24. Marine Macrophytes: Biosorbents

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Biosorption is a biological method suggested as a cheaper and more effective technique for heavy metal ion removal and recapture from aqueous solutions such as industrial wastewater. A large range of biomass, principally bacteria, algae, seagrasses, crab shells, yeasts, and fungi have received increasing attention for heavy metal ion removal and recovery. In particular, through this method, nonliving aquatic macrophytes (i. e., macroalgae and seagrasses) can be used for heavy metal removal due to their large availability, easy regeneration, and low costs. In recent years, macrophytes have been shown to be able to remove pollutants by surface adsorption or by bioaccumulation, incorporating metals into their tissues or storing them in a bound form. The type of biomass used in the treatment procedure can make a significant difference for the removal of pollutants. Furthermore, the knowledge of metal kinetic biosorption parameters for nonliving macrophytes has become crucial for treatment design to improve the efficiency of metal removal in artificial systems for their recycling. In this chapter, the properties of dried macrophytes in the treatment of wastewater and their cell wall and cuticle compartments are described. Moreover, the procedures for biosorption experiments are analyzed.

24.1 Marine Macrophytes

The term *macrophytes* indicates aquatic organisms capable of performing oxygenic photosynthesis that are sufficiently large-sized to be observed with the bare eye. They typically grow submerged or floating in riverine, estuarine, and marine coastal environments, where they play a key role as primary producers. Marine macrophytes form the bulk of the photosynthesizing biomass in coastal habitats; they belong to two different groups, indicated with the common names of algae and seagrasses.

24.1.1 The Algae

The term algae designates the complex of all oxygenic photosynthesizers other than embryophyte plants [24.1]. As such, algae represent a heterogeneous assemblage of organisms belonging to many separate evolutionary lineages, which in modern classifications are distributed in no less than four eukaryotic supergroups (Archeplastida, Chromalveolata, Excavata, Rhizaria) [24.2]. Due to such vast evolutionary diversity, different groups of algae exhibit striking differences in terms of morphology, ultrastructure, ecology, biochemistry, and physiology.

The benthic macroalgae, or seaweeds, are the most common macrophytes on marine shores where stable substrata exist (natural rock, artificial reefs, concrete, or wood pillars). They belong to three different groups, empirically recognized on the basis of thallus color: the red algae (phylum Rhodophyta), the brown algae (phylum Heterokontophyta, class Phaeophyceae), and the green algae (phylum Chlorophyta, class Ulvophyceae). Although they co-exist in the same coastal habitats, these three groups are phylogenetically distinct and are characterized by different biochemical and physiological traits. This has important implications with regard to their biosorption properties.

24.1.2 Marine Benthic Green Algae (Chlorophyta, Ulvophyceae)

The Viridaeplantae, formed by the embryophyte plants and the complex of algae generally called green algae, is one of the most diverse evolutionary lineages within the eukaryotic supergroup Archaeplastida [24.3, 4]. Molecular and ultrastructural studies conducted in the last 20 years suggest that the Viridaeplantae evolved from a unicellular flagellate ancestor, from which an early separation in two lineages took place [24.3, 4]. One of these, the Streptophyta, radiated in freshwater environments and eventually gave rise to terrestrial embryophyte plants. The other, the Chlorophyta, had a more complex evolutionary history and gave rise to several different groups of green algae. The class Ulvophyceae is the most diverse of these groups in terms of thallus complexity and cellular sophistication, and includes the vast majority of the green benthic macroalgae (about 1600 species) [24.4].

Fig. 24.1 Thallus of *Ulva compressa* (green algae) from Portonovo, Ancona, Adriatic sea (Italy). Scale bar: 2 cm

Species of Ulvophyceae occur in all oceans and are most abundant in the intertidal and shallow subtidal zones of rocky shores. They mostly consist of pluricellular macrophytes (up to 1 m in size) growing attached to stable substrata, but some species (particularly in the genus *Ulva*) (Fig. 24.1, *Ulva compressa* Linnaeus) can get detached and survive floating for a long time. Their morphologies range from microscopic unicells to macroscopic multicellular plants, and giant-celled organisms with unique cellular and physiological characteristics [24.4]. Four main cytomorphological types are recognized in this class [24.5]: nonmotile uninucleate cells (e.g., *Oltmansiellopsis*); multicellular filaments or blades composed of uninucleate cells (e.g., *Ulva*); multicellular filaments composed of multinucleate cells, with nuclei organized in regularly spaced cytoplasmic domains (e.g., *Cladophora, Chaetomorpha*); siphonous thalli, i. e., consisting of a single giant tubular cell, which often contains thousands to millions of nuclei (e.g., *Acetabularia*, *Caulerpa*, *Codium*, and *Halimeda*).

