PRECONCENTRATION OF HEAVY METALS IN ENVIRONMENTAL SAMPLES BY BIOSORPTION AND DETERMINATION BY ATOMIC SPECTROMETRY

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Ayşegül ŞEKER

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We approve the thesis of **Ayşegül ŞEKER**

Date of Signature

..... **18 July 2007**
Assoc. Prof. Dr. Ahmet E. EROĞLU
Supervisor
Department of Chemisty
İzmir Institute of Technology

..... **18 July 2007**
Assoc. Prof. Dr. Talal SHAHWAN
Co-Supervisor
Department of Chemisty
İzmir Institute of Technology

..... **18 July 2007**
Prof. Dr. Tamerkan ÖZGEN
Department of Chemisty
İzmir Institute of Technology

..... **18 July 2007**
Assoc. Prof. Dr. Meltem Conk DALAY
Department of Bioengineering
Ege University

..... **18 July 2007**
Assoc. Prof. Dr. Nusret ERTAŞ
Division of Analytical Chemistry
Gazi University

..... **18 July 2007**
Assoc. Prof. Dr. Serdar ÖZÇELİK
Department of Chemisty
İzmir Institute of Technology

..... **18 July 2007**
Prof. Dr. Levent ARTOK
Head of Department
İzmir Institute of Technology

.....
Prof. Dr. M. Barış ÖZERDEM
Head of the Graduate School

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ABSTRACT

PRECONCENTRATION OF HEAVY METALS IN ENVIRONMENTAL SAMPLES BY BIOSORPTION AND DETERMINATION BY ATOMIC SPECTROMETRY

In the assessment of environmental quality, one of the priorities must be given to the determination of heavy metals. In the present study, *Spirulina platensis,* a cyanobacteria (or blue-green alga) was suggested to be used as a biosorbent prior to the atomic spectrometric determination of Pb, Cd and Ni in some environmental samples. For this purpose, the parameters which might be effective on the biosorption were investigated such as pH, time, initial metal ion concentration, biosorbent amount, temperature, kinetics of sorption, repetitive reactivity and ionic competition.

According to the sorption kinetics, results obeyed well the pseudo second-order model. Freundlich, Dubinin Radushkevich and Temkin isotherm models were applied in describing the equilibrium partition of the ions. Freundlich isotherm was applied to describe the design of a single-stage batch sorption system. Thermodynamic parameters (ΔG^0, ΔH^0 and ΔS^0) were calculated and the sorption process was found to be largely driven towards the products and it had an endothermic nature. Faster adsorption kinetics was observed for Pb^{2+} ions in comparison to Cd^{2+} and Ni^{2+} ions. Based on kinetic modeling, the apparent activation energy, E_a, was calculated to be 44 $kJmol^{-1}$, -16 $kJmol^{-1}$ and 54 $kJmol^{-1}$ for Pb^{2+}, $Cd^{2+}$and Ni^{2+}, respectively.

The measurements of the repetitive reusability of *Spirulina platensis* indicated a large capacity towards the three metal ions. Sorption activities in a three metal ion system were studied and at an initial metal concentration of 100.0 mgL^{-1}, % Pb^{2+} was found to be still high (85%).However, it decreased to less than 20% for Cd^{2+} and Ni^{2+} indicating the relative selectivity of the biosorbent towards Pb^{2+}.

Finally, the use of *Spirulina platensis*, in its natural form or after being immobilized onto various matrices (alginate, silicate, carboxymethylcellulose and polysulfone) was planned for the separation of heavy metals from the sample matrix.

ÖZET

ÇEVRESEL ÖRNEKLERDEKİ AĞIR METALLERİN BİYOSORPSİYON YÖNTEMİYLE ÖN-DERİŞTİRİLMESİ VE ATOMİK SPEKTROMETRİ İLE TAYİNİ

Çevre kalitesinin değerlendirilmesi açısından önceliğe sahip alanlardan biri de ağır metal kirliliğinin belirlenmesidir. Bu çalışmada, bir siyanobakteri olan *Spirulina platensis*'in, çevre numunelerindeki Pb, Cd, Ni'nin atomik spektrometrik yöntemlerle tayin edilmesi öncesinde, biyosorbent olarak kullanılması hedeflenmektedir. Bu amaçla, biyosorpsiyona etki edebilecek pH, çalkalama zamanı, metal iyonları başlangıç derişimi, biyosorbent miktarı, sıcaklık, sorpsiyon kinetiği, biyosorbentin tekrar kullanılabilirliği ve iyon aktivitesi gibi parametreler incelenmiştir.

Kinetik sonuçlar tutunma tepkimesinin ikinci-dereceden hız denklemine uyduğunu göstermektedir. Ayrıca Freundlich, Dubinin Radushkevich ve Temkin izoterm modelleri uygulanmış, sorpsiyon sistem tasarımını tanımlamak amacıyla Freundlich izotermi kullanılmıştır. Termodinamik parametreler (ΔG^0, ΔS^0 ve ΔH^0) hesaplanmış ve sorpsiyonun ürünler yönünde ve endotermik olduğu belirlenmiştir. Pb^{2+}'nın sorpsiyon kinetiğinin Cd^{2+} ve Ni^{2+}'ya oranla daha hızlı olduğu belirlenmiştir. Buna göre E_a değerleri hesaplanmış ve Pb^{2+}, Cd^{2+} and Ni^{2+} için sırasıyla 44 $kJmol^{-1}$, -16 $kJmol^{-1}$ and 54 $kJmol^{-1}$ olarak bulunmuştur. Biyosorbentin tekrar kullanılabilirliği test edilmiş ve *Spirulina platensis*'in üç metal iyonuna karşı yüksek kapasiteye sahip olduğu saptanmıştır. Söz konusu metal iyonlarının bir arada olduğu sistemler incelendiğinde, 100.0 mgL^{-1} başlangıç derişiminde Pb^{2+} sorpsiyonunun hala yüksek olduğu (%85), ancak derişim arttıkça Cd^{2+} ve Ni^{2+} için sorpsiyon değerlerinin %20'nin altına düştüğü görülmüştür. Bu sonuç, *Spirulina platensis*'in Pb^{2+}'ya karşı göreceli olarak daha seçici davrandığını göstermektedir.

Sonuç olarak, *Spirulina platensis*'in serbest halde ve çeşitli katı yüzeylere (aljinat, silikat, karboksimetilselüloz ve polisülfon) sabitlenmiş olarak ağır metallerin numune matriksinden ayrılmasında kullanılması planlanmıştır.

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CHAPTER 1

INTRODUCTION

1.1. Heavy Metals

The term heavy metal refers to any metallic element that has a relatively high density and is toxic or poisonous at low concentrations. Examples of heavy metals include mercury (Hg), cadmium (Cd), arsenic (As), chromium (Cr)and lead (Pb).

Heavy metals are natural components of the Earth's crust. They cannot be degraded or destroyed. To a small extent they enter our bodies via food, drinking water and air. As trace elements, some heavy metals (e.g. copper, selenium, zinc) are essential to maintain the metabolism of the human body. However, at higher concentrations they can lead to poisoning. Heavy metal poisoning could result, for instance, from drinking-water contamination (e.g. lead pipes), high ambient air concentrations near emission sources, or intake via the food chain.

Heavy metals are dangerous because they tend to bioaccumulate. Bioaccumulation means an increase in the concentration of a chemical in a biological organism over time, compared to its concentration in the environment. Compounds can accumulate in living organisms any time after being taken up and stored faster than they are broken down (metabolized) or excreted.

Heavy metals can enter a water supply by industrial and consumer waste, or even from acidic rain breaking down soils and releasing heavy metals into streams, lakes, riversand groundwater (WEB_1 2007).

1.1.1. Cadmium

Cadmium is a natural element in the earth’s crust. It is usually found as a mineral combined with other elements such as oxygen (cadmium oxide), chlorine (cadmium chloride), or sulfur (cadmium sulfate, cadmium sulfide). All soils and rocks, including coal and mineral fertilizers, contain some cadmium. Cadmium does not corrode easily and has many uses, including batteries, pigments, metal coatings and plastics.

Cadmium enters air from mining, industry and burning coal and household wastes. Its particles in air can travel long distances before falling down to the ground or water. It enters water and soil from waste disposal and spills or leaks at hazardous waste sites. It binds strongly to soil particles. Some cadmium dissolves in water. It doesn't break down in the environment, but can change forms. Fish, plants and animals take up cadmium from the environment. Cadmium stays in the body for a very long time and can build up from many years of exposure to low levels.

Cadmium exposes to human via breathing contaminated workplace air (battery manufacturing, metal soldering or welding), eating foods containing cadmium, low levels in all foods (highest in shellfish, liverand kidney meats), breathing cadmium in cigarette smoke (doubles the average daily intake), drinking contaminated water, and breathing contaminated air near the burning of fossil fuels or municipal waste.

Breathing high levels of cadmium severely damages the lungs and can cause death. Eating food or drinking water with very high levels severely irritates the stomach, leading to vomiting and diarrhea. Long-term exposure to lower levels of cadmium in air, food, or water leads to a buildup of cadmium in the kidneys and possible kidney disease. Other long-term effects are lung damage and fragile bones. Animals given cadmium in food or water had high blood pressure, iron-poor blood, liver diseaseand nerve or brain damage.

The EPA has set a limit of 5 parts of cadmium per billion parts (5 ppb) of drinking water (WEB_2 2007). EPA doesn't allow cadmium in pesticides. The Food and Drug Administration (FDA) limits the amount of cadmium in food colors to 15 parts per million (15 ppm). The Occupational Safety and Health Administration (OSHA) limits workplace air to 100 $\mu g/m^3$ as cadmium fumes and 200 $\mu g/m^3$ as cadmium dust.

1.1.2. Lead

Lead is a naturally occurring bluish-gray metal found in small amounts in the earth's crust. Lead can be found in all parts of our environment. Much of it comes from human activities including burning fossil fuels, mining and manufacturing. Lead has many different uses. It is used in the production of batteries, ammunition, metal products (solder and pipes) and devices to shield X-rays. Because of health concerns,

lead from gasoline, paints and ceramic products, caulkingand pipe solder has been dramatically reduced in recent years.

Lead itself does not break down, but its compounds are changed by sunlight, airand water. When lead is released into the air, it may travel long distances before settling down to the ground. Once lead falls onto soil, it usually sticks to soil particles. Movement of lead from soil into groundwater will depend on the type of lead compound and the characteristics of the soil.

Lead exposes to human via eating food or drinking water that contains lead. Water pipes in some older houses may contain lead solder and lead can leach out into the water.

The effects of lead are the same whether it enters the body through breathing or swallowing. Lead can affect almost every organ and system in the body. The main target for lead toxicity is the nervous system, both in adults and children. Long-term exposure of adults can result in decreased performance in some tests that measure functions of the nervous system. It may also cause weakness in fingers, wrists, or ankles. Lead exposure also causes small increases in blood pressure, particularly in middle-aged and older people and can cause anemia. Exposure to high lead levels can severely damage the brain and kidneys in adults or children and ultimately cause death. In pregnant women, high levels of exposure to lead may cause miscarriage. High level exposure in men can damage the organs responsible for sperm production. EPA limits lead in drinking water to 15 μg per liter (WEB_3 2007).

1.1.3. Nickel

Nickel is a very abundant natural element. Pure nickel is a hard, silvery-white metal. Nickel can be combined with other metals, such as iron, copper, chromium and zinc, to form alloys. These alloys are used to make coins, jewelry and items such as valves and heat exchangers. Most nickel is used to make stainless steel. Nickel can combine with other elements such as chlorine, sulfur and oxygen to form nickel compounds. Many nickel compounds dissolve fairly easily in water and have a green color. Nickel compounds are used for nickel plating, to color ceramics, to make some batteries and as chemical catalysts. Nickel is found in all soil and is emitted from

volcanoes. Nickel is also found in meteorites and on the ocean floor. Nickel and its compounds have no characteristic odor or taste.

Nickel is released into the atmosphere by industries that make or use nickel, nickel alloys, or nickel compounds. It is also released into the atmosphere by oil-burning power plants, coal-burning power plants and trash incinerators. In the air, it attaches to small particles of dust that settle down to the ground or are taken out of the air in rain or snow; this usually takes many days. Nickel released in industrial waste water ends up in soil or sediment where it strongly attaches to particles containing iron or manganese. Nickel does not appear to accumulate in fish or in other animals used as food.

Nickel exposes to human via eating food containing nickel, which is the major source of exposure for most people; by skin contact with soil, bath or shower water, or metals containing nickel, as well as by handling coins or touching jewelry containing nickel. Human may also be exposed to nickel by drinking water that contains small amounts of nickel, by breathing air or smoking tobacco containing nickel. Higher exposure may occur in industries that process or use nickel.

The most common harmful health effect of nickel in humans is an allergic reaction. Approximately 10-20% of the population is sensitive to nickel. People can become sensitive to nickel when jewelry or other things containing it are in direct contact with the skin for a long time. Once a person is sensitized to nickel, further contact with the metal may produce a reaction. The most common reaction is a skin rash at the site of contact. The skin rash may also occur at a site away from the site of contact. Less frequently, some people who are sensitive to nickel have asthma attacks following exposure to nickel. Some sensitized people react when they consume food or water containing nickel or breathe dust containing it. People working in nickel refineries or nickel-processing plants have experienced chronic bronchitis and reduced lung function. These persons breathed amounts of nickel much higher than levels found normally in the environment. Workers who drank water containing high amounts of nickel had stomach ache and suffered adverse effects to their blood and kidneys. Damage to the lung and nasal cavity has been observed in rats and mice breathing nickel compounds. Eating or drinking large amounts of nickel has caused lung disease in dogs and rats and has affected the stomach, blood, liver, kidneys and immune system in rats and mice, as well as their reproduction and development.

The EPA recommends that drinking water should contain less than 0.1 mg/L. To protect workers, the Occupational Safety and Health Administration (OSHA) has set a limit of 1 mg/m^3 of air for metallic nickel and nickel compounds in workplace air during an 8-hour workday, 40-hour workweek (WEB_4 2007).

1.2. Heavy Metal Pollution

Toxic heavy metal contamination of the environment is a significant worldwide phenomenon. It is well perceived that there is a permissible limit of each metal above which they are generally toxic and and some are hazardous.

Traditional technologies for the removal of heavy metals, such as ion exchange or lime precipitation, are often known to be ineffective or very expensive when used for the reduction of heavy metal ions at very low concentrations. For this reason, new technologies are said to be required which can reduce heavy metal concentrations to environmentally acceptable levels at affordable costs. One of these alternative techologies is biosorption which is defined as the accumulation and concentration of pollutants from aqueous solutions by the use of biological materials, thus allowing the recovery and/or environmentally acceptable disposal of the pollutants could greatly contribute to the achievement of this goal (Madrid and Camara 1997).

1.3. Biosorption of Heavy Metals

It was only in the 1990s that a new scientific area developed that could help to recover heavy metals: biosorption. The first reports described how abundant biological materials could be used to remove, at very low cost, even small amounts of toxic heavy metals from industrial effluents. Metal-sequestering properties of non-viable biomass provide a basis for a new approach to remove heavy metals when they occur at low concentrations (Volesky 1990). Note that metals can be removed from solution only when they are appropriately immobilized, the procedure of metal removal from aqueous solutions often leading to effectively concentrating the metal. That aspect of biosorption makes the eventual recovery of this waste metal easier and economical.

The assessment of the metal-binding capacity of some types of biomass has gained momentum since 1985 as reported (Volesky and Holan 1995). Indeed, some

biomass types are very effective in accumulating heavy metals. Availability is a major factor to be taken into account to select biomass for clean-up purposes. The economy of environmental remediation dictates that the biomass must come from nature or even has to be a waste material. Seaweeds, molds, yeasts, bacteria, crabshells, among other kinds of biomass, have been tested for metal biosorption with very encouraging results.

Some biosorbents can bind and collect a wide range of heavy metals with no specific priority, whereas others are specific for certain types of metals (Hosea et al. 1980, Volesky and Kuyucak 1988). When choosing the biomass for metal biosorption experiments, its origin is a major factor to be taken into account. Biomass can come from (i) industrial wastes which should be obtained free of charge; (ii) organisms easily available in large amounts in nature; and (iii) organisms of quick growth, especially cultivated or propagated for biosorption purposes. Cost effectiveness is the main attraction of metal biosorption and it should be kept that way. Not only should microbial biomass be used directly, but biosorbents derived from it in a simple process should be most low-priced for economical metal-removal process applications. If, for any reason, by-products of fermentation processes would not be available, biosorbents could be produced by using relatively unsophisticated and low-cost culture propagation techniques. Nutrients from readily available and inexpensive sources such as carbohydrate-rich industrial wastewaters, which often pose pollution/treatment problems, such as food, dairy and starch industries, might be conveniently used. On the contrary, the costs of biosorbents especially produced could be higher and affect negatively the overall economy of their application (Kuyucak 1990, Volesky 1987, Yerushalmi 1990).

