found to occur in two distinct phases, the first step was shown to be a rapid uptake not dependent on light to the clay-cell material and a second slower step which was inhibited by metabolic inhibitors. This data indicated a role for cell and energy dependent uptake, which was pH and salt dependent. The data indicated that the clay adsorption played a significant role supplemented by a slower binding step and accumulation by the cyanobacteria. The practical ability to remove caesium using *Rhodobacter* was analysed from contaminated mud in Japan after the Fukushima accident. Approximately 90% of the Cs found in the mud in a swimming pool could be removed by immobilized cells in a 3 day period [75]. The treatment was repeated 3 times and efficiencies remained high with 84% of the remaining material being sorbed on the second treatment and a further 78% sorbed on the third batch treatment. Here Cs attachment was not altered by nitric acid treatment below pH 2 indicating a strong sediment attachment whereas cell sorption showed major utility. This study indicated the potential for cell sorption in dealing with certain tightly attached radionuclides.

3.3. Reactor configurations for biosorption

Use of diverse biomass material as a biosorption candidate has been infrequently examined. Free biomass, such as microbial cells suffers from a number of disadvantages, including low

mechanical strength, the small size of individual microbial cells and the difficulty of separating cells once they have been utilised to adsorb metals in liquid effluents. Several processes using biomass immobilisation have been investigated to overcome these disadvantages. Immobilisation of biomass in bio-towers, trickle filters, airlift reactors or rotating systems where microbial biofilms play a key role have been examined [76]. As the immobilised biomass grows and its size increases, there is natural expansion and leakage of the biomass, which can then be collected as a microbial sludge. Provided the metals in the wastewater do not have a deleterious effect on the biofilm or other co- habiting organisms, this system can work well. The advantage of rotating immobilised systems, in the case of cyanobacteria, would be that they can still be exposed to light, as opposed to bio-tower systems. Moving sand bed reactors have also been used [77] to develop consortia to treat mixed metal pollutant effluents, which could also provide enough light for cyanobacterial consortia. Technologies and processes for metal recovery are reviewed in [78].

Dried or dead cells may absorb more metals than live cells and for this reason encapsulation of biomass may be advantageous [79], which would mean the utilisation of different process configurations. Although dead cells or biomass can be used, there is little data on the relative merits when compared to live cells. Generally, in addition to metallic pollution, natural waste materials may contain other substances that need remediation, and thus having live biomass may, on occasion, be more advantageous. It is envisaged however that should biosoption be employed at scale then some form of continuous flow through system would need to be employed. Many variables need to be considered; including biomass concentration, pollutant metal concentration, pH of the system, and flow rate. As such studies have been carried out at small laboratory scale there is little data available on large scale systems particularly with cyanobacteria.

Metals absorbed by EPS or biomass are often required to undergo elution in subsequent processes. The nature of such elution processes is dependent on whether the biomass needs to be reused or recycled. Acid or alkali desorption can generally be used for elution [1]. For particular cases, such as precious metal recovery, selective desorption may be used. In the case of radionucleotide recovery, this can occur via combustion and ash removal. In other cases simple liquid extraction may be used on occasion with a variety of solvents. The desorption procedures utilised are thus dependent on the metal, its value and whether the biomass will be reused.

4. Other biodegradative reactions associated with *Synechocystis*

The genome sequences of a number of *Synechocystis* strains have now been determined but there appears to be few genes associated with biodegradation in this organism. As the organism utilises a phototrophic metabolism, it appears to lacks transport systems for organic materials in general as it synthesises most of what it needs. Interrogation of the KEGG database [80] for metabolic activities associated with *Synechocystis* has revealed that the organism does not possess metabolic pathways to degrade organic pollutants. This may not be a general feature of all *Synechocystis* genera as there have been reports that some species may possess

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degradative abilities. *Synechocystis aquatilis* has been reported to degrade 85% of n-octadecane and 90% of pristine added to growth medium [81]. However other than this strain, which indeed may be unique, there appears to be limited metabolic degradative capability within the genus, particularly with respect to organic compounds.

5. Conclusion

Although there have been many studies on the biosorption potential of cyanobacteria, there remains some way to go before their potential may be realised. Many laboratory based studies do not translate to the field. This may be a factor of the altered physicochemical environment, the competition for binding sites for metals when there are mixed metal species present, or the presence of other competing substances in the polluting water. The debate between use of live and dead cells is also open, with some metals showing both EPS binding and bioaccumulation. Bioaccumulation of the metal species may thus be favoured by the use of live biomass. When live cells are used the uptake tends to be bi-phasic, with initial rapid uptake occurring followed by a slower metabolism driven accumulation [74, 79]. Live cells additionally may display the potential to mutate, become more resistant to the metals and adapt to increase metal loadings in the longer term. Indeed there is a trend to seek out specific metal resistant species as biosorbants, in many cases to verify their potential as a start point for further study [82]. Some studies support the use of dead or dried cells, which often show greater metal binding capacity and may be particularly important if the biomass is to be reused a number of times. There have also been attempts to utilise mixed consortia, using organisms with varying and mixed metal sorption capacities [83]. Such consortia, which may develop naturally in response to the metal loading, are difficult to characterise and members are often transient, making the assignment of roles to particular genera or species difficult.

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