The Ulvophyceae possess chlorophylls a and b and a characteristic set of accessory pigments including the xanthophylls lutein, zeaxanthin, violaxanthin, antheraxanthin, and neoxanthin [24.6]. Siphonein and siphonoxanthin are found in the siphonous representatives. The main reserve polysaccharide is starch, which occurs as grains. The chloroplasts are surrounded by two membranes and may contain one or more pyrenoids. Flagellate cells possess two, four, or numerous flagella, which are similar in structure and in the basal part exhibit a transition zone with stellate structure. Details in the ultrastructure of the flagellar apparatus and mitosis differentiate the Ulvophyceae from other classes of green algae.

24.1.3 The Red Algae (Rhodophyta)

The Rhodophyta are one of the most ancient groups of photosynthetic eukaryotes (fossils of *Bangiomorpha pubescens*, considered the oldest red alga, are approximately 1.2 billion years old [24.7]). This phylum includes about 6200 species, which occur primarily in marine environments (150 species are found in freshwater). Red algae are ubiquitous on rocky shores of all oceans and represent the dominant group in seaweed floras throughout the world. They are also the benthic macroalgae with the widest ecological range, occurring at all levels of the photic zone. Some species occur in

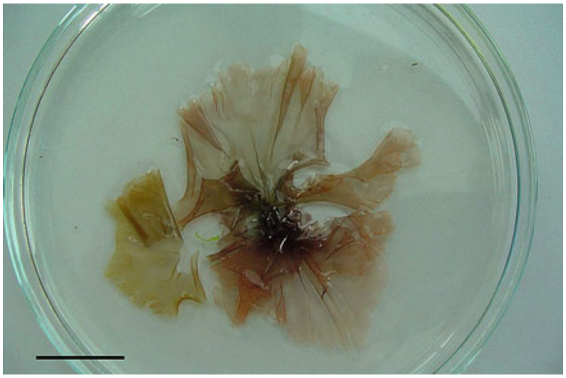

Fig. 24.2 Thallus of *Porphyra leucosticta* (red algae) from Portonovo, Ancona, Adriatic sea (Italy). Scale bar: 2 cm

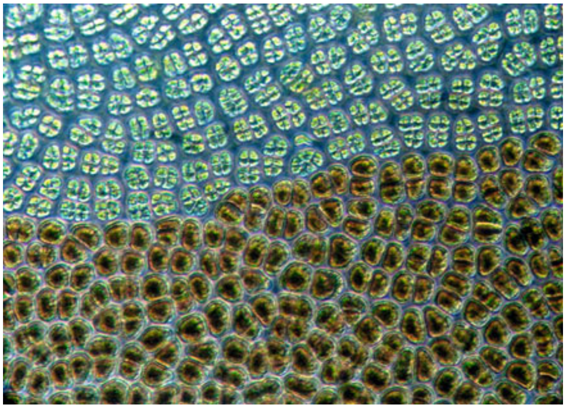

Fig. 24.3 Vegetative and reproductive cells of the thallus of *Porphyra leucosticta* (red algae) from Portonovo, Ancona, Adriatic sea (Italy). Magnification: 40×

the upper intertidal zone, where they are able to tolerate prolonged desiccation (e.g., members of the genus *Porphyra*) (Figs. 24.2, 24.3, *Porphyra leucosticta* Thuret). Most Rhodophyta, however, occur in subtidal habitats, and some species can reach considerable depths. The deepest-living benthic algae known are coralline red algae recorded at −268 m depth off the shores of Bahamas, where the available light irradiance was 0.001% as at the surface [24.8]. From a morphological point of view, this group is equally diverse and includes spherical unicells, thin uniseriate filaments, expanded blades with variable shape, encrusting forms growing attached to the substratum, and corticated macrophytes with parenchymatous structure [24.9].

Chlorophyll a is the only chlorophyll present in this group. Accessory pigments include phycobiliproteins (phycoerythrin, phycocyanin, allophycyanin), carotenoids (α-carotene, β-carotene), and xanthophylls (lutein, zeaxanthin, antheraxanthin, violaxanthin). The main reserve product is a polysaccharide called floridean starch, which is accumulated in grains in the cytoplasm. The chloroplasts are enveloped by two membranes and contains numerous parallel thylakoids, arranged singly and not stacked; numerous phycobilisomes (structures formed by phycobiliproteins) occur on the surface of the thylakoids. A distinctive feature of the red algae is the complete absence of flagella and centrioles in all stages of the life history; the male gametes, called spermatia, are small and spherical and are carried by water currents to the female gamete (called carpogonium). The cytokinesis is brought about by the development of a cleavage furrow that in the majority of the species does not complete the separation between adjacent cells and leaves a protoplasmic connection called pit connection.