Whereas the use of synthetic ion exchangers can be considered a mature technology, biosorption is in its developmental stages and further improvement in both performance and costs can be expected (Volesky 1999). Biosorbents are prepared from the naturally abundant or waste biomass of mainly algae, fungi or bacteria that have been killed by washing biomass with acids or bases, or even both, before final drying and granulation (Brierley J.A. 1990, Kratochvil et al. 1997). Figure 1.1. schematically summarizes alternative process pathways to produce biosorbent materials which are effective and durable in repeated long-term applications aimed mainly at removing metals from large quantities of toxic industrial metal-bearing effluents. Whereas the preparation of biomass is an extremely important aspect, this type of process development is based on trial-and-error routines but obviously challenging because of

the many biomass raw materials. Abundant natural materials, particularly of cellulosic nature, have been suggested as potential biosorbents for heavy metals. It is mainly the bacterial cell wall that contains chemical compounds with sites capable of passively sequestering metals (Remacle 1990). Numerous chemical groups have been suggested to contribute to biosorption metal binding by either whole organisms such as algae (Crist et al. 1981, Greene et al. 1987) and bacteria (Brierley C.L. 1990, Mann 1990) or by molecules such as biopolymers (Hunt 1986, Macaskie and Dean 1990). These groups comprise hydroxyl, carbonyl, carboxyl, sulfhydryl, thioether, sulfonate, amine, imine, amide, imidazole, phosphonate and phosphodiester groups. The importance of any given group for biosorption of a certain metal by a certain biomass depends on factors such as the number of sites in the biosorbent material, the accessibility of the sites, the chemical state of the site (i.e. availability)and affinity between site and metal (i.e. binding strength).

For covalent metal binding even an already occupied site is theoretically available. The extent to which the site can be used by a given metal depends on its binding strength and concentration as compared to the metal already occupying the site. For electrostatic metal binding, a site is only available if the metal is ionized.

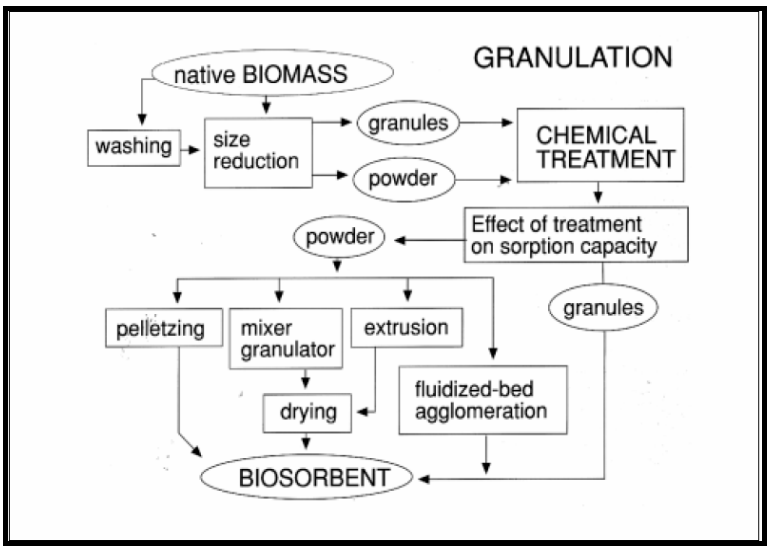


Figure 1.1. Schematic diagram of processing different types of microbial biomass into usable biosorption materials (Vieira and Volesky 2000).

1.4. The Use of Algae as Metal Biosorbents

The use of various types of algae and living, dead, immobilized and algal derived products which can be used as metal biosorbents must be described. This involves metal biosorption using photosynthetic microorganisms, both unicellular microalgae and cyanobacteria (formerly known as the blue-green algae), as well as multicellular macroalgae.

Large quantities of metals can be accumulated by a variety of processes dependent and independent on metabolism. Both living and dead biomass as well as cellular products such as polysaccharides can be used for metal removal. Such algal biomass can be used in a 'natural form' free in solution or immobilized by various techniques on to a solid support or to produce granules for a metal removal/recovery process. Algal biosorption techniques/processes can be used to remove toxic metals and/or radionuclides from liquid effluents before their safe discharge in addition to their use as a recovery process for metals of value. In the majority of cases biosorption of metals can be considered as rapid metabolism-independent ionic and covalent binding to a particular structure of the cell surfaces, although initial binding to the cell surface may be followed by further inorganic deposition of increased amounts of metal, possibly up to 50% of dry weight (Gadd 1990).

> ‘In addition, precipitation or crystallisation of metals may occur within and around cell walls as well as the production by algae of metal binding polysaccharides and siderophores: these are processes which could be considered as biosorption, but may in some cases be dependent on cell metabolism and are thus better termed bioaccumulation.
> Active metal uptake by energy-dependent transport systems should not and will not, be considered as biosorption. An important feature of biosorption is that dead cells can be responsible for binding and accumulation of metals. Remaining cell debris such as cell walls can still represent a potent biosorbent’(Volesky 1990).

So far there has been little commercial exploitation of algal biosorption for metal removal or recovery processes, despite the wealth of information and studies on

such algal-based processes and, in many cases, the availability of abundant and relatively inexpensive algal biomass for use in future metal biosorption processes (Kuyucak 1990).

1.5. Factors Affecting the Biosorption of Metals by Algae

Previously biosorption of metals by algae has been described as being dependent on the algal species used and differences in the cell wall composition of the species. However, other factors affecting the biosorption of metals by algal biomass should be considered, such as cell size and morphology, pH of external media, cation and anion concentration of external media, metal speciation, temperature and physiology of the algal biomass used (Forster and Wase 1997).

Cell size and morphology: Differences observed in the amounts of metal biosorbed by different algal species can be explained by differences in cell size and shape between species, in addition to cell wall structure differences. The greater the cell surface area to dry weight ratio, the greater the amount of metal biosorbed by a cell surface per unit dry weight (Kuyucak 1990).

pH of external medium: The pH of metals by algae is dependent on the pH of the external medium. Generally there is increased biosorption of cationic metal species with increasing pH values. The isoelectric point of most algal cell walls lies between pH 3 and 4, thus, the net overall charge on the cell wall under low pH conditions promotes easier access of anions to positively charged binding sites as the pH is decreased below the isoelectric point (Forster and Wase 1997).

Cation and anion concentration of external media: Concentrations of cations depress the biosorption of a metal of interest or the biosorption of other cations. Such effects can be explained in terms of competition between ions for the same binding sites on the algal biomass. Studies of the effect and and competition have led to the selectivity of biosorption based on the biosorption observed for mixtures of metal cations at the same concentration. The selectivity in biosorption can be explained by

binding sites on algae having preferences for ‘hard’ or ‘soft’ metal ions (Grene and Darnall 1990).

Metal speciation: The charge of a metal species will affect how much metal is biosorbed and how pH and competing ions affect biosorption (Forster and Wase 1997).

Temperature: In most biosorption studies with algae, temperature is kept usually close to that of the temperature of the laboratory during experiments (Forster and Wase 1997).

Physiological state of algal biomass: This greatly affects the amount of metal biosorbed by algal biomass. There is a large difference between metal biosorbed by dead and live algal biomass. In most studies biosorption of metals is greater with dead biomass as compared to an equal amount of live biomass. When algal biomass is in dead state the cells are permeable and allows metals to enter and bind on internal components and surfaces of the cell as well as the external surface, thus increasing the metal uptake (Horukoshi et al.1979).

1.6. Biosorption Mechanisms

Biosorption is considered as a potential strategy for the removal of metals from solutions for toxic metal removal, but also for precious metal recovery. Microbial biomass consists of small particles with low density, poor mechanical strength and little rigidity. Contacting large volumes of metal-bearing aqueous solutions with microbial biomass in conventional unit processing operations is not practical, largely because of solid/liquid separation problems (Brierley C.L. and Brierley J.A. 1993). The immobilization of the biomass in solid structures are told to create a material with the right size, mechanical strength, rigidity and porosity necessary for use in columns. Immobilization can also yield beads or granules that can be stripped off metals, reactivated and re-used in a manner similar to ion exchange resins and activated carbons (Brierley C.L. and Brierley J.A. 1993). The possibility of using the biosorbent material in subsequent adsorption-desorption cycles would also substantially improve the economics of biomass technical applications (Tsezos 1984). The economics of the

process is also improved by using waste biomass instead of purposely produced biomass. The recovery of the adsorbed metal could be achieved by the use of an appropriate eluent capable of effectively stripping the metal from the biomass and bringing it back to solution, yielding a concentrated solution of the metal of interest.

The complexity of the microorganism's structure implies that there are many ways for the metal to be captured by the cell. Biosorption mechanisms are therefore various and in some cases they are still not very well understood. Figure 1.2. shows schematically the various biosorption mechanisms. Transport of the metal across the cell membrane yields intracellular accumulation, which is dependent on the cells' metabolism. This implies that this kind of biosorption may take place only with viable cells. It is often associated with an active defense system of microorganisms, which react in the presence of a toxic metal. Obviously, in this case biosorption is not immediate, since it requires the time for the reaction of the microorganism.

In the case of physicochemical interaction between the metal and functional groups of the cell surface, based on physical adsorption, ion exchange and complexation, have cell surface sorption may occur which is not dependent on the metabolism (Figure 1.2). Cell walls of microbial biomass, mainly composed of polysaccharides, proteins and lipids, offer particularly abundant metal-binding functional groups, such as carboxylate, hydroxyl, sulphate, phosphate and amino groups. This physicochemical phenomenon of metal biosorption, non-metabolism dependent, is relatively rapid and can be reversible (Kuyucak and Volesky 1988). In the presence of such a mechanism, which fortunately is the most common, biomass has all the chemical characteristics of an ion exchange resin or of an activated carbon, implying many advantages in the industrial application of biosorption. In the case of precipitation, the classification is not unique.

In fact the precipitation of the metal may take place both in solution and on the cell surface (Ercole et al. 1994). Furthermore, it may be dependent on the cells' metabolism if, in the presence of toxic metals, the microorganism produces compounds which favour the precipitation process. On the other hand, precipitation may not be dependent on the cells' metabolism, occurring after a chemical interaction between the metal and the cell surface.

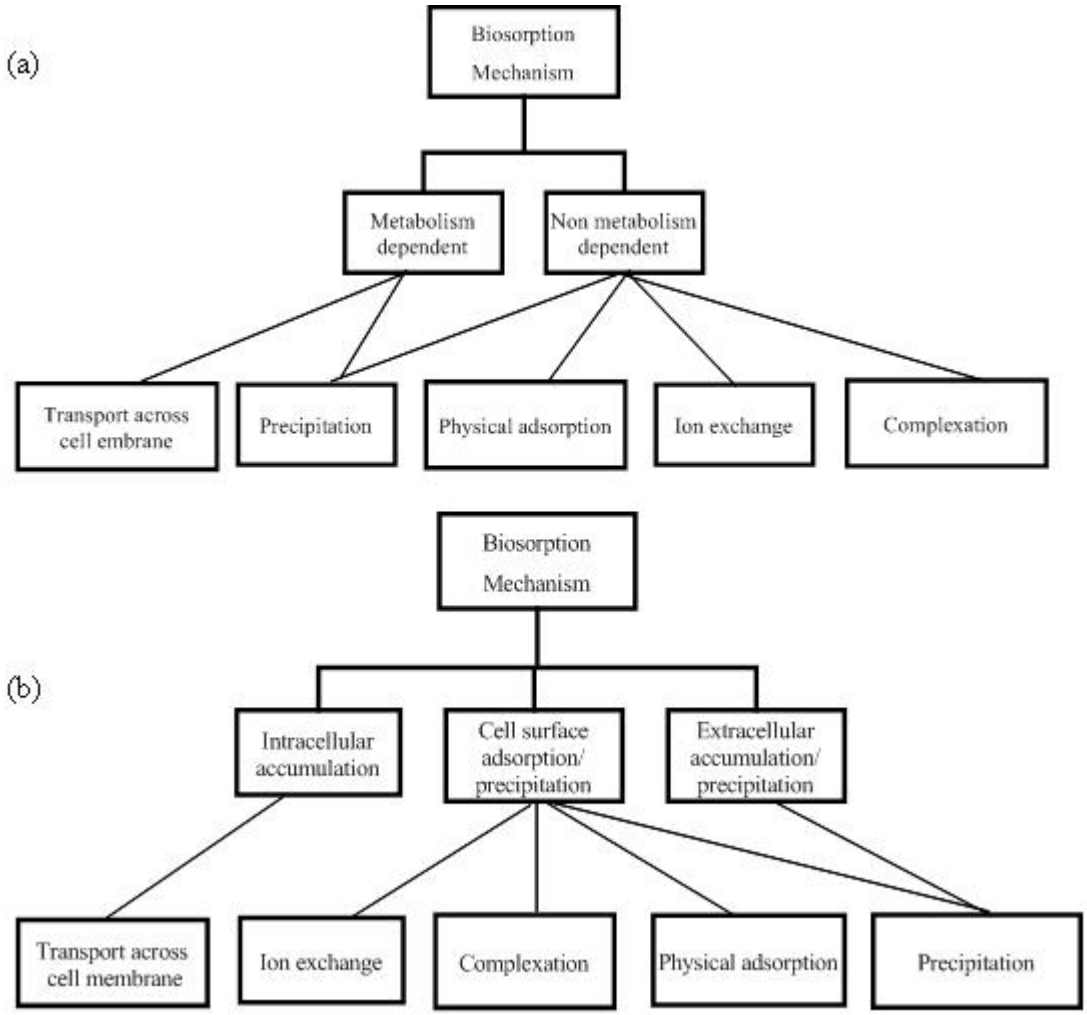


Figure 1.1. Biosorption mechanisms. (a) Classified according to the dependence on the cells' metabolism (b) Classified according to the location where the metal removed is found (Veglio and Beolchini 1997)

1.6.1. Transport Across Cell Membrane

As mentioned above, this phenomenon is associated with cell metabolism. Unfortunately, it is the toxicity of some elements which does not allow investigation of biosorption in the presence of high metal concentrations. In fact, little information is available about this kind of mechanism. Heavy metal transport across microbial cell membranes may be mediated by the same mechanism used to convey metabolically essential ions, such as potassium, magnesium and sodium. The metal transport system may become confused by the presence of heavy metal ions of the same charge and ionic radius (Brierley 1990).

1.6.2. Physical Adsorption

In this category phenomena associated with the presence of van der Waals' forces are included (Crowell 1966). It is hypothesized that uranium, cadmium, zinc, copper and cobalt biosorption by dead biomass of algae, fungi and yeasts takes place through electrostatic interactions between ions in solution and cells walls (Kuyucak and Volesky 1988). Physical adsorption is furthermore responsible for the biosorption of copper, nickel, zinc, cadmium and lead by *Rhizopus arrhizus* (Zhou and Kiff 1991, Fourest and Roux 1992).

1.6.3. Ion Exchange

Cell walls of microorganisms contain polysaccharides as basic building blocks. The ion exchange properties of natural polysaccharides have been studied in detail and it is a well established fact that bivalent metal ions exchange with counter ions of the polysaccharides. Alginates of marine algae usually occur as natural salts of K^{+}, Na^{+}, Ca^{2+} and/or Mg^{2+}. These metallic ions can exchange with the counter ions such as Co^{2+}, Cu^{2+}, Cd^{2+} and Zn^{2+}, resulting in the biosorptive uptake of the metals (Tsezos and Volesky 1982).

1.6.4. Complexation

The metal removal from solution may also take place through complex formation on the cell surface after interaction between the metal and active groups. Metal ions can bind to unidentate ligands or through chelation (Cabral 1992).

1.6.5. Precipitation

As mentioned above, precipitation may be either dependent or independent on the cellular metabolism of it. In the former case, the metal removal from solution is often associated with an active defense system of microorganisms (Veglio and Beolchini 1997).

1.7. Biosorption by Free Cells

The term *free cells* indicates non-immobilized microorganisms, which are free in aqueous solutions. Contacting large volumes of metal-bearing aqueous solutions with microbial biomass in conventional unit processing operations is not practical, largely due to solid/liquid separation problems. However, the investigation of the performance of free cells is fundamental for the industrial application of biosorption, because it gives information about the equilibrium of the process. This is necessary for the design of equipment. The uptake is usually measured by the parameter q (milligrams of metal accumulated per gram of biosorbent material).

A global analysis of the results found in literature suggest the following about the influence of operating conditions on the equilibrium of the biosorption process:

1. Temperature seems not to influence biosorption performances in the range 20-35°C (Aksu et al. 1992).

2. pH seems to be the most important factor in the biosorption process: it affects the solution chemistry of the metals, the activity of functional groups in the biomass and the competition of metallic ions (Tsezos and Volesky 1981).

3. Biomass concentration in solution seems to influence the specific uptake: for lower values of biomass concentrations there is an increase in the specific uptake (Fourest and Roux 1992). It was suggested that an increase in biomass concentration leads to interference between binding sites. Fourest and Roux invalidated this hypothesis, attributing the responsibility of the specific uptake decrease to metal concentration shortage in solution (Gadd et al. 1988). This factor would, however, need to be taken into account in any application of biomass as adsorbent.

4. Biosorption is, in some cases, selective. This aspect has to be investigated in detail. Biosorption is mainly applied to treat waste water containing metal ions and the removal of one metal may be influenced by the presence of other metals (Veglio and Beolchini 1997).

1.8. Biosorption by Immobilized Cells

Immobilization of the biomass in solid structures creates a material with the right size, mechanical strength, rigidity and porosity necessary for use in unit operations typical of chemical engineering. Various techniques are used for the biomass immobilization. The principal techniques found in the literature for the application of biosorption are based on adsorption on inert supports, on entrapment in a polymeric matrix, on covalent bonds to vector compounds, or on cells cross-linking.

The free cells generally have low mechanical strength and small particle size; therefore excessive hydrostatic pressures are required to generate suitable flow rates. High pressures can cause disintegration of free biomass. These problems can be avoided by the use of immobilized free cell systems. Immobilized biomass offers many advantages including better reusability, high biomass loading and minimal clogging in continous flow systems.

Cell immobilization is a general term that describes many different forms of cell attachment or entrapment. The physical entrapment of organisms inside a polymeric matrix, generally described as a gel, is one of the most widely used techniques for immobilization because a polymetric matrices can be made as beads with optimize mechanical strength, rigidity and porosity characteristics (Lu and Wilkins 1995). Either natural biopolymers (polysaccharides, alginate, agar) or synthetic polymer (polyethers, polyacrylates) can be used as gel-forming agents. Alginate gel and silica gel are two commonly used entrapment matrices.

Figure 1.3. shows schematically the various techniques found in literature for biomass immobilization. Examples of each technique are reported as follows.

Adsorption on inert supports: Support materials are introduced into the tower fermenter prior to sterilization and inoculation with starter culture and are left inside the continuous culture for a period of time, after which a film of microorganisms is apparent on the support surfaces.

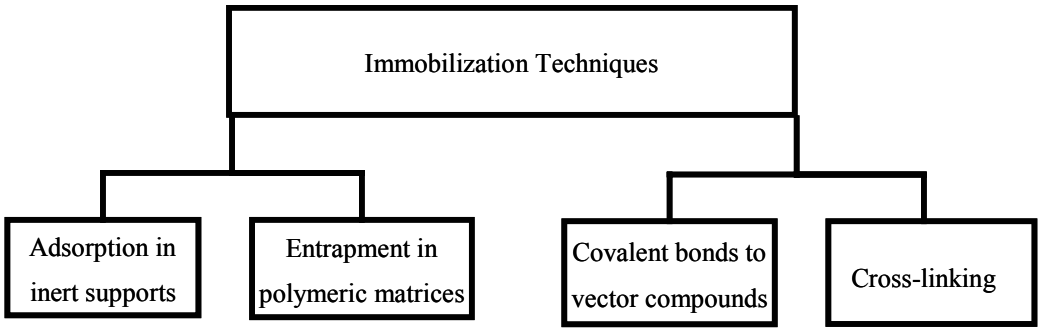


Figure 1.3. Immobilization techniques.
(Source: Veglio and Beolchini 1997)

Entrapment in polymeric matrices*:* The polymers used are: calcium alginate (Babu et al. 1993, Costa and Leite 1991) polyacrylamide (Macaskie et al. 1987, Michel et al. 1986), polysulfone (Jeffers 1991) and polyethylenimine (Brierley C.L. and Brierley J.A 1993). The materials obtained from the immobilization in calcium alginate and polyacrylamide are in the form of gel particles. Those obtained from immobilization in polysulfone and polyethylenimine proved to be the strongest.

Covalent bonds to vector compounds: The most common vector compound is silica gel. The material obtained is in the form of gel particles. This technique is used above all for algae immobilization (Holan et al. 1993).

Cross-linking: The addition of a cross-linker leads to the formation of stable cellular aggregates. This technique was found to be used above to immobilize algae. The most common cross-linkers are: formaldehyde, glutaric dialdehyde, divinylsulfone and formaldehyde-urea mixtures (Holan et al. 1993).

1.9. Immobilized Algae and Derived Products

Microalgae or macroalgal fragments are said to be used being immobilized in a matrix which develops many processes for the biosorption of metals. Two types of immobilization strategy are reported which can be adopted with microalgae, either active or passive entrapment. Active entrapment is stated to involve the culturing of the required biomass before its entrapment within a polymeric matrix, whereas passive methods depend on microalgal growth to invade the matrix (Robinson et al. 1986). Natural polymers used for entrapment are known such as kappacarrageenan or

alginates. These are said to have the advantage that they tend not to be toxic toward the algal cells which remain viable within the matrix whilst artificial polymers and matrices such as acrylamide, polyurethane or silica may be toxic towards the cells. However, as regards metal biosorption this is not a problem since it is a passive process. Such active methods are reported to be used to produce a biosorptive material in the form of beads, granules or pellets. Passive immobilization methods are stated to include the use of polyurethane foam matrices, china clay particles, glass beads or other inert matrices which are colonised by the algae (Robinson et al. 1986). It has been expressesd that many systems using immobilized microalgae suffer from poor cell retention. Such cell leakage on a commercial scale could cause severe problems. In addition to this problem, many of the immobilized systems, e.g. alginate or polyacrylamide (Nakajima et al. 1982, Garnham et al. 1992c), are told to tend not to be very robust and fall apart under certain conditions, many of which are found in industrial environments.

> 'Macroalgae are often dried before use, homogenised to a particular size and cross-linked with formaldehyde as well as being actively immobilized before use. Dry algal biomass before use, either powdered or in chunks or granules, will tend to swell on wetting, breaking up and making handling difficult (Volesky 1990), thus a support matrix is often essential to processes utilising it.'

The loss of biomass from an immobilized system is thought to be less than that from one based on free cells, which will in turn reduce the cost (Ashley and Roach 1989)

1.10. Production of Algal Biomass for Metal Removal

Systems have been developed utilizing actively growing algae in ponds or lagoons for wastewater treatment and metal removal where the metal concentrations encountered are not toxic to the algal biomass (Hammouda et al. 1995). Filamentous algae and cyanobacteria, such as *Cladophora*, *Spirogyra*, *Rhizodonium* and *Oscillatoria*, are told to remove copper, zinc, lead and manganese that allowed discharge of the effluent from a lead mine (Gale and Wixon 1979). For these systems no biomass is

reported to be produced as such - once the algae are established they grow within the system, photosynthesising and utilising the nutrients in the effluent - thus the cost of biomass production in such systems is zero. However, a practical limitation of these systems is that algal growth is inhibited by high metal concentrations or when significant amounts of metal ions are biosorbed by the algae (Bedell 1990). In addition, such systems tend to perform best in warm, sunny climates where algal growth is encouraged (Wong and Chan 1990). Metal removal systems which use pre-grown algal biomass or algal biomass harvested from the natural environment avoid the problem of maintaining growth under adverse circumstances and have received much attention as they are perceived as being more viable processes to be utilized by industry, especially in temperate climates. Such processes, however, usually involve the production, harvesting, storage and transportation of biomass, all of which incur costs.

Only eight microalgae have been reported to be grown on a commercial scale. These are *Spirulina, Chlorella, Scenedesmus*, *Phaedactylum, Botryococeus*, *Chlamydomonas*, *Dunaliella*- and *Porphyridium* (Bedell 1990). They are told to be usually grown for the production of animal feed, chemicals/biochemicals or fertilizer, or as a food source for humans, *Chlorella* and *Spirulina* have also been grown for use in a metal removal process. This is because they are the cheapest to grow and give the greatest yield and not because of their metal biosorption qualities. The cost of production of 1 Ib (0.45 kg) of these microalgae is approximately 7US$ (Bedell 1990). Many algae used in laboratory-scale processes biosorb more metal and perform better in metal removal processes than *Chlorella* and *Spirulina*, but have not been grown on a large scale as this would be more costly. The eight genera can be obtained cheaply as waste products from other processes and no cost is incurred, apart from processing and transportation of the biomass. However, once the value of such biomass for metal removal was recognized, the value and cost would rise.

For the optimum growth and production of microalgal biomass for metal biosorption, optimum pH, temperature, mineral concentration, CO_2, and illumination must be maintained. Sunlight and temperature tend to be the two limiting factors to outdoor algal production. The optimum temperature for growth is dependent on the algal species, but fluctuation above or below these optimum temperatures of as little as 3°C will affect the yield of algae (Payer et al. 1980). If all growth factors are optimal, the yield of microalgal biomass is dependent solely on the seasonal light flux. In addition to pond systems, specialized outdoor and indoor reactors for the cultivation of

microalgae are being developed which may increase biomass yields and reduce costs (Chrismadha and Borowitzka 1994). An artificial light source can be used to culture microalgae, often in conjunction with a reactor normally used for the production of microbial biomass but with a light transmitting culture vessel. This is a costly way of producing microalgae just for a metal biosorption process and would probably make any metal removal process uneconomical.

An obvious problem with the cultivation of microalgae in ponds/lagoons is stated to be that cultures are subject to contamination by other algal species, bacteria and fungi, However, if a culture can be maintained at a temperature greater than 35°C and a pH value either > pH 9 or < pH 4, less contamination will occur (Bedell 1990). Usually, a medium is used which has been established as the most suitable for a particular algal species, although such a medium may be complex and expensive, thus increasing the cost of the algal biomass. Various studies have been reported to be still developed for cheaper production of algal biomass (Oron et al. 1979).

1.11. Commercial Algal Biosorption

A range of separate technologies is reported to be available for metal recovery from process liquors or waste streams (Eccles and Holroyd 1993).These include: adsorption to materials such as activated charcoal or entrapment within zeolitic structures, biological mechanisims such as biosorption, bioprecipitation, intracellular accumulation and oxidation or reduction reactions mediated by biological systems, electrochemical methods or metal reduction to soluble forms, use of specific or non-specific ion-exchange materials, precipitation with lime or caustic soda. However, most of these technologies may have limited tolerance to suspenden solids which cause fouling. Fouling can also occur due to the interaction of adsorbents, membranes and ion-exchange resins with organic molecules. All the systems have a limited ability to have wide range of metal concentrations and, especially, dilute metal streams which can cause significant environmentalic problems.

Algal biosorption systems can overcome several of these limitations. An algal-based biosorption system should be suitable for a wide range of metal concentrations from 100 ppm to 100 ppb level or even less and should also allow a range of metal selectivities to be obtained (Eccles and Holroyd 1993). The full cost of an algal based

biosorption metal recovery system are not well documented, although Volesky (1990) compared the cost of algal-based biosorbents with those of other types of metal adsorbents. He described the cost of specifically cultered marine microalgae biosorbent as ranging from 7US$-l lb and an ion-exchange resin cost 7US$-14 lb (1lb=0.45kg).

To summarise, algal biosorption systems have not been commercialized even though they possess several advantages over currently available technologies for metal separation such as, selectivity for heavy metals and cost-effectivenes against alternative processes. One reason why algal biosorption systems have not been widely used in industry might be the lack of knowledge about the engineering of such systems. Engineers may like to adopt ion-exchange methods which they understand better than biological-based systems. Thus it is suggested that it is necessary to adopt a multidisciplinary approach in which chemists, biologists and engineers work together (Forster and Wase 1997).

Immobilizing biomass in a compact, form such as pellets or granules avoids the disadvantages of free cells, i.e. small particle size, low mechanical strength and low density. Hence, systems based on uniform granules of optimum size, is believed to be more advantageous. The loss of biomass from an immobilized system is thought to be less than that from one based on free cells (Ashley and Roach 1989).

1.12. Main Analytical Applications of Biological Organisms

The use of biosorption as an analytical tool for metal speciation and preconcentration is a recent phenomenon. Microorganisms, potentially the cell walls have a variety of functional sites which can be active in metal binding. These are amine and carboxyl groups from amino acids and polysaccharides, sulfohydryl groups and unmethylated pectins. Different metal ions can be separated selectively by changing experimental conditions (Madrid and Camara 1997).

1.13. Characteristics of Cyanobacteria

Cyanobacteria are aquatic and photosynthetic, that is they live in the water and can produce their own food. Because they are bacteria, they are quite small and usually unicellular, though they often grow in colonies large enough to see. Because of its

photosynthetic nature, cyanobacteria are often called ‘‘blue-green algae’’. They get their name from the bluish pigment phycocyanin, which is used to capture light for photosynthesis. They also contain chlorophyll a, the photosynthetic pigment that plants use.

Cyanobacteria may be single-celled or colonical. Depending on the conditions, colonies may form filaments, sheets or hollow bells. They are found in almost every habitat, from oceans to fresh water to bare rock to soil. In short they have no one habitat and you can find them almost anywhere in the world. One of the most common example of cyanobacteria is *Spirulina* (WEB_5 2007).

1.13.1. *Spirulina*: overwiev

Spirulina is a blue-green algae (cyanobacterium) belonging to the family of Oscillatoriaceae and forms unbranched, multicellular helicoidal filaments with a length of about 5-10μm broad (Hedenskog and Hofsten 1970). *Spirulina platensis*, a member of blue-green algae, is an alternative source of protein for human food and feed purposes. Other than protein, it involves polysaccharides, lipids and vitamins within (Ciferri 1983). Its chemical composition includes proteins (55-70%), carbohydrates (15-25%), essential fatty acids (18%) vitamins, minerals and pigments. These contain a variety of functional groups such as carboxyl, hydroxyl, sulphate and other charged groups which are responsible for metal binding (Li et al. 2006).

Spirulina cell wall is reported to form by four numbered layers, from the inner most outward as: LI, LII, LIII and LIV. All these layers are very weak, except layer LII made up of peptidoglycan, a substance that gives the wall its rigidity (Ciferri 1983). The LI layer contains β-1,2-glucan, a polysaccharide not very digestible by human beings. However, the low concentration (<1%) of this layer, and the protein and lipo-polysaccharide nature of the LII layer are responsible for the easy human digestion of *Spirulina* (Balloni et al. 1980).

Proteins: *Spirulina* has high protein content (60%-70% of its dry weight), (Ciferri 1983). It is useful in human nutrition, due to the high quality and quantity of its protein. The nutritive value of a protein is related to the quality of amino acids, digestibility coefficient, as well as by its biological value (Dillon and Phan 1993, Richmond 1992). *Spirulina* contains essential amino acids; the highest values are

leucine (10.9% of total amino acids), valine (7.5%)and isoleucine (6.8%), (Cohen 1997). Denaturation of *Spirulina* protein is observed when algae are heated above 67 ºC, at neutral aqueous solution. Hydrophobic regions interaction during heating and hydrogen bonds formation during cooling are aggregation and gelation factors of *Spirulina* protein (Chronakis 2001).

Vitamins: Among food, *Spirulina* has a relative high provitamin A concentration (Belay 1997). An excessive dose of β-carotene may be toxic, but when the β-carotene is ingested from the *Spirulina* or another vegetable it is usually harmless since the human organism only converts into vitamin A the quantity it needs (Henrikson 1994). *Spirulina* is a very rich source in vitamin B_{12} and that is a reason why these cyanobacteria is of great value for people needing supplements in the treatment of pernicious anemia (Richmond 1992, Becker 1984, Belay 1997).

Lipids: *Spirulina* contains 4-7% lipids. *Spirulina* has essential fatty acids: linoleic acid (LA) and γ-linolenic acid (GLA) (Othes and Pire 2001). The latter is claimed to have medicinal properties and is required for arachidonic acid and prostaglandin synthesis (Dubacq and Pham-Quoc 1993). GLA lowers low-density lipoprotein, being 170-fold more effective than LA (Cohen 1997).

Minerals: Iron in some nutritional complements is not appropriately absorbed. Iron in *Spirulina* is 60% better absorbed than ferrous sulfate and other complement. (Pyufoulhoux et al. 2001).

Carbohydrate: *Spirulina platensis* contains about 13.6% carbohydrates; some of these are glucose, rhamnose, mannose, xylose and galactose (Shekharam et al. 1987). *Spirulina* does not have cellulose in its cell wall, a feature that makes it an appropriate and important foodstuff for people with problems of poor intestinal absorption and geriatric patients (Richmond 1992). A new high molecular weight polysaccharide, with immunostimulatory activity has been isolated from *Spirulina* and is called “Immulina”. This highly water-soluble polysaccharide represents between 0.5% and 2.0% (w/w) of the dry microalgae (Pugh et al. 2001).

Nucleic acids content: One of the main concerns about the consumption of microorganisms is their high content of nucleic acids that may cause disease such as gout. *Spirulina* contains 2.2%-3.5% of RNA and 0.6 %-1% of DNA, which represents less than 5% of these acids, based on dry weight. These values are smaller than those of other microalgae like *Chlorella* and *Scenedesmus* (Ciferri 1983).