24.1.4 The Brown Algae (Heterokontophyta, Phaeophyceae)

The class Pheophyceae, or brown algae, includes approximately 1800 species distributed almost entirely in marine environments (with a few freshwater species). Although they coexist with red algae and ulvophyte green algae, they are not closely related to these groups and belong to a different eukaryotic supergroup, the Chromalveolata [24.10]. They are believed to have arisen between 200 and 150 mya [24.11].

The Phaeophyceae are present in all seas of the world, but are most diverse and abundant in cold seas. This group includes the largest-sized seaweeds and

Fig. 24.4 Thallus of *Cystoseira barbata* (brown algae) from Portonovo, Ancona, Adriatic sea (Italy). Scale bar: 4 cm

Fig. 24.5 Thallus of *Dictyota dichotoma* (brown algae) from Portonovo, Ancona, Adriatic sea (Italy). Scale bar: 2 cm

Fig. 24.6 Transverse section of the thallus of *Dictyota dichotoma* (brown algae) from Portonovo, Ancona, Adriatic sea (Italy). Magnification: 40×

represents a major component of the algal biomass on many rocky shores. In many temperate regions the lower part of the intertidal zone is occupied by dense canopies of brown algae of the order Fucales (*Cystoseira* in the Mediterranean, *Fucus* and *Ascophyllum* in the North Atlantic, *Hormosira* in Australia and New Zealand) (Fig. 24.4, and *Cystoseirabarbata* (Stackhouse) C. Agardh). Species of the order Laminariales (mainly of the genera *Laminaria*, *Ecklonia*, *Lessonia*, and *Macrocystis*) can reach 70 m in length and form large submerged forests in cold seas of both hemispheres.

Brown algae exhibit a great variation in habit, ranging from thin, uniseriate filaments to complex pseudoparenchymatous thalli (Figs. 24.5, 24.6, *Dictyota dichotoma* (Hudson) J.V. Lamouroux). They possess chlorophylls a and c (in the forms c_1, c_2, and c_3), and β-carotene and fucoxanthin as main accessory pigments. The main storage polysaccharide is chrysolaminaran, a β-1,3 glucan deposited in liquid form in special vacuoles. The chloroplasts are surrounded by four membranes, two of which are formed by endoplasmic reticulum; the thylakoids are packed in stacks of three parallel to each other and a peripheral thylakoid called girdle lamella usually encloses the others. The flagellate cells possess two flagella which differ in length and structure; one is long, pleuronematic (i. e., provided with thin tripartite tubular hairs), and directed forward; the other is short, smooth, and possesses a basal swelling in proximity of which the photoreceptor apparatus of the cell is located.

24.1.5 Seagrasses

The phylum Magnoliophyta includes the plants generally known as angiosperms, or flowering plants. This is the most specialized and successful group of vascular plants, which is currently represented by about 275 000 species and dominates the vegetation of most terrestrial ecosystems. The oldest angiosperm fossils date back approximately 135 mya, with the major lineages evolving between 130 and 90 mya [24.12]. Between 100 and 65 mya some lineages of angiosperms moved back to aquatic environments, and a few of them acquired the capability to live in seawater. Marine angiosperms, or seagrasses, are represented today by 60 species belonging to 13 genera and 4 families (Cymodoceaceae, Hydrocharitaceae, Posidoniaceae, Zosteraceae). As vascular plants, they have a body that can be divided into roots, stem, and leaves, and a vascular apparatus

Fig. 24.7 Thallus of *Cymodocea nodosa* from Gabicce, Pesaro and Urbino, Adriatic sea (Italy). Scale bar: 4 cm

for the transportation of nutrients, water, and gases. Overall all species of marine angiosperms have developed a similar habit, characterized by a prostrate stem (rhizome) supporting bundles of ribbon-like leaves with parallel veins connected by short transversal veins (Fig. 24.7, *Cymodocea nodosa* (Ucria) Asch.).

24.2 Heavy Metals: Definition and Toxicity

Heavy metals such as arsenic (As), cadmium (Cd), chrome (Cr), copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn), have a specific gravity (i. e., measure of density) at least five times that of water. Because of their solubility in the water environments, heavy metals can be absorbed by living organisms [24.13–15]. It is known that in small quantities heavy metals are essential for human nutrition, however, when consumed in high amounts they can cause health problems. Indeed, elements like iron, copper, nickel, manganese, selenium, and zinc are common in nature and are normally ingested with food (foodstuffs, fruits, vegetables, multivitamin products) [24.16–18]. The anthropogenic impact on the environment has increased the presence of heavy metals, which is of major concern because of their toxicity and threat to plant and animal life. The main anthropogenic sources of heavy metals released into the environment are:

a) Acid mine drainage (AMD), caused by mining operations
b) Electroplating industry waste solutions (e.g., zinc plating, nickel plating, chrome plating, oxidation)
c) Coal-burning applications for power generation and
d) Nuclear technology for power generation (uranium mining/processing and special waste generation).