Pigments: Some natural pigments are found in *Spirulina*. These pigments are responsible for the characteristic colors of certain flamingo species that consume these cyanobacteria in the African Valley. This knowledge has promoted the use of this microorganism as a source of pigmentation for fish, eggs (Ciferri 1983, Saxena et al. 1983, Henrikson 1994) and chicken. *Spirulina* also increases the yellowness and redness of broiled chicken due to accumulation of zeaxanthin (Toyomizu et al. 2001).

A fundamental aspect of *Spirulina* biology is its life cycle (Figure 1.4.) due to the taxonomic, physiologic and cultivation implications (Richmond 1984).

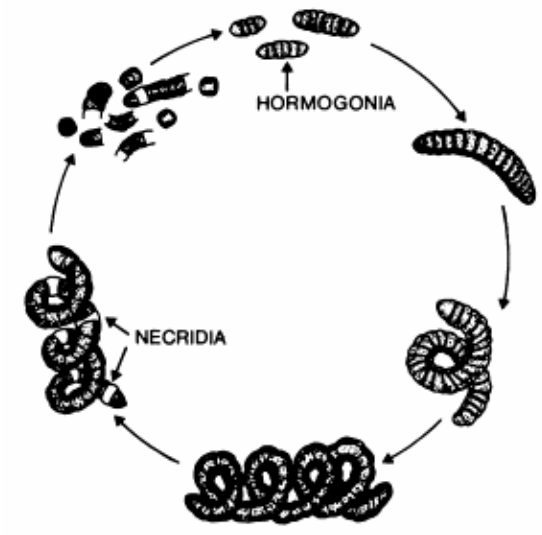


Figure 1.4. Life cycle of *Spirulina.*
(Source: Ciferri 1983)

This period is summarized in three fundamental stages: *trichomes* fragmentation, hormogonia cells enlargement and maturation processes and trichome elongation. The mature trichomes are divided into several small filaments or hormogonia through previous formation of specialized cells, necridium cells, in which the cell material is reabsorbed allowing fragmentation. The number of cells in the hormogonias is

increased by binary fission. For this process, the trichomes grow lengthwise and take their helical form (Balloni et al. 1980).

Biological approaches, especially the use of biosorbents, have been evaluated as an alternative to these techniques (Forster and Wase 1997). Within this context, algae was reported to be one of the usable biosorbents by virtue of their low cost, relatively large surface area, high binding affinity and metal recovery (Volesky 1986, Veglio and Beolchini 1997). In line with this trend in biotechnology, the usage of *Spirulina platensis* as a biosorbent is being viewed as a proper choice due primarily to its fast growth (Mosulishvili 2002).

1.14. Aim of this Work

The aim of this study was initially to find out the biosorption characteristics of *Spirulina platensis* for the removal of Pb, Cd and Ni from waters. Experiments were performed as a function of pH, initial metal concentration, desorption condition, biosorption time and biosorbent amount. Within this context, the mechanism of biosorption were tried to be evaluated.

In addition, some aspects of the kinetic and thermodynamic nature of the sorption of Pb^{2+}, $Cd^{2+}$and Ni^{2+} by *Spirulina platensis* were also examined. Furthermore, the competitive biosorption of the three metal ions was studied, and the repetitive application of the biosorbent for five successive times was investigated.

Another purpose was to immobilize the biosorbent onto suitable matrices (alginate, carboxymethylcellulose, silica gel and polysulfone) in order to develop a new sorbent which would led to make biosorption process easier.

CHAPTER 2

MATERIALS AND METHODS

2.1. Biosorbent Preparation

The cyanobacterium, *Spirulina platensis* (Ege-Macc 31), was obtained from the culture collection of Ege University Microalgae Culture Collection, Ege-Macc. It was grown in Zarrouk's Medium under constant light (3000 lux) and temperature (25 ± 1 ^{0}C). The culture was then filtered by Whatman No:1 filter paper and washed with deionized water to remove the growing medium. Harvested cells were stored at -24 ^{0}C and lyophilized accordingly with Christ alpha 1-4 Ld instrument. Lyophilized biomass was obtained in powder form and used as a biosorbent material in the experiments.

2.2. Characterization of Biosorbent

2.2.1. SEM, Optical Microscope, IR, TGA and Elemental Analysis

Optical image was obtained by using a trinocular light microscope (Olympus CH40). The texture of the biosorbent was examined using Scanning Electron Microscope (SEM) using Philips XL-30S FEG type instrument. Prior to analysis, the solid sample was sprinkled onto C tapes which were adhesive and supported on metallic discs. Images of the sample surfaces were recorded at different magnifications.

Both the optical image and the SEM micro image show that *Spirulina platensis* has a spiral shape as seen from the characteristic morphology (Figure 2.1).

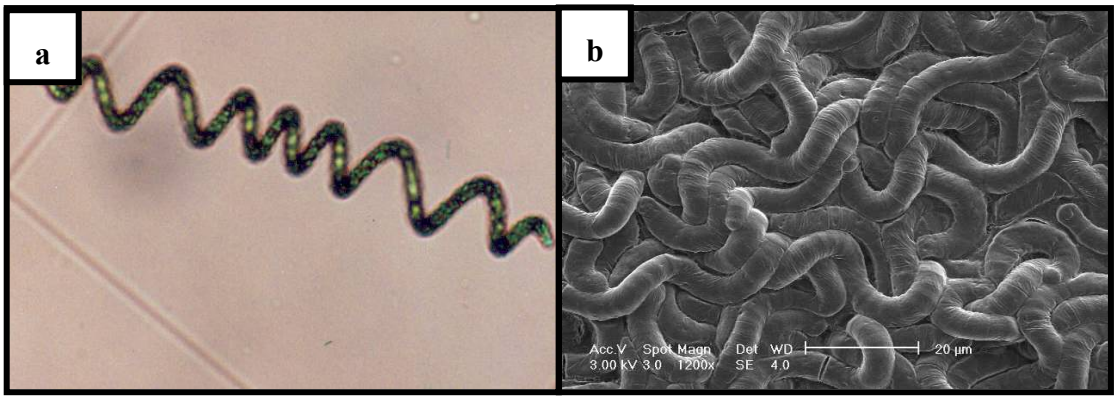


Figure 2.1. Typical images of *Spirulina platensis* (a) Optical microscope (100x) (b) Scanning Electron Microscope (1200x)

The elemental composition of the alga was determined with LECO-932 elemental analyzer. The carbon, hydrogen and nitrogen content of the sample were determined from the quantities of CO_2, H_2O and N_2O produced by the combustion of dried solid biosorbent. According to the results *Spirulina platensis* mainly involves 43.10 % C, 9.90 % N, 6.47 % H and 2.89 % S by mass. IR spectrum of biomass was obtained by using Nicolet Magna 550 FTIR instrument. The IR spectrum was presented in Figure 2.2. *Spirulina platensis* has been reported to contain functional groups like sulphonic, carboxylic (fatty acids and amino acids), phosphate, amide, hydroxyl (polysaccharide) and so on (Campanella et al. 1998).

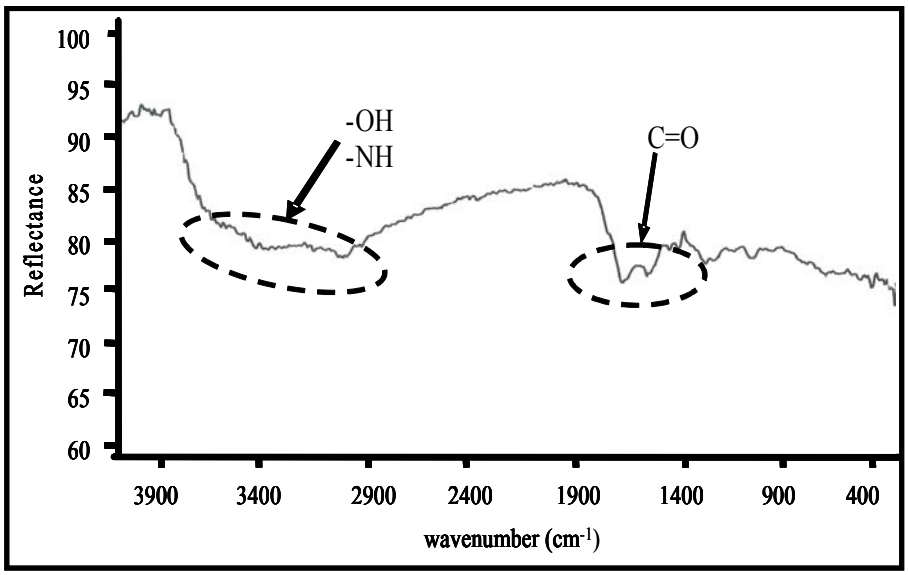


Figure 2.2. IR spectrum of *Spirulina platensis*

Table 2.1. Functional groups in lyophilized *Spirulina platensis*.
(Source: Rathinam et al. 2004)

Functional group	Standard Wavenumber (Skoog et al.. 1997) (cm^{-1})	Wavenumber from the results (cm^{-1})	Relative quantity[a]
Hydroxyl O-H	3250-3700	3200-3600	1
Carboxyl COOH	2400-3300	3200-3600	1
Amine NH_2	3300-3500	3200-3600	1
C-O	1050-1300	1000-1200	2
Sulfonyl S=O	1040-1200	1000-1200	2
Carbonyl C=O	1670-1780	1600-1750	3
Alkyl C-H	550-650	500	4
Carboxylic acid	2500-3100	2900-3000	5
Alcohol	3400-3460	2900-3000	5

[a]The quantity in the order from large to small (1 is the most abundant and 5 is the least)

The very broad band at ~3300 cm^{-1} in *Spirulina platensis* is due to the hydrogen-bonded OH group. This is also coupled with stretching vibrations of the NH_2 moiety. The very strong absorption at ~1040 cm^{-1} is assigned to stretching of C-O in polysaccharides.

This has possibly contribution from SO and PO stretching vibrations (Loukidou et al. 2004). The peaks at ~ 1530 cm^{-1} and ~ 1430 cm^{-1} in *Spirulina platensis* are assigned to ν_{asy} COO- and ν_{sym} COO- respectively. The band around 1600 cm^{-1} may be attributed to coupled vibrations of C=O and C=N stretching and NH_2 bending arising from the amino acids/amides present in the biomass.

Thermo gravimetric analysis (TGA) was performed by Perkin Elmer Diamond Tg/DTA instrument. The solid samples were heated from 25°C to 800°C by a heating rate of 10 ^{0}C in the presence of N_2 atmosphere. Results showed that the biosorbent underwent four steps decomposition process during heating from 25 to 800°C. The maximum weight loss was observed between 240-345°C and 345-730°C (Figure 2.3). In the initial step (~8% weight loss), which was small in the range in 25–110°C could be attributed to the loss of adsorbed water molecules. Maximum weight loss due to degradation was observed in the last two steps, i.e., between 240 - 345°C and 345 - 730°C indicating that the organic fraction of the studied biosorbent got decomposed in this range.

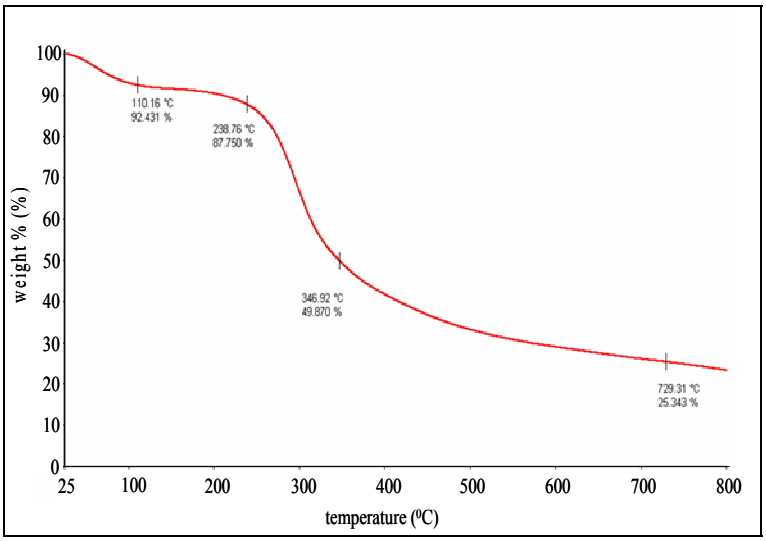


Figure 2.3. TGA analysis of *Spirulina platensis*

2.3. Chemicals and Reagents

All chemicals were of analytical reagent grade. Ultra pure water (18 MΩ) was used throughout the study. Glassware and plastic ware were cleaned by soaking in 10 % (v/v) nitric acid and rinsed with distilled water prior to use.

1. Standard Pb(II) stock solution (4000 mgL^{-1}): prepared by dissolving 1.599 g of $Pb(NO_3)_2$ (Riedel, 99 %) in 1% HNO_3 (Merck) and diluting to 250.0 mL with ultra pure water.
2. Standard Cd(II) stock solution (4000 mgL^{-1}): prepared by dissolving 1.630 g $CdCl_2$ (Fluka, >99), in 1% HNO_3 (Merck) and diluting to 250.0 mL with ultra pure water.
3. Standard Ni(II) stock solution (4000 mgL^{-1}): prepared by dissolving 3.539 g $NiCl_2.6H_2O$ (Carlo Erba, 99%), in 1% HNO_3 (Merck) and diluting to 250.0 mL with ultra pure water.
4. Calibration standards: Lower concentration standards were prepared daily from their stock standard solutions.
5. pH adjustment: NH_3 (Merck) (0.1-1.0 M) and HNO_3 (Merck) (0.1-1.0 M) were used.
6. H_2SO_4 solution (5%): prepared from 96% H_2SO_4 (Merck) stock solution.

7. HCl solution (0.5M): prepared from 37% 10.4 mL HCl (Merck) stock solution by diluting to 250.0 mL with ultra pure water.

8. $CaCl_2$ (0.5M): prepared by dissolving 13.872 g $CaCl_2$ (Fluka) and diluting to 250.0 mL with ultra pure water.

9. $BaCl_2$ (0.1M): prepared by dissolving 2.083 g $BaCl_2$ (Fluka), diluting to 100.0 mL with ultra pure water.

10. Sodium alginate solution (4% w/v): prepared by dissolving 4.000g sodium alginate (Sigma-Aldrich) and diluting to 100.0 mL with ultra pure water.

11. Carboxymethylcellulose (1% w/v): prepared by dissolving 1.000 g carboxymethylcellulose (Sigma-Aldrich) and diluting to 100.0 mL with ultra pure water.

12. Sodium silicate (6% w/v): prepared from 4.320 mL (d=1.39 gmL^{-1}) Sodium silicate (Sigma-Aldrich) stock solution by diluting to 250.0 mL with ultra pure water.

13. $FeCl_3$ (0.2 M): prepared by dissolving 3.240g $FeCl_3$ (Riedel) and diluting to 100.0 mL with ultra pure water.

14. Polysulfone (100 gL^{-1}): prepared by dissolving 10.000 g polysulfone (Sigma-Aldrich) and diluting to 100.0 mL with dimethylformamide (DMF) (Merck) solution.

15. Ethanol (50%): prepared by diluting 50.0 mL ethanol (Merck) to 100.0 mL with ultra pure water

2.4. Instrumentation and Apparatus

In batch adsorption experiments, GFL 1083 water bath shaker equipped with a microprocessor thermostat was used to control the temperature. The pH measurements were performed by using Ino Lab Level 1 pH meter.

In the determination of Pb, Cd and Ni an atomic absorption spectrometer (AAS), Thermo Elemental Solaar M6 Series with an air-acetylene burner was used in all measurements. Single element hallow cathode lamps were used at the wavelengths of 217.0, 228.8 and 232.0 nm for Pb, Cd and Ni, respectively. A deuterium hollow cathode lamp was used for background correction in all determinations.

2.5. Biosorption Studies

For biosorption studies, the effect of initial metal concentration, pH, shaking time and biosorbent amount on sorption were investigated.

The experiments were conducted in 50 mL polyethylene centrifuge tubes containing 10 mL of metal solutions in the concentration of interest. The pH of the solutions was adjusted with dilute NH_3 and HNO_3. In biosorption studies, 10 mg biosorbent was used. The temperature was set to 25^0C in all experiments unless stated otherwise. The mixtures were then mixed using GFL 1083 water bath shaker equipped with microprocessor thermostat. At the end of each mixing period the biomass was removed by filtration and the filtrate was analyzed using the flame atomic absorption spectrometer.

2.6. Desorption Studies

Desorption was also studied to investigate the possible recoveries. 10.0 mL of 100 mgL^{-1} metal solutions were added with 10 mg of biosorbent. After shaking and filtration, the sorbed amounts were tried to be desorbed with 10 mL of 0.1 M of nitric acid, hydrochloric acid and sodium citrate. Each metal concentration in the filtrate was measured as described earlier and expressed as percent desorption against the metal biosorption by the cells.

2.7. Immobilization Studies

In addition to the experiments performed with the free *Spirulina platensis*, immobilization of the biosorbent to the various matrices was applied and these experiments were also repeated. In the case of free cell usage, it was thought that there would appear some problems like expansion of biosorbent, resistance to flow and clogging columns. For the expectation of avoiding these possible problems, new biosorbents formed by immobilization was thought to have homogenous particle size, increased mechanical strength and thus, to be more appropriate for the column studies.

2.7.1. Immobilization of *Spirulina platensis* into Sodium Alginate

Alginate has been reported to be most extensively used for immobilization of algal cell wall as other kinds of biomass. It is extracted form algae as water-soluble sodium salt. Alginates are linear unbranched polymers containing β-(1→4)-linked D-mannuoric acid (M) and α-(1→4)-linked L-guluronic acid (G) residues (Figure 2.4).

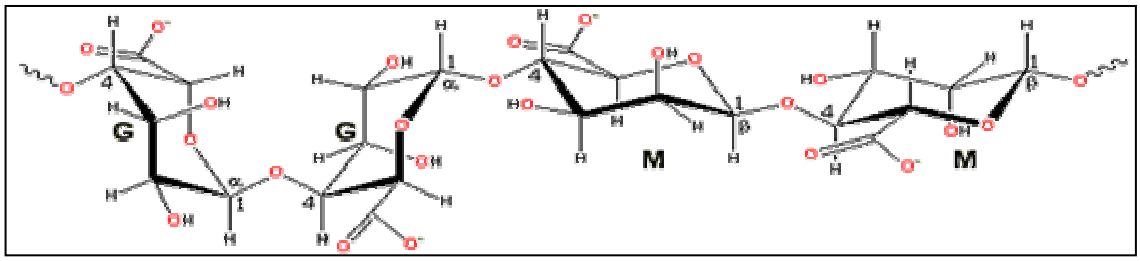


Figure 2.4. Structure of alginate

They are covalently linked together in different sequences or blocks. When calcium replaces sodium, ionic cross-linking between carboxylic acid groups occurs giving a gelatinous substance. It may be noted that alginate beads are stable when the pH is between 5-9. A considerable loss of alginate is reported at low pH below 3 and above 9. At pH 11, alginate beads most often are said to be broken. Also treatment of alginate-immobilized biomass may result in shrinking of biomass with acidic solution loaded beads (Mehta and Gaur 2005).

In the immobilization of *Spirulina platensis* by alginate, one gram of dry weight of cyanobacterial biomass was suspended in 25 mL of deionized water and then mixed with 25 mL of 4 % w/v sodium alginate. The mixture was dropped with the aid of a peristaltic pump into 0.5 M of $CaCl_2$ forming beads around 1.0 mm. The $CaCl_2$ solution was cured overnight, rinsed with deionized water, soaked in 0.5 M HCl solution for more than 24 h and rinsed again with deionized water (Lu and Wilkins 1995).

2.7.2. Immobilization of *Spirulina platensis* into Sodium Silicate

Silica gel has also been used for entrapment of algal cells or biomass. Silica gel is reported to be prepared by decreasing the pH of alkali silicate to less than 10 (Rangsayatorn et al. 2004). The solubility of silica is then reduced to form gel. As silica

begins to gel, cells in silica are trapped in porous gel that is three dimensional SiO_2 network, of which is 60 % of water. The silica surface is quite complex and contains more than one hydroxyl group.

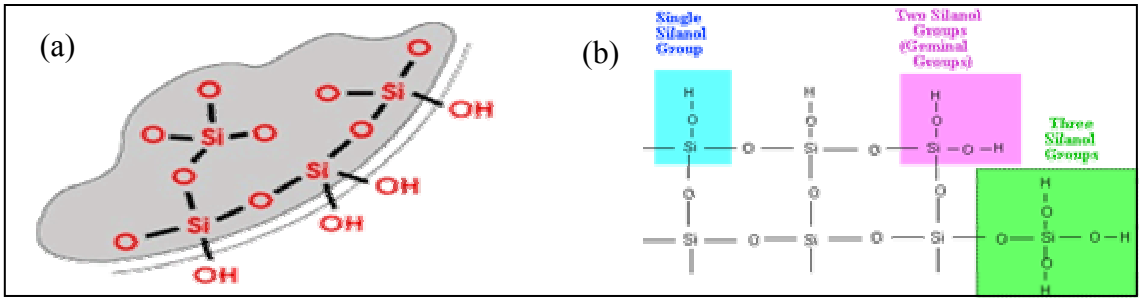


Figure 2.5. (a) Structure of silica gel (b) Types of silanol groups

There are three types of hydroxyl group in silica gel which is shown in Figure 2.5b. The first is a single hydroxyl group attached to a silicon atom which has three siloxane bonds joining it to the gel matrix. The second one is one of two hydroxyl groups attached to the same silicon atom which, in turn, is joined to the matrix by only two siloxane bonds. The third is one of three hydroxyl groups attached to a silicon atom which is now only joined to the silica matrix by only s single siloxane bond (Rangsayatorn et al. 2004).

In the immobilization of *Spirulina platensis* to silica gel, an amount of 3.0 mL of 5% H_2SO_4 was mixed with sufficient 6% sodium silicate (Na_2SiO_3) solution to raise the pH to 2.0. An amount of 0.2 g sorbent was added to silica solution and stirred for 15 minutes. The pH was then raised slowly by the addition of 6% sodium silicate to reach pH 7.0. The polymer gel was washed with water thoroughly until the addition of barium chloride ($BaCl_2$) resulted in no white precipitate due to the presence of sulphate. The polymer gel with immobilized *Spirulina platensis* was dried overnight at 45^0C. The gel was crushed and grounded with a mortar and sieved to remove the particles smaller than 125 μm.

2.7.3. Immobilization of *Spirulina platensis* into Carboxymethylcellulose

Sodium carboxymethylcellulose (CMC) is known to be a water-soluble polymer. The hydrophilic character of cellulose is obtained by carboxymethylation and this also

provides functional carboxylic groups on the cellulose derivative for cross-linking via trivalent metal ions. The structure of CMC was shown in Figure 2.6.

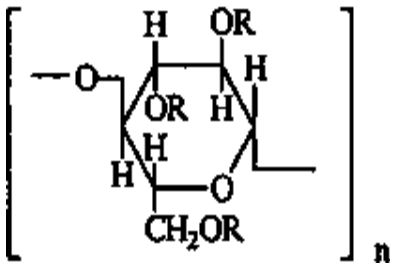


Figure 2.6. Structure of carboxymethylcellulose

CMC can simply be converted into a hydrogel via chelation using salts of polyvalent cations such as ferric chloride and aluminum chloride (Dervari and Hasırcı 1996). In the present work, CMC beads were prepared by cross-linking with trivalent ferric ions. Fe(III) ions have strong affinity for electron-rich 'bases' such as carboxylic, phosphate and sulfate oxygen (Ianuccelli et al.1993). The presence of carboxylic groups on the carboxymethylcellulose molecules provides a binding side for Fe(III) ions. Cross-linking should be a combination of metal coordination and ion exchange interaction between the carboxylic oxygen of CMC molecules and trivalent ferric ions. As a result of these interactions, CMC droplets precipitated in the bead form in aqueous ferric chloride solution. The water content of the CMC beads was 92%. Their high water content make them highly permeable, which is advantageous for some biotechnological uses because of their enhanced degradability.

The immobilization of *Spirulina platensis* via entrapment was carried out as follows: CMC sodium salt solution was dissolved in deionized water and then it was mixed with *Spirulina platensis* (0.2 g in 5.0 mL deionized water). The mixture was introduced into 0.2 M ferric chloride solution with a peristaltic pump. The biomass entrapped beads (~1mm) were cured in this solution for 30 minutes and then washed with deionized water. The preparations were then maintained in an oven at 45 ^{0}C overnight, ground to powder and sieved to remove the particles smaller than 125 μm.

2.7.4. Immobilization of *Spirulina platensis* into Polysulfone Matrix

Polysulfone is a rigid, high-temperature resistant, amorphous thermoplastic that can be thermoformed into a wide variety of shapes. It is composed of phenylene units linked by three different chemical groups: isopropylidene, ether and sulfone. Each group contributes specific properties to the polymer.

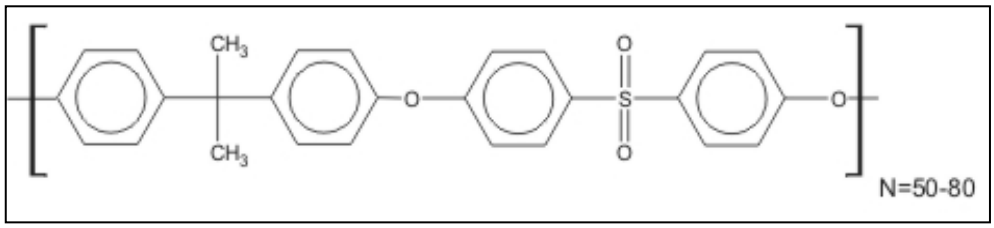


Figure 2.7. Structure of polysulfone

The most distinctive feature of the backbone chain is the diphenylene sulfone group (Figure 2.7). The influence of this group on the molecule results in its thermal stability, oxidation resistance, and rigidity, even a televated temperatures (WEB_6 2007).

In the immobilization of *Spirulina platensis*, biomass entrapment in polysulfone beads was carried out. The lyophilized biomass was grounded and mixed with 100 gL^{-1} polysulfone dissolved in dimethylformamide (DMF). The mixture was stirred for 1 h at room temperature and then the resulting slurry was dripped through a micropipette into 50% (v/v) ethanol. Durable, spherical beads containing 50% biomass by weight were immediately formed by a phase interversion process. The beads formed were washed thoroughly with deionized water and dried at room temperature (Blanco et al. 1999).

2.8. Kinetics of Sorption

Kinetic studies of sorption on *Spirulina platensis* were carried out at the initial metal concentrations of 100.0 mgL^{-1} and 500.0 mgL^{-1} for times of contact that ranged from 5 seconds to 240 minutes. The experiments were conducted at two preset temperatures; namely 25^0C and 50^0C. 10.0 mL solution volume and 50.0 mg dose of biosorbent were used in all experiments.

CHAPTER 3

RESULTS AND DISCUSSION

3.1. Effect of Parameters on Biosorption

Biosorption of heavy metals by algae may be affected by several factors, including concentration of metal, the amount of biomass, pH, temperature and metabolic stage of the organism. In the present study, effects of initial metal concentration, pH, shaking time and biosorbent amount on sorption were checked. These experiments were performed as discussed earlier in part 2.5.

3.1.1. Effect of Initial Metal Concentration on Pb(II), Cd(II) and Ni(II) Biosorption

Sorption and removal of heavy metals by algal biosorbents largely depend on the initial concentration of heavy metals in the solution. Metal sorption initially increases in metal concentration in the solutionand then becoming saturated after a certain concentration of metal. Also algal cell surface has several kinds of functional groups with varying affinity for ionic species (Aloysius et al. 1999).

Sorption yields determined at different concentrations (10.0-50.0-100.0-250.0-500.0-1000.0 mgL^{-1}) for each metal ion were compared in Figure 3.1. The initial metal ion concentration remarkably influenced the equilibrium metal uptake .The higher the initial concentration, the larger the amount of metal ion taken up. The increases in loading capacities of biosorbents with the increase of metal ion may be due to higher probability of collision between metal ions and the biosorbents. Also, when the algal biomass is in dead state, the cells are permeable and allow the metals to enter and bind to internal components and surfaces as well as the external surface, thus increasing the metal uptake. Lead is known to represent the best adsorption among most metals. This suggests that metal sorption uptake depends not only on the nature of biomass (functional groups, structure, porosity and permeability) but also on physicochemical parameters of the solution used (pH, temperature, presence of other cations and anions,

ionic strength) and, of course, on the properties of metals and their variety of chemical forms (Mehta and Gaur 2005).

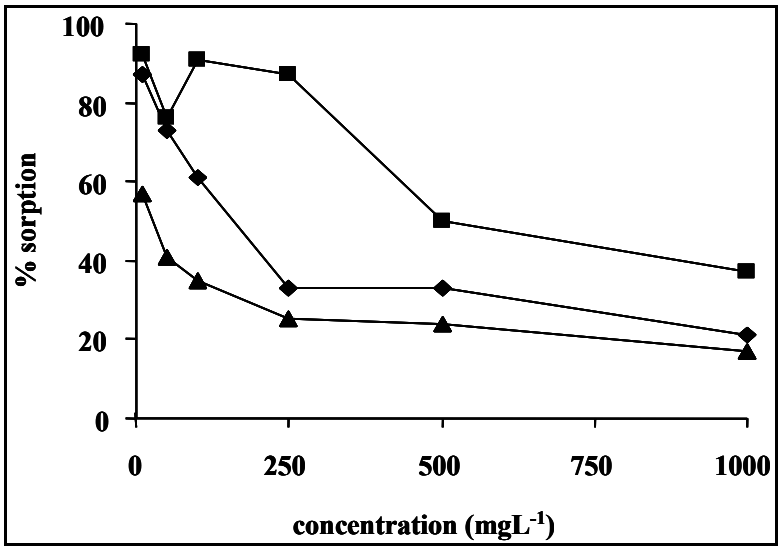


Figure 3.1. Percentage uptake of *Spirulina platensis* for (■) Pb, Cd (♦) and Ni (▲) as a function of initial metal concentration (biosorbent amount: 10.0 mg, solution pH: 6.0, shaking time: 60 min.)

Attending to the last factor, metals can be classified in different classes: (Duffus 2002) Class A or hard acids, Class B or soft acids, or borderline, depending on their observed affinity for different ligands. In general, there is a relatively sharp separation between Class A and borderline metal ions, but the difference between borderline and Class B is less clearly defined. This classification of metals indicates the form of bonding in their complexes. Class A or nonpolarizible metal ions, preferentially form complexes with similar nonpolarizible ligands and the bonding in these complexes is mainly displaced and mobile. Class B metal ions preferentially bind to polarizible, soft ligands to give rather more covalent bonding; the metal ions are difficult to displace. In the case of heavy metals under study, lead and cadmium belong to the Class B and nickel to the borderline. The high lead sorption uptake could be explained as due to stronger bonding of this metal with functional groups of the biomass.

3.1.2. Effect of Initial pH on Pb(II), Cd(II) and Ni(II) Biosorption

pH is one of the most important factors that affect the biosorption process. Most of the studies have shown that the sorption of metal ions in batch as well in continuous system is a function of pH of the solution. Therefore efforts have been made to find the

optimum pH for maximizing metal removal with algae. The optimum different pH for the metals could be due to the nature of their chemical interaction with the algal cell. At pH 5.0 algal cells have generally a net negative charge on the surface that favors the binding of metal ions to the surface ligands. At pH below isoelectric point, cells have positive charge that inhibits the approach of positively charged ions (Mehta and Gaur 2005).

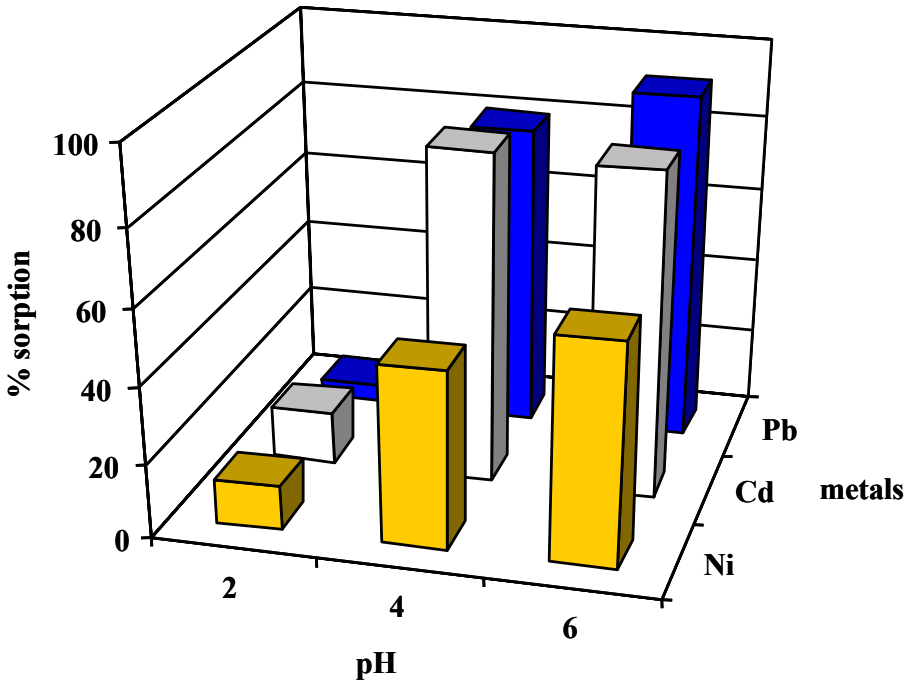


Figure 3.2. Percentage uptake of *Spirulina platensis* for Pb, Cd and Ni as a function of pH (biosorbent amount: 10.0 mg, initial metal concentration 10.0 mgL^{-1}, solution volume: 10.0 mL, shaking time: 60 min.)