Moreover, heavy metals are common in industrial applications like the manufacture of batteries, textile dyes, pesticides, alloys, steel, and others. The risks for human health and the toxicity associated with heavy metals are now well known [24.19–21]. Heavy metals in significant quantities are released into freshwater bodies and seas. These substances can be accumulated and biomagnified along sediment, water, and aquatic food chains, resulting in fatal effects in fish populations, and for humans. They become toxic when they are not metabolized by organisms and accumulate in the soft tissues [24.19, 20]. The Agency for Toxic Substances and Disease Registry (ATSDR), based in Atlanta (Georgia), is one of the largest federal public health agencies of the USA. It provides trusted health information to prevent harmful exposures and diseases related to toxic substances.

24.3 Biosorption

Biosorption is a method that can be used for the removal of pollutants from wastewater, especially those that are not easily biodegradable like heavy metals and industrial dyes. Furthermore, this process can remove low heavy metal concentrations as an inexpensive, simple and effective alternative to conventional methods like activated carbon [24.22–26]. The recapture of heavy metals from industrial wastewater is of dual interest: (i) the prevention of threats to health (metal toxicity) [24.19–21], and (ii) society is realizing the importance of recycling these substances for metal costs [24.27–29]. When the concentrations of wastewater contaminants are above $100\,\mathrm{mg/L}$, the biological treatment processes for removing heavy metals are the most effective [24.30]. The treatments used are precipitation, electrodialysis, and reverse osmosis, which become costly and difficult to manage at low metal concentrations ($< 100\,\mathrm{mg/L}$) [24.31, 32]. The potential of metal biosorption by biomass materials has been well established in the last 15 years. This technique uses a variety of biological materials for binding of these pollutants, including fungi [24.33], bacteria [24.34–36], plants [24.37], macroalgae [24.24–26, 38], microalgae [24.39, 40], and agricultural and industrial wastes [24.41–43]. For economic reasons, particular interest must be given to large biomass types, either generated as a waste by-product of large-scale industrial fermentations or certain metal binding macrophytes found in large quantities in the sea. Recent studies showed that aquatic macrophytes (i. e., seaweeds and seagrasses) can be used as potential biosorbents for the removal of heavy metals [24.24–26, 38, 44–46]. This is considered one of the most promising types of biosorbents in view of its high capacity for uptake, its low cost, and its abundance in many parts of the world. Indeed, a comprehensive collection of algae for food and algal phycocolloids (agar, carrageenans, alginates) is in excess of 3 million tons per year, with a potential estimated at around 2.6 million collected for red algae and 16 million for brown algae [24.47].

The biosorbent ability of the macrophytes resides in the structure of the cell walls of seaweeds and the cuticle of seagrasses, which are responsible for this phenomenon. Indeed, in biosorption, the ability of active sites on the surface of macrophytes to bind and concentrate heavy metals from even the most dilute aqueous solutions is exploited. The cell wall intervenes in the mechanisms of biosorption through physicochemical processes like ion exchange, complexation, microprecipitation, and electrostatic interactions. Specifically, in solution heavy metals can form ions (cations or anions), which create a passive binding with nonliving biomass through a mechanism that is not metabolically controlled, in contrast to bioaccumulation by living cells [24.48–50]. The use of dried nonliving macrophytes as adsorbent material has considerable advantages, such as:

a) High efficiency in detoxification of diluted effluents
b) Minimizing the volume of waste, biological and/or chemical
c) No demand for nutrients, and
d) Low cost.

An additional advantage is represented by the possible recovery of heavy metals: once the binding of metals reaches saturation, the biomass can be regenerated with acid solutions and/or hydroxyl, which release small volumes of heavy metal concentrates [24.51–53]. These may be subsequently treated with techniques of co-precipitation, flocculation, and electro-deposition.

24.3.1 Cell Wall of the Algae

The cell wall is the portion of the algal cell directly involved in biosorption; its chemical structure has a major effect on the mechanisms by which this phenomenon takes place. The electrostatic attraction and the complexation of the groups present on the surface of the cell wall with the metal in solution play a key role in this regard [24.25]. The polysaccharides present in the cell walls of algae contain chemical groups such as carboxyl, hydroxyl, and sulfate, which may act as binding sites [24.54]. They are effective ion exchangers and, therefore, important sites for complexation of metal cations [24.55, 56]. Proteins, lipids and, occasionally, nucleic acids may also be present on the surface of the cell walls. These molecules, however, occur mainly in the plasma membrane and in the cytoplasm; they are, therefore, bound to metal ions through their functional groups (aminic, carboxylic, imidazolic, thiolic, thioesteric, nitrogen, and oxygen in peptidic bonds) mostly within the cell [24.57]. In all algae and plants the cell wall consists of a fibrillar fraction, which is responsible of the mechanical strength of the wall, and an amorphous fraction, which provides flexibility. The composition and relative amounts of these two fractions vary in different taxonomic groups [24.6].