The comparative biosorption of Pb(II), Cd(II) and Ni(II) ions with *Spirulina platensis* was investigated as a function of initial pH. The results were given in Figure 3.2. As the pH increases the sorption yield increases as well. Higher pH values were not preferred to be studied due to the possible precipitates of metal hydroxides.

Since a majority of metal binding groups of algae are acidic (e.g.,carboxyl), their availibility is pH dependent. These groups generate a negatively charged surface at acidic pH and electrostatic interaction between cationic species and the cell surface are responsible for metal biosorption. A decreased metal sorption by algae has been frequently observed at extremely low pH (<2). High concentration of H^+ ions at low pH decrease the metal sorption by excluding them from their binding to ligands on the cell

surface (Mehta and Guar 2001a). Therefore as the pH decreases further, the sorption yield also decreases.

3.1.3. Effect of Shaking Time on Pb(II), Cd(II) and Ni(II) Biosorption

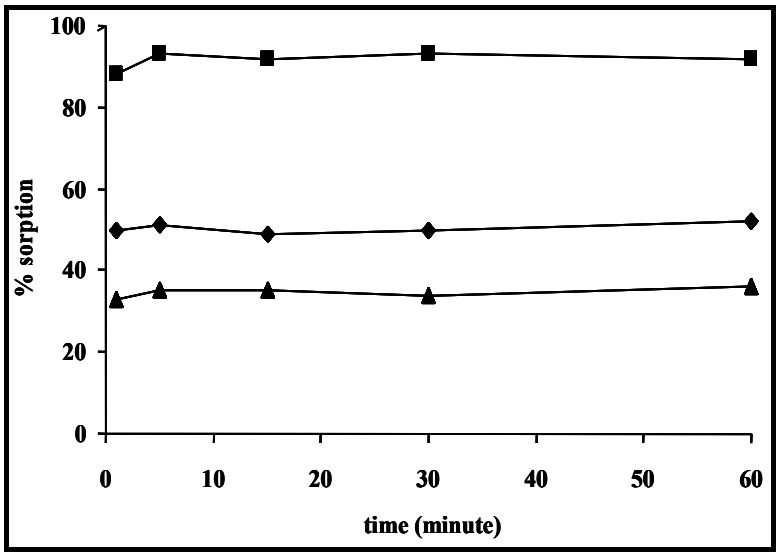


Figure 3.3. Percentage uptake of *Spirulina platensis* for (■) Pb, Cd (♦) and Ni (▲) as a function of time (biosorbent amount: 10.0 mg, initial metal concentration 10.0 mgL^{-1}, solution volume: 10.0mL, pH: 6.0).

To determine the time required for sorption equilibrium to be reached, sorption experiments were carried out over 1 hour and samples were analyzed at regular intervals. Typical results are shown in Figure 3.3. It is clear that the uptake of all three metal ions by *Spirulina platensis* was rapid and within 5 minutes of contact, biosorption reached its maximum and then the system seemed to approach in saturation in 1 hour.

3.1.4. Effect of Biosorbent Amount on Pb(II), Cd(II) and Ni(II) Biosorption

The amount of metal ion recovered from a solution is affected by the amount of biomass. The effect of biomass amount on the affinity for metal binding was determined. The results are given in Figure 3.4. It was found that there was a certain decrease in Pb sorption capacity with increasing biomass amount, especially after 50.0 mg dose.

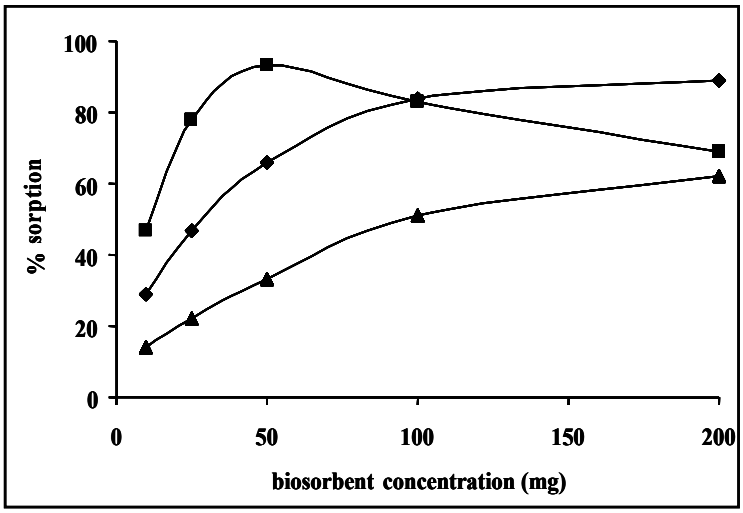


Figure 3.4. Percentage uptake of *Spirulina platensis* for (■) Pb, Cd (♦) and Ni (▲) as a function of biosorbent concentration (initial metal concentration: 100.0 mgL^{-1}, solution volume: 10.0 mL, pH: 6.0, shaking time: 60 min.)

Probable explanations for such a relationship between biomass concentration and sorption may be the limited availability of metal, increased electrostatic interactions, interference between metal binding sites and reduced mixing at higher biomass concentrations (Brady and Duncan 1994, Singleton and Simmons1996, Fogarty et al. 1999). While an increased biomass concentration has a negative effect on the sorption capacity of a biosorbent, the total metal removed (% of initial concentration) by a biosorbent is higher at higher biomass concentrations as in the case of Cd and Ni. However, there is no straightforward relationship between biomass concentration and metal removal.

3.1.5. Desorption Studies

In order to make biosorption process feasible for industrial application, provision must be made to regenerate the biomass for the repeated use. Metal sorbed on biomass can be desorbed by a suitable eluent or desorbing solution, thus biomass can be used in multiple sorption-desorption cycles. One of the most common methods for desorption of heavy metals from the biomass could be the lowering of pH. Lowering the pH of the metal-loaded biomass suspension causes displacement of heavy metal cations by protons from the binding sites. Figure 3.5 shows the results of desorption process. In desorption experiments different eluents were used. 0.1 M HCl and 0.1M HNO_3 solutions were able to desorb Pb and Cd ions from the microalga better than 0.1M Na-citrate. Biomass was damaged at extremely low pH values.

Although 1.0 M HNO_3 removed almost all metal ions bound by the biomass as shown in Figure 3.5b, it damaged the algal cells and caused a significant decrease in the sorption capacity in the further use of the biosorbent. However, new biosorbent could be used at each time since it has relatively low cost.

It is interesting that Ni could be desorbed hardly even when 1M eluents were used at lower initial metal concentrations. This might be due to the fact that Ni sorption mechanism could be different than those of the other metal ions.

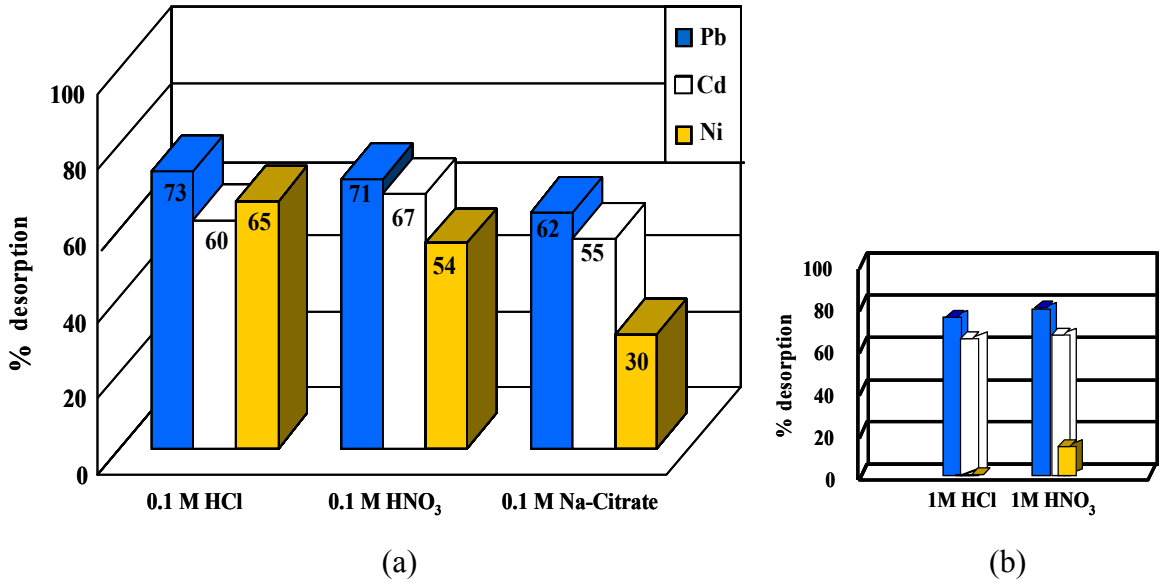
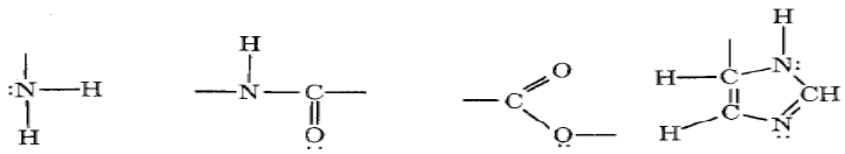


Figure 3.5. Desorption results for *Spirulina platensis* with various eluents. (biosorbent amount: 10.0 mg, initial metal concentration 100.0 mgL^{-1} for (a) 10.0 mgL^{-1} for (b), solution volume: 10.0mL, pH: 6.0)

As indicated before *Spirulina platensis* cell composition is reported to have high protein content. Amino acids in the proteins could provide such functional groups as shown as:



The amino and carboxyl groups, the imidazole of histidine and the nitrogen and oxygen of the peptide bond could be available for characteristic coordination bonding with metallic ions (Crist et al. 1981).

Ni(II) is a borderline metal ion capable of forming stable complexes with both hard (oxygen) and soft (nitrogen, sulfur) donors. Available information on the binding modes of

proteins and data for nickel peptide complexes indicate, however, that imidazole of histidine and thiol of cysteine should be thermodynamically preferred by Ni(II) among donor groups provided by protein-building amino acids (Halcrow and Christouc 1994). Therefore, it might be more difficult to desorb Ni(II) ions from the biosorbent.

3.2. Sorption Kinetics

According to the kinetic experiments, results showed that the time required to reach equilibrium was in all cases less than one hour for each of Pb^{2+}, Cd^{2+} and Ni^{2+} ions. The experimental and theoretical variations of the sorbed amounts of Pb^{2+}, Cd^{2+}, Ni^{2+} on *Spirulina platensis* at temperatures of 25^0C and 50^0C is provided in Figure 3.6 a,b,c, respectively.

The kinetic plots for the three cations are demonstrated in Figure 3.6. The experimental kinetic data showed best correlation with the pseudo second order rate equation given by the equation (Shahwan et al. 2005):

$$\frac{t}{q_t} = \left(\frac{1}{k_2.q_e^2}\right) + \left(\frac{1}{q_e}\right).t \qquad (3.1)$$

where q_t is the concentration of sorbed ion on the solid at time t (μmol g^{-1}) , q_e is the concentration of sorbed ion at equilibrium (μmol L^{-1}) and k_2 is the pseudo second order rate constant (μg $\mu mol^{-1} min^{-1}$), respectively. The values of q_t in the equation above were calculated using the mass balance equation:

$$q = (C_0 - C_l).\frac{V}{M} \qquad (3.2)$$

where C_0 is the initial metal concentration (μmol L^{-1}), C is the equilibrium concentration of metal solution (μmol L^{-1}), V is the volume of solution (L) and M is the mass of the solid (g).

Figure 3.6 shows the kinetic plots as predicted by the pseudo second order kinetics. These plots were constructed using the model parameters obtained from the linear regression analysis. The linear regression plots of t/q_t against t (insets in Figure 3.6) provided the rate constants, k_2, which are given in Table 3.1, together with q_e and the linear correlation coefficients, R.

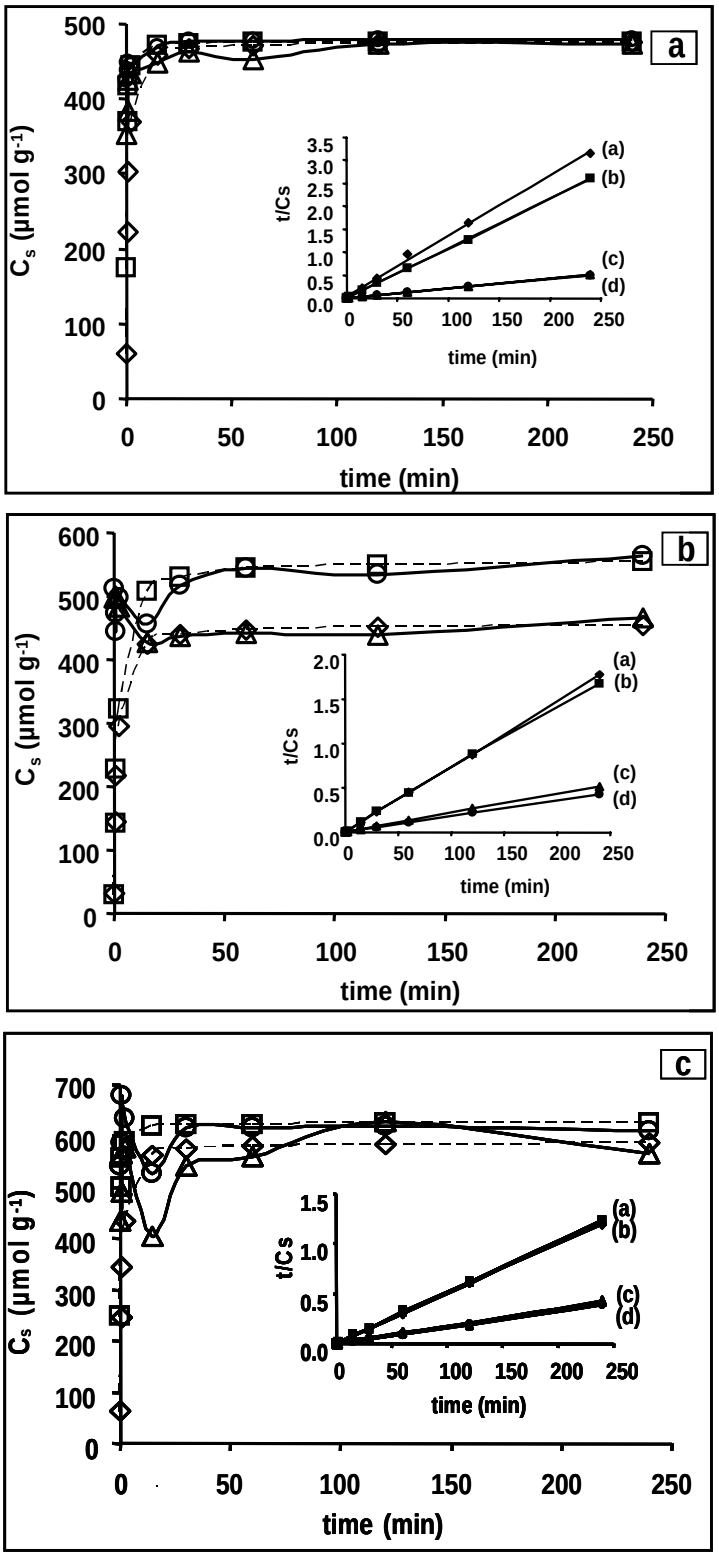


Figure 3.6. Theoretical and experimental variations of the sorbed amount of (a) Pb^{2+}, (b) Cd^{2+} and (c) Ni^{2+} (µmol g^{-1}) with time at 25^{0}C and 50^{0}C on *Spirulina platensis*. (-Δ-25^{0}C 500.0 mg L^{-1} experimental, -○- 50^{0}C 500.0 mg L^{-1}experimental, -◊- 25^{0}C 500.0 mg L^{-1} theoretical, -□- 50^{0}C 500.0 mg L^{-1} theoretical). The insets show the linear regression plots (a) 25^{0}C 100.0 mg L^{-1}, (b) 50^{0}C 100.0 mg L^{-1},(c) 25^{0}C 500.0 mg L^{-1}, (d) 50^{0}C 500.0 mg L^{-1}.

The values which are tabulated at two different temperatures indicate that while the k_2 values increased as temperature was increased in the cases of Pb^{2+} and Ni^{2+}, a decrease in the k_2 value was observed in the case of Cd^{2+} sorption. as shown in Table 3.1. This means that, unlike Pb^{2+} and Ni^{2+}, the increase in temperature is causing a delay in the attainment of equilibrium for Cd^{2+}. Generally speaking, the effect of temperature on the rate constants in liquid-solid sorption systems is likely a complicated issue. Literature resources have reported many cases in which the increase in temperature caused a decrease in the rate constants of different adsorbate ions (Horsfall and Spiff 2005). From a physicochemical perspective that is based on the behavior of gases, the rate constant is expected to usually increase as the temperature is increased (Levine 3rd edition). This is usually caused by the fact that the increase in temperature, in a medium where the intermolecular forces are very weak, leads to an increase in the kinetic energy of gas molecules/atoms and thus enhances the rate of reactions. In liquid-solid sorption systems, however, the situation is much more complicated as the behavior of ions in solution or on the solid would be subject to factors like the inter-ionic forces, the hydration energy, the availability of sorption sites and the relative stability of sorbed ions at these sites (Shahwan et al. 2006).

Table 3.1. Kinetic parameters obtained from linear fits of the experimental data for Pb^{2+}, Cd^{2+}, Ni^{2+} biosorption on *Spirulina platensis* (Metal ion concentrations: 500.0 mg L^{-1}, pH= 6.0, solution volume : 10.0 mL and amount of biomass : 50.0 mg).

Sample	**T (^{0}C)**	**$[q]_e$ ($\mu molg^{-1}$)**	**k_2 ($g\mu mol^{-1}min^{-1}$)**	**R**	**h ($\mu molg^{-1}min^{-1}$)**	**E_a ($kJmol^{-1}$)**
Pb^{2+}-loaded *S. platensis*	25	476	3.68×10^{-3}	>0,9999	8.33×10^2	+ 44
	50	476	1.47×10^{-2}	0,9999	3.33×10^3	
Cd^{2+}-loaded *S. platensis*	25	455	2.01×10^{-3}	0,9991	4.16×10^2	- 16
	50	556	1.24×10^{-3}	0,9993	3.83×10^2	
Ni^{2+}-loaded *S. platensis*	25	588	2.41×10^{-3}	0.9969	8.33×10^2	+ 54
	50	625	1.28×10^{-2}	0.9998	5.00×10^3	

The sign of the observed energy of activation, E_a, which are also provided in Table 3.1, reflect the effect of temperature on the rate constants. According to literature resources, when the value of E_a is between 8.4 and 83.7 kjmoL^{-1}, the adsorption is said to be activated chemical type which means that the rate varies with temperature according to finite activation energy in the Arrhenius equation (Aksu 2002 and Smith 1981). However, since the values of E_a are not purely determined by the intrinsic sorption step, but might include also contributions from ionic dehydration effects in addition to plausible contributions dependent on the nature of the adsorbent, it is unlikely to make mechanistic speculations about the sorption process.

3.3. Thermodynamic Parameters

The experimental data obtained at different temperatures were used in calculating the thermodynamic parameters of sorption; ΔH^0, $\Delta G^0$and ΔS^0 utilizing the well-known equations:

$$\Delta G^0 = -RT \ln R_d \tag{3.3}$$

$$\Delta H^0 = R \ln \frac{R_d(T_2)}{R_d(T_1)} \left[\frac{T_1 T_2}{T_2 - T_1} \right] \tag{3.4}$$

$$\Delta S^0 = \frac{\Delta H^0 - \Delta G^0}{T} \tag{3.5}$$

The Gibbs free energy indicates the degree of spontaneity of the sorption process and the higher negative value reflects more energetically favorable sorption.

The distribution ratio, R_d (mLg^{-1}), is defined as q_e/C_e and reflects the extent of distribution of the adsorbate ion between the solid and liquid phases at equilibrium. R_d is valid at a particular initial concentration and reaction conditions. The obtained 'apparent' values of ΔH^0, ΔS^0 and ΔG^0 of sorption for each metal ion uptake on the biosorbent are summarized in Table 3.2.

Table 3.2. Values of ΔH^0, ΔS^0 and ΔG^0 calculated from the sorption data of Pb^{2+}, Cd^{2+}, Ni^{2+} on *Spirulina platensis* (Metal ion concentrations: 500.0 mg L^{-1}, pH: 6.0, solution volume: 10.0 mL and amount of biomass: 50.0 mg).

Sample	**ΔH^0 ($kJmol^{-1}$)**	**ΔS^0 ($Jmol^{-1}K^{-1}$)**	**ΔG^0 ($kJmol^{-1}$)**	
			298 K	**323 K**
Pb^{2+} - *S. platensis*	36	194	-21.8	-26.6
Cd^{2+}- *S. platensis*	16	97.7	-13.1	-15.6
Ni^{2+} - *S. platensis*	5	53.7	-11.4	-12.7

The ΔH^0 values demonstrate that the sorption process is of endothermic nature in all cases. This behavior indicate that higher temperatures are more preferred for higher sorption, with the sequence of preference being $Pb^{2+} > Cd^{2+} > Ni^{2+}$. The calculated values are described as 'apparent' values because they include plausible energy contribution from the adsorbent and forces of dehydration of the adsorbate ions in addition to the energy associated with the intrinsic adsorption step. Negative standard Gibbs energy changes depict that the sorption reactions are largely driven towards the products, with the same sequence of preference as given above.

The calculated values of ΔS^0 refer to the entropy change of the adsorption system (not the total entropy change), which can have positive or negative values. As shown in Table 3.2, positive values are reported in all cases. Positive values mean, theoretically, that more disorder is associated with the adsorption process. This might be intuitively explained based on the increase in the dehydration steps of the adsorbate ions, which are known to possess relatively high energies of salvation and thus are stabilized by water sheaths in the absence of the adsorbent.

3.4. Sorption Isotherm Models

The relation between the amount adsorbed and the concentration is known as the adsorption isotherm. Adsorption equilibrium data are typically plotted in the form of an adsorption isotherm with the mass adsorbed on the y-axis and the mass in the fluid on the x-axis at constant temperature.

Sorption isotherms are mathematical models that describe the distribution of the sorbate specie among liquid and solid phases, based on a set of assumptions that are

related to the heterogeneity/homogeneity of the solid surface, the type of coverage and the possibility of interaction between the sorbate specie. Thus they are important from the view point of chemical design. The data of Pb^{2+}, $Cd^{2+}$and Ni^{2+} biosorption on *Spirulina platensis* was examined by using three types of the most common isotherms; Freundlich, Dubinin-Radushkevich (D-R) and Temkin isotherm models

Freundlich Isotherm: A brief empirical equation often used to represent adsorption data is called the Freundlich equation. The Freundlich isotherm describes physical adsorption from liquids. The empirically derived Freundlich isotherm is defined as follows.

$$q_e = K_f \cdot C_e^{\,n} \tag{3.6}$$

where; q_e : amount adsorbate adsorbed per unit weight of adsorbent at equilibrium

C_e : equilibrium concentration of adsorbate in solution after adsorption

K_f : empirical Freundlich constant or capacity factor (mg/g), (mol/L)

n : the Freundlich exponent. (Vadivelan and Kumar 2005)

The exponent n is an index of the diversity of free energies associated with the sorption of the solute by multiple components of a heterogeneous sorbent. When n=1, the isotherm is linear and system has a constant free energy at all sorbate concentrations. When n<1, the isotherm is concave and sorbates are bound with weaker and weaker free energies, finally, when n>1, the isotherm is convex and more sorbate presence in the sorbent enhance the free energies of further sorption (Schwarzenbach 2003).

The good fit of Freundlich isotherm to an adsorption system means there is almost no limit to the amount adsorbed and there is a multilayer adsorption. The applicability of the Freundlich equation to a particular case is tested by plotting $log\ q_e$ against $log\ C_e$ from the logarithmic form of Equation 3.4.

$$log\ q_e = log\ K_f + n\ log\ C_e \tag{3.7}$$

such a plot should yield a straight line with intercept equal to $log\ K_f$ and slope equal to n.

Non-linear fits of sorption data of Pb^{2+}, Cd^{2+} and Ni^{2+} are given in Figure 3.7. The values of Freundlich constants, "k" and "n", are obtained from linear fits of sorption data and given in Table 3.3. The "n" values indicate that the biosorption process is highly nonlinear, while the "k" values show that the affinity of the biosorbent towards the adsorbate ions follows the sequence $Ni^{2+} > Pb^{2+} > Cd^{2+}$.

Table 3.3. Freundlich parameters n, k obtained from the plots of Pb^{2+}, Cd^{2+}, Ni^{2+} sorbed by *Spirulina platensis* at 25^0C (Amount of biomass: 10.0 mg, pH: 6.0, solution volume: 10.0 mL).

Sample	**Freundlich constants**		
	n	**K**	**R**
Pb^{2+} - *S. platensis*	0.2174	0.00419	0.8554
Cd^{2+} - *S. platensis*	0.2169	0.00254	0.9308
Ni^{2+} - *S. platensis*	0.4785	0.0162	0.9201

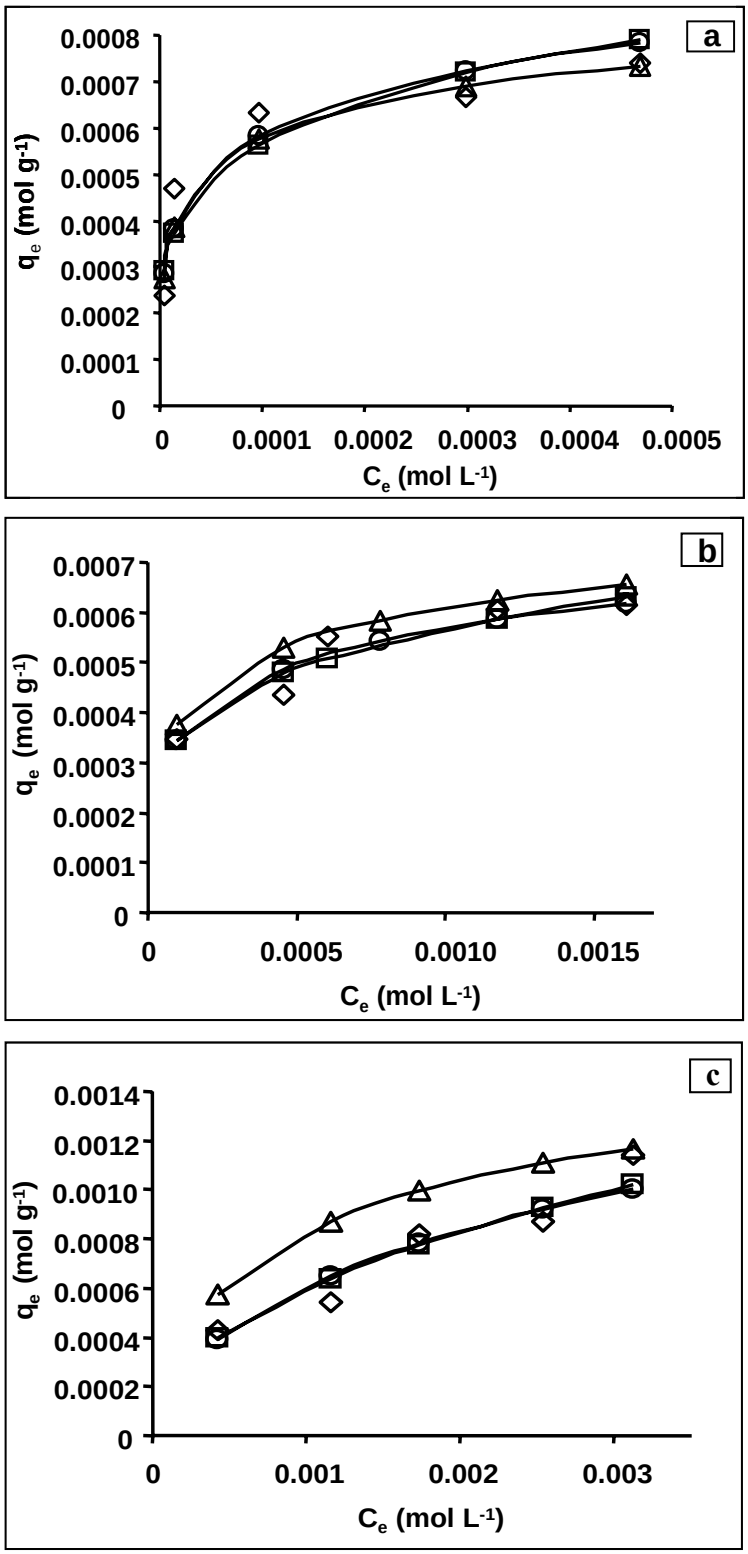


Figure 3.7. Non-linear fits of isotherm models for (a) $Pb^{2+,}$ (b) Cd^{2+} and (c) Ni^{2+} sorbed by *Spirulina platensis* (-◊- Experimental, -□- Freundlich, -Δ- Temkin -○- D-R isotherms)

Dubinin-Radushkevich (D-R) Isotherm: This model is good at low concentration ranges and can be used to describe sorption on both homogeneous and heterogeneous surfaces (Shahwan and Erten 2002).

The D-R isotherm is defined as follows (Yurdakoç et al. 2004),

$$q_e = q_m \exp(-K\varepsilon^2) \tag{3.8}$$

where qe: amount adsorbed per unit weight of solid (mole/g)
qm: sorption capacity of adsorbent per unit weight (mole/g)
K: constant related to the energy of sorption (mol^2/kJ_2)
ε: Polanyi potential = RTln(1+ 1/(Ce)) (kJ/mole)
Ce: equilibrium concentration of solute in solution (mole/g)
R: gas constant (kJ/mole K)
T: absolute temperature (K)

The linear form of the equation may be obtained by rearrangement:

$$\ln q_e = \ln q_m - K\varepsilon^2 \tag{3.9}$$

By plotting ln q_e versus ε^2, K and ln q_m can be calculated from the slope and intercept, respectively. The magnitude of D-R parameter, q_m, corresponds to the sorption monolayer capacity and K gives information about sorption energy E, perceived as the amount of energy required to transfer one mole of the adsorbate ion from infinity in the bulk of the solution to the site of sorption. It is calculated using D-R constant, K, according to the relation $E = (2K)^{-1/2}$ .The values of q_m and K are evaluated from the intercepts and slopes of plot of log q vs. ε^2. The obtained constants are given in Table 3.4.

Table 3.4. D-R parameters, K, q_m and E obtained from the plots of Pb^{2+}, Cd^{2+}, Ni^{2+} sorbed by *Spirulina platensis* at 25^0C (Amount of biomass: 10.0 mg, pH: 6.0, solution volume: 10.0 mL)

Sample	**D-R constants**			**R**
	K (mol kJ^{-1})^2	**q_m (mol g^{-1})**	**E (kJ mol^{-1})**	
Pb^{2+} - *S. platensis*	0.0018	0.0015	16.7	0.8869
Cd^{2+} - *S. platensis*	0.0022	0.0011	15.1	0.9463
Ni^{2+} - *S. platensis*	0.0057	0.0032	9.4	0.9022

Temkin isotherm: This model is given as:

$$q_e = B\ln (K_T C_e) \tag{3.10}$$

It can be expressed in the linear form as:

$$q_e = B\ln K_T + B\ln C_e \tag{3.11}$$

where B = RT/b

A plot of q versus lnC enables the determination of the isotherm constants B and K_T from the slope and the intercept, respectively. Temkin constants are given in Table 5. K_T is the equilibrium binding constant corresponding to the maximum binding energy and constant B is related to the heat of adsorption.

Table 3.5. Temkin parameters K_T and B obtained from the plots of Pb^{2+}, Cd^{2+}, Ni^{2+} sorbed by *Spirulina platensis* at 25^0C (Amount of biomass: 10.0 mg, solution volume: 10.0 mL)

Sample	**Temkin constants**		**R**
	K_T (L mol^{-1})	**B**	
Pb^{2+} - *S. platensis*	$3.3x10^6$	0.0001	0.9333
Cd^{2+} - *S. platensis*	$4.4x10^5$	0.0001	0.9367
Ni^{2+} - *S. platensis*	$1.6x10^4$	0.0003	0.8523

3.5. Design of Batch Sorption from Isotherm Data

The sorption isotherm relations were applied to predict the design of single-stage batch sorption systems (Ho and McKay 2000, Vadivelan and Kumar 2005). In a batch operation with a solution volume V (L), the initial concentration of the adsorbate is decreased from C_0 to C_l (mol L^{-1}).