Chlorophyta (Green Algae)

The composition of the cell wall of the Ulvophyceae shows some variation between different orders (in some cases even between different life history stages in the same species) [24.6]. In many groups, the fibrillar component forms an internal layer that lies directly against the plasmalemma, whereas the amorphous portion is predominantly on the outer side of the wall, forming a slimy outer layer and containing relatively few and randomly orientated microfibrils [24.6]. The fibrillar layer consists of microfibrils with diameters varying between 3 and 35 nm. In many ulvophytes, the microfibrils consist of crystalline cellulose and are arranged in numerous parallel layers; in orders with siphonous construction (Bryopsidales and Dasycladales), however, the most abundant constituents are mannans, xylans, and glucans. The structural component is embedded in an amorphous, mucilaginous matrix, which also varies in different orders. This may be formed by acidic, partially sulfated heteropolysaccharides (as in the case of the Ulvales) or highly sulfated arabinogalactans (e.g., in the Cladophorales) [24.6]. Polysaccharides extracted from *Ulva* (order Ulvales) contain 16% sulfates and 15–19% uronic acid [24.58]. Several genera of siphonous ulvophytes (*Acetabularia*, *Avrainvillea*, *Penicillus*, *Halimeda*, *Udotea*) are encrusted with calcium carbonate, which is deposited on the cell wall in the form of aragonite crystals. Members of these genera are among the most important contributors to calcium carbonate deposition on tropical reefs.

Rhodophyta (Red Algae)

In the red algae the fibrillar fraction is constituted by cellulose, which is deposited in the form of microfibrils arranged to produce an irregular felt-like network [24.6, 59] immersed in the amorphous matrix. In some genera, such as *Bangia* and *Porphyra*, cellulose is replaced by xylans and mannans. The amorphous matrix forms up to 70% of the dry weight of the wall and consists of mucilaginous material that may be extracted with hot water. It is constituted by several types of highly hydrophilic sulfated polygalactans, polymers of β-(1 → 4) galactose, and α-(1 → 3) linked 3,6 anhydrogalactose, which are mostly combined to form two types of polysaccharides: agars and carrageenans. The presence of D-galactose and anhydro-D-galactose distinguishes the more highly sulfated carrageenans from the less highly sulfated agars (anhydro-L-galactose) [24.60]. Agar and carrageenans have colloidal properties and for this reason are widely used in many industrial applications [24.61]. Carrageenans consist of a repeating disaccharide backbone: β-(1 → 4)-D-galactopyranosyl-α-(1 → 3)-D-galactopyranosyl. There are 17 different types of carrageenans, which occur in different species and can also vary within a species. Agars are formed by alternating 3-O-linked β-D-galactopyranose and 4-O-linked 3,6 anhydro-α-L-galactopyranose, but the repetition can be interrupted by blocks of repeated units of either one of the two constituents [24.60].

In all species of the order Corallinales and some species belonging to other orders (Nemaliales, Peyssonneliales), the cell wall is encrusted by calcium carbonate. In the Corallinales this compound is present primarily as crystals of calcite (rhomboid crystals), whereas the calcified members of other orders deposit it mainly as crystals of aragonite (orthorhombic crystals) [24.59]. In other red algal genera (e.g., *Mazzaella*) the surface of the alga is covered by a continuous layer of proteinaceous material [24.60]. This cuticle is neither homologous or biochemically similar to the cuticle of vascular plants.

Phaeophyceae (Brown Algae)

In this group the fibrillar portion of the wall is formed by a felt-like network of cellulose, which is probably strengthened by insoluble alginates [24.6]. The amorphous portion constitutes most of the wall mass and is composed of alginates and fucans. Alginates are salts of alginic acid, a polymer formed by monomers of two acid sugars (D-mannuronic acid and L-guluronic acid) connected by β-1,4 bonds. The residues can form salts with various cations, such as Ca^{++}, Mg^{++}, Na^{+} (this is the predominant form of alginates linked with the cellulose fibrils in the fibrillar portion). Fucans (or fucoidans, or ascophyllans) are sulfated polysaccharides containing, in addition to the monosaccharide L-fucose, varying proportions of galactose, mannose, xylose, and glucuronic acid [24.6]. The amorphous portion is responsible for the elasticity of the cell walls and contributes to prevent desiccation of the thallus during emersion.

Alginates confer flexibility upon the thallus, help prevent desiccation, and function in ion exchange [24.60]. They are used extensively in industry because of their colloidal properties. In alginates, the relative proportion of mannuronic acid and guluronic acid vary, depending on species, age of the thallus, tissue, time of the year, and geographic location. The sequence and the relative amounts of these two sugars produce different chemical structures, and, therefore, different physical properties of these polysaccharides. Haug showed that the affinity of some metal cations (Pb^{2+}, Cu^{2+}, Cd^{2+}, Zn^{2+}) for alginates differs in relation to the relative amounts of the two sugars, increasing with the content of guluronic acid. The high specificity of alginates containing high amounts of guluronic acid for bivalent cations is explained by their zig-zag structure. In the presence of these ions, the alginates develop a network-like arrangement by dimerization of polyguluronic sequences, which allows the pairing of two chains and the formation of spaces suitable to accommodate Ca^{++} and other bivalent cations similar to Ca^{++} in size. These spaces are produced by the grouping of polyguluronic chains determined by the alignment of carboxylates and other atoms of oxygens of the guluronic units. This structure is known as the egg-box model.

Alginates contain three different functional groups to which metal cations may bind, carboxyl (−COO), ether (C−O−C), and hydroxyl (−OH). Carboxyl groups are usually the most abundant acidic groups in brown algae, and most metals (Cd, Co, Cu, Fe, Ni, Pb) are adsorbed at pH close to the pH of dissociation of carboxyl groups in these algae (pK_0 close to 5) [24.62]. The second most abundant acidic group is the sulfonic acid of fucoidans [24.25]; this compound occurs both in the amorphous matrix and in close association with the cellulose microfibrils in the innermost portion of the wall [24.63–65]. Sulfonic acid plays a secondary role in the bond with metals, except when this happens at low pH. This is also the case for the hydroxyl groups present in all polysaccharides, which are negatively charged only at $pH > 10$ [24.25].

Proteins represent a minor component in the cell wall of brown algae [24.66]. This implies that the carboxyl groups of the alginates are more abundant than the carboxyl groups and the amine groups of proteins and represent the majority of the binding sites [24.58]. The role of the carboxyl groups in adsorption processes has been clearly demonstrated by the uptake of Cd and

Table 24.1 Binding groups for biosorption in macrophyte

Binding group	Chemical formula	Ligand atom	Occurrence in biomolecules	Phylum
Amine	$-NH_2$	Hydrogen	Protein Metallothionein	Chlorophyta Magnoliophyta
Carboxyl	$-COOH$	Oxygen	Cutin Alginic acid	Magnoliophyta Heterokontophyta
Carbonyl	$>C{=}O$	Oxygen	Alginic acid Protein Alginic acid	Heterokontophyta Chlorophyta Heterokontophyta
Hydroxyl	$-OH$	Oxygen	Polysaccharides Cutin Agarose (agar), carrageenan Fucoidan (sulfated polysaccharide) Agaropectin (agar), carrageenan	Chlorophyta Magnoliophyta Rhodophyta Heterokontophyta
Sulfonate	$-SO_2{=}O$	Oxygen	(Sulfated polysaccharides), porphyran, furcellaran (sulfated galactans)	Rhodophyta
Sulfhydryl (thiol)	$-SH$	Sulfur	Metallothionein	Magnoliophyta

Pb by *Sargassum* following partial or complete esterification of carboxyl sites [24.62].

24.3.2 Seagrass Cuticles

As all vascular plants, the seagrasses possess a wall of microfibrils of cellulose embedded in a matrix that consists mainly of pectins and cross-linking glycans [24.12]. The surface of the leaves is covered by a layer of polymeric material called cuticle, which consists of two types of polymers: cutin and cuticular waxes. This layer serves to avoid water loss, reduce penetration by chemicals and pathogenic organisms, and perhaps prevent adhesion of organ surfaces during development [24.12]. Since seagrasses live mostly submerged, they do not need protection against desiccation (which is the main function of cuticle); in these plants the cuticle is, therefore, thinner than in terrestrial angiosperms and the stomata are absent. The thin cuticle also facilitates uptake of ions and carbon; seagrasses are able to uptake nutrients and carbon directly through the leaves. In order to facilitate the exchange of solutes and gases, the cuticle of seagrasses is porous and perforated [24.67].

The cuticle forms an external layer around the surface of the leaves. Cutin and cuticular waxes, the main constituents of the cuticle, are large molecules with a complex tridimensional arrangement. Cutin is a complex compound formed by omega hydroxy acids and their derivatives interlinked via ester bonds and forming a polyester polymer of indeterminate size [24.68, 69]. Waxes are esters of fatty acids and long-chain alcohols; within the cutin network they exist in an amorphous state, but at the outer surface they crystallize to form plates and protrusions that may be very elaborate [24.12]. The cuticle may also contain several nonlipidic components such as carbohydrates and phenols [24.70]. It is also known that the polymeric matrix includes a considerable amount of dissociable groups [24.71] and is a polyelectrolyte with an isoelectric point around 3 [24.71].

24.3.3 Macrophytes: Key Chemical Functional Groups

Biosorption is based on the ability of biological materials to sequester metals [24.25, 53] through chemical-physical mechanisms [24.72] ranging from electrostatic forces, or Van der Waals interaction to ionic and covalent bonds [24.32]. Macrophytes show several chemical groups that have been suggested as being responsible for biosorption of heavy metals, such as carboxyl, hydroxyl, carbonyl, sulfhydryl, and sulfonate (Table 24.1) [24.24, 25, 38, 73, 74]. In particular, the matrix of the brown algal (phylum Heterokontophyta) cell wall consists mainly of alginic acid with carboxyl groups and a smaller amount of sulfated polysaccharides, such as fucoidan with sulfonic acid. In red algae (phylum Rhodophyta) the amorphous portion is formed by a number of sulfated galactans such as carrageenan, agar, furcellaran, porphyran. Green algae (phylum Chlorophyta) may have an external capsule that is composed of protein or polysaccharides or both (Table 24.1) [24.60]. In the leaves of seagrasses, carboxylic groups present on the cutin layer are probably responsible for the binding of heavy metal ions [24.24]. The biosorption ability of different chemical groups depends on many factors: the number of sites on the adsorbent material, accessibility of the site, their chemical status (i. e., availability), and the affinity between site and metal ions (i. e., bond strength) [24.25, 58, 75]. So it is important to understand what kind of interaction takes place between the functional groups present on the adsorbent material and ionic metal speciation in solutions; MEDUSA software can be used for this [24.76].

24.3.4 Chemical and Physical Mechanisms

Mechanisms involved in the biosorption process have a chemical-physical nature. More specifically, heavy metal chelation depends on the constituents of the outer layers of macroalgae and seagrasses. The biosorption capacity of different chemical groups depends on several factors: the number of sites on the adsorbent material, accessibility, their chemical status (availability), and the affinity between site and metal (bond strength) [24.77]. In particular:

1) Key functional groups (Table 24.1)
2) Ion-exchange processes, and
3) Ionic speciation of metals

have a fundamental role in biosorption studies. The carboxylic groups are abundant in brown algae, due to the presence of alginic acid, and in the cutin of seagrasses. The adsorption capacity of the macrophytes is directly associated with the presence of these sites on cell walls and on the cuticles of the nonliving biomass. Ionic speciation of metals in solution is important for metal uptake and frequently depends on the pH of the sorption system. The MEDUSA software [24.76] creates theoretical predictions of ionic dissociation of metals as a function of pH. The most common metals investigated, such as Pb^{2+}, Cd^{2+}, Cu^{2+}, Zn^{2+} and Fe^{2+}, show a maximal or nearly maximal sequestration at pH near the apparent dissociation constant of carboxylic acids ($R{-}COOH$), which indicates adsorption on carboxyl groups. The role of carboxylic groups in the adsorption process has been widely demonstrated through the adsorption capacity of lead and other metals cations by dried biomass of *Cystoseira*, *Scytosiphon*, *Sargassum*, *Ascophyllum* (brown algae), *Posidonia*, and *Cymodocea* (seagrasses) following partial or complete esterification of the carboxylic sites [24.24, 45, 75, 78–81]. The other acidic functional group that is important in biosorption activity is the sulfonate group of fucoidans (brown algae) and sulfated polysaccharides (red algae) (Table 24.1). Sulfonic acid groups have an important role when metal binding occurs at low pH values. Hydroxyl groups present in all macrophytes studied except brown algae (Table 24.1) become negatively charged at high pH values (about $pH > 10$), and consequently have a minor role at low pH [24.25, 75, 80]. Concerning heavy metals that have negative ionic speciation in solution, such as As(V), recent studies have demonstrated that red algae (phylum Rhodophyta), which are known to be bad binders of cationic metals, showed very good arsenic sorption performance at pH values higher than 7 [24.38].

Ion-exchange is an essential mechanism at the base of biosorption. It is the process in which ions are exchanged between a solution and an insoluble solid, usually a resin. During the biosorption activity, dried macrophytes can be viewed as natural ion-exchange materials that contain weakly acidic and basic groups (Table 24.1). In the pH range 2.5–5, the binding of heavy-metal cations is determined primarily by the state of dissociation of the weakly acidic groups (acid–base equilibrium theory) [24.25, 41]. In studies of biosorption, maintaining a low pH (around pH 4.5–5) in the sorption system, microprecipitation of metals and their contribution to the uptake are avoided. pH control in the system is important both for its effects on the configuration of the active ion-exchange sites and for the ionic state of the metals in the solution. In solution, at low pH the concentration of protons is high, and the ion-exchange sites, therefore, become solidly protonated. This allows displacing the metals sequestered on the biosorbent by a simple acidic wash. The regeneration of the biosorbent biomaterial allows its multiple recycling, further increasing the economy of its use [24.82, 83]. Consequently, the use of a biosorbent such as dried algae and seagrasses in wastewater treatment often depends not only on the biosorptive capacity, but also on how well the biosorbent can be regenerated and recycled.

24.4 Biosorption Experiments: Procedure

24.4.1 Biosorption: Kinetic Curves

The kinetic curves of adsorption describe the time evolution of the concentration of metal in solution, in the presence of the adsorbent biomaterial. A stock solution containing a known amount of metal salts is prepared to test the ability of the adsorbent macrophytes. Dried biomass of the macrophytesis is suitably fragmented to increase the adsorbing surface area and is placed in a beaker with deionized water. The pH of the solution is adjusted using solutions of NaOH and HCl (in general, 0.1 M) according to the experimental plan followed. Indeed, pH check control is important to avoid metal precipitation in solution. Subsequently, a stock solution with metals is added according to experimental conditions. Aliquot amounts of solution are then periodically sampled to determine the concentration of metal. In general, the kinetic curves are set for investigation of different pH values.

24.4.2 The Langmuir Adsorption Isotherm

The equilibrium isotherms represent the concentration of the metal on the solid (q, mg/g) as a function of the respective concentration in solution at equilibrium (C_{eq}, mg/L). The determination of isotherms is essential to define the capacity of the adsorption of metal ions by the adsorbent material, such as dried macrophytes. For the determination of curves, a known amount of dried and fragmented biomass is added to a solution of deionized water with varying concentrations of metals established from the experimental plan. The pH of the solutions is adjusted to predetermined values with solutions of NaOH (general 0.1 M) and HCl (general 0.1 M) [24.24, 38]. 1 mL aliquots of test solution are sampled periodically and centrifuged for metal determination. Metal uptake, q ($\mathrm{mg\,g^{-1}}$), is calculated as the difference in metal concentration in the aqueous phase before and after sorption, according to (24.1)

$$q = \frac{V\left(C_{\mathrm{i}} - C_{\mathrm{eq}}\right)}{W}\,, \tag{24.1}$$

where V is the volume of metal solution (L), C_{i} and C_{eq} are the initial and equilibrium concentration of metal in solution (mg/L), respectively, and W is the mass of dry macrophytes (g).

Through regression analysis, the Langmuir adsorption isotherm [24.84] given in (24.2) was adapted to the experimental data

$$q = \frac{q_{\max} b C_{\mathrm{eq}}}{1 + b C_{\mathrm{eq}}}\,, \tag{24.2}$$

where $q_{\max}$ (mg/g) and b (L/mg) are the Langmuir constants, which represent the maximum adsorption capacity and the affinity between the metal ion and adsorbent dried biomass, respectively.

24.5 Conclusions

Macrophytes represent particularly efficient biosorbents compared with other biomass types for the treatment of wastewater [24.24, 26, 37, 38, 45, 46]. Furthermore, due to their economic value in many food, cosmetic, and pharmaceutical applications, there is a large bulk of information about their cell wall structure and biochemical composition of the thalli [24.85–89]. Brown algae, in particular the orders Laminariales and Fucales, are the most important algae with respect to biosorption because of the abundance of their cell wall matrix polysaccharides and extracellular polymers (Table 24.1). Alginates and fucoidans (sulfated mucopolysaccharides) are mainly responsible for the natural ion exchange ability of brown algae. In fact, previous studies support the possibility that the biosorption process consists essentially of ion exchange, which involves the carboxyl group in the alginate polymer and sulfonic acid in the fucoidan [24.24, 26, 38]. The cuticle of seagrasses is also rich in carboxyl groups, which are involved in the bond with the protonated metal, while red and green algae have a lower capacity to immobilize protonated metals due of the presence of groups other than carboxylic ones (Table 24.1). In general, it is assumed that the number of binding sites identified decreases in the order brown algae > seagrasses > green algae > red algae.

Moreover, recent literature confirms that the structure of the thallus affects the adsorption performance of the algae [24.24–26, 38, 45]. *Pennesi* et al. [24.24] concluded that the sorption abilities of the red algae *Porphyra* and *Polysiphonia* might be related to the simple structure of the thallus (i. e., monostromatic blades and uncorticated filaments, respectively) compared to species of *Gracilaria*, which possess a more complex, parenchymatous thallus. Accordingly, it may be supposed that there is a direct relationship between the complexity of the thallus and the adsorption of lead [24.90].

As a whole, these results have opened new perspectives for the utilization of marine macrophytes as low-cost sorbents in the removal of heavy metals from wastewater; this is a key step towards the implementation of biosorption technology in industrial and environmental remediation.

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