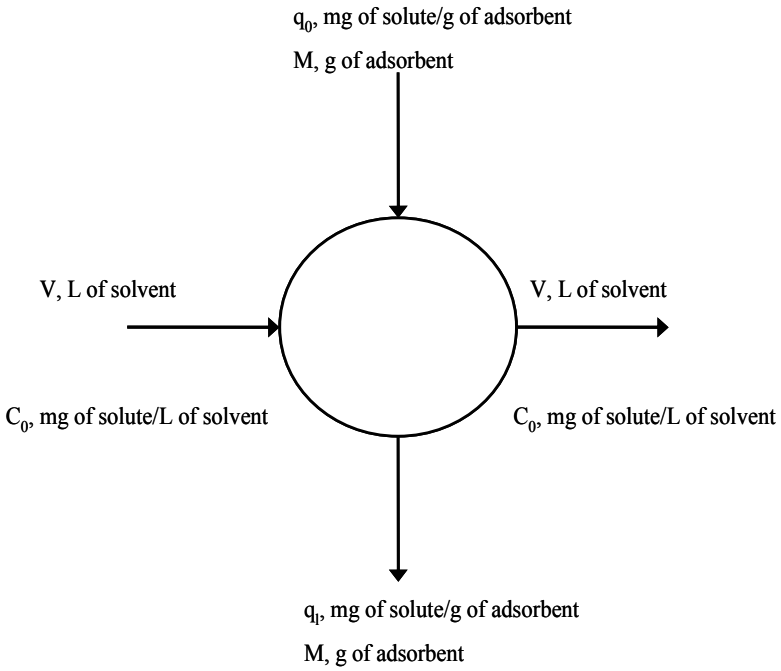


Figure 3.8. Single stage batch adsorber design

At the same time the amount of adsorbate on the adsorbent of mass M will increase from q_o to q_l. The mass balance equation for the sorption system can be written as:

$$V (C_0-C_l) = M (q_o-q_l) = M\, q_l \qquad (3.12)$$

Under equilibrium conditions, $C_l \rightarrow C_e$ and $q_l \rightarrow q_e$

Since the adsorption isotherm studies confirm that the equilibrium data for each metal ion correlated well with Freundlich isotherm, the isotherm equation can be used to calculate q_l in the mass balance equation. This yields:

$$M/V = (C_0-C_l)/q_e = (C_0-C)/(K_f C_e^{\,n}) \qquad (3.13)$$

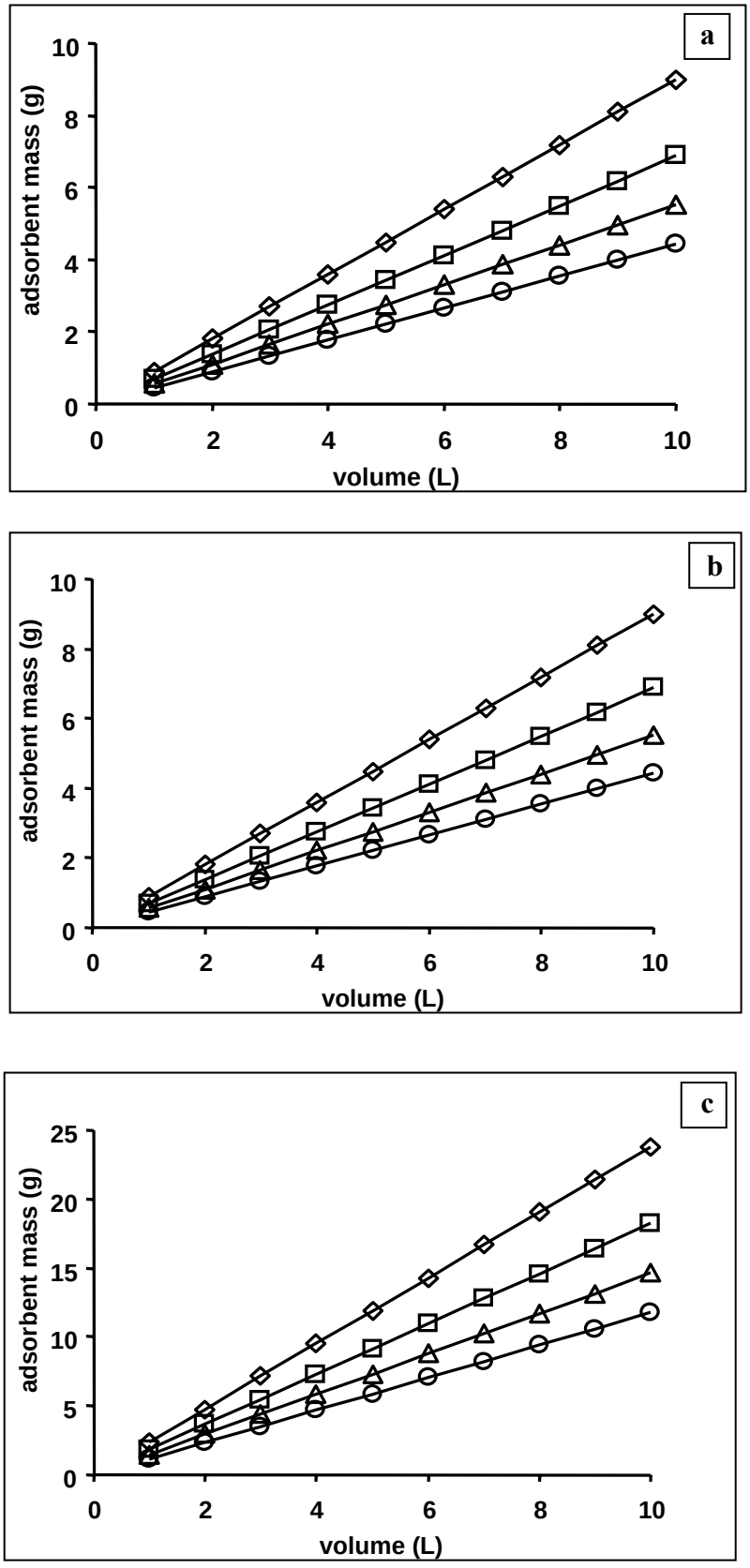


Figure 3.9. Predicted amount of biosorbent (M) against sample volume (L) for 60-90 % removal of (a) Pb^{2+} (b) Cd^{2+} and (c) Ni^{2+} (Metal ion concentration : 100.0 mg L^{-1}, -◊- 90 % removal, -□- 80 % removal, -○- 70 % removal, -Δ- 60 % removal).

The equation can be used to predict the amount of adsorbent required to achieve a specific percentage removal for a given initial metal ion concentration and solution volume. Figure 3.9 shows the plots of predicted amount of biosorbent required to obtain 60-90 % removal at the initial metal ion concentration of 100.0 mg L^{-1} and at different solution volumes of (1-10 L).

3.6. Sorption Activities in a Three Metal Ion System: Competitive Biosorption

Competitive biosorption was investigated at three different initial concentrations of Pb^{2+}, Cd^{2+} and Ni^{2+}; namely, 10.0, 50.0and 100.0 mg L^{-1} contacted each time with 10.0 mg doses of the biomass with a solution volume of 10.0 mL. The results of competitive runs, expressed as % biosorption, are provided in Table 3.6, in line with the data that were obtained for biosorption of single components.

Table 3.6. Comparison of the amount of Pb^{2+}, Cd^{2+}, Ni^{2+} sequestered from multi-metal ion solutions by S*pirulina platensis* (Amount of biomass: 10.0 mg, sample volume: 10.0 mL, n=3).

Initial concentration of Pb^{2+}+ Cd^{2+}+ Ni^{2+}	**% Pb^{2+}sorption**		**% Cd^{2+}sorption**		**% Ni^{2+}sorption**	
	single	**mixed**	**single**	**mixed**	**single**	**mixed**
10.0 mg L^{-1}	99 ± 1	97 ± 2	99 ± 1	93 ± 2	95 ± 0	87 ± 2
50.0 mg L^{-1}	99 ± 0	95 ± 2	82 ± 2	36 ± 2	64 ± 3	34 ± 2
100.0 mg L^{-1}	75 ± 3	85 ± 1	54 ± 0	15 ± 2	31 ± 6	19 ± 2

In general, the presence of competing ions leads to a decrease in the biosorption. The extent of this decrease is, however, dependent on the concentrationand is also different for different ions. As the initial concentration is increased, less biosorption is observed, as expected. The most prominent result here is the apparent relative selectivity of the biosorbent towards Pb^{2+} ions, as can be seen at the ion concentrations of 50.0 and 100.0 mgL^{-1}.

3.7. Reusability

The repetitive reactivity of the biosorbent was studied for the three metal ions at the initial concentration of 10.0 mgL^{-1}. In these experiments the amount of the biosorbent was 10.0 mg, solution volume was 10.0 mL and each sample was tried repetitively for five successive runs. The results are shown in Figure 3.10. As seen, almost a total removal of Pb^{2+} ions can still be achieved even at the fifth trial of the same sample. In comparison to this, a steady decrease in the ability of sorption towards Ni^{2+} and Cd^{2+} was observed. However, this decrease is not steep, the thing that emphasizes the high capacity of the biosorbent at the applied conditions.

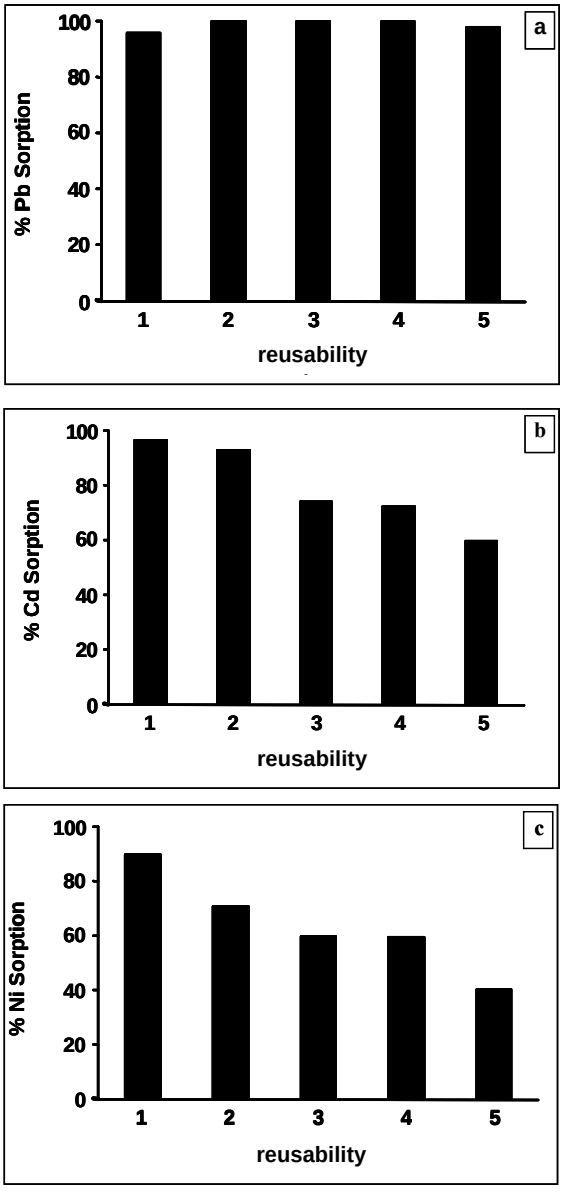


Figure 3.10. Repetitive adsorption data for (a) Pb^{2+} (b) Cd^{2+} and (c) Ni^{2+}

3.8. Immobilization of *Spirulina platensis* into Sodium Alginate

Immobilization of *Spirulina platensis* by sodium alginate showed a decrease in metal uptake capacities compared to free *Spirulina platensis* SEM images of the biosorbent in question give important clues about this. Ca-alinate gel was formed by covering *Spirulina platensis* completely according to the procedure applied. Therefore it is thought that this might decrease the sorption properties of functional groups belonging to biosorbent or cause a delay in sorption. The EDX spectra taken from the surfaces support this. It was seen that there was no great difference of N percents (3.66 and 5.41 respectively) between the alginate (Figure 3.11d) and immobilized *Spirulina platensis* (Figure 3.11e)

Particularly at low concentrations, SEM-EDX spectra were thought not to give quantitative information, N peaks were thought if there were functional groups available on the surface (*Spirulina platensis* was reported to contain amino acid and protein about 50-70%). Change in the structure of cell wall during immobilization means likely to be responsible for decreased metal sorption capacity of the immobilized biosorbent. It is also probable that the part of the cell surface might be shilded by the gel matrix making functional sites for metal binding unavailable.

For the immobilization cell system to be effective, the process of immobilization must not cause structural, physiological and metabolic damage to the cell. Immobilized biomass in the form of beads should not expand or swell during metal removal (Brouers et al. 1989). One of the most important goals of immobilization was to use the new formed sorbents in column systems. For this purpose, micro and midi-columns were prepared. However, since there appeared expansion and swelling of the new biosorbent when filled in both micro and midi columns; it was understood that it was not suitable for the flow injection systems.

It was pointed out that there was a decrease in the metal uptake capacity when immobilized *Spirulina platensis* was used. If SEM images were examined, it was seen that alginate matrix covered the biosorbent completely when the biosorbent amount was little. For this reason, new biosorbents were prepared by changing the biosorbent amounts (Figure 3.12), the experiments were repeated and the metal uptake properties of these new biosorbents were investigated. The aim of this work was to see if there was

an increase in the sorption by optimizing the biosorbent amount immobilized. The results obtained might be accepted that they were close to each other.

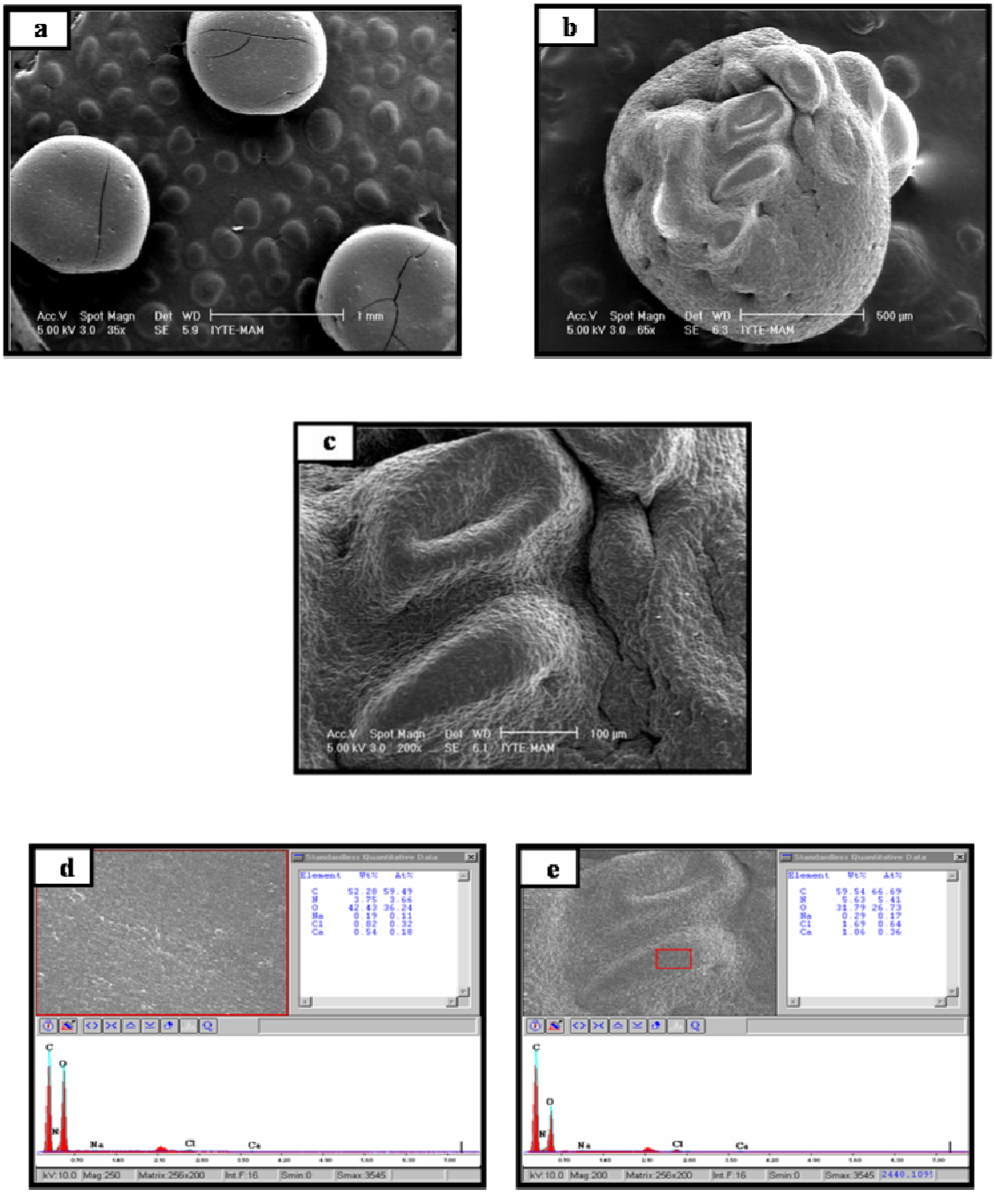


Figure 3.11. The immobilization of *Spirulina platensis* into alginate matrix I. (a) The SEM images of alginate beads (35x) (b and c) The SEM images of immobilized *Spirulina platensis* into alginate matrix (65x and 200x) (d) EDX spectrum of alginate surface (e) EDX spectrum of immobilized *Spirulina platensis* into alginate matrix (These are the typical spectra; same distribution was observed from the other points on the surface).

Table 3.7. Percent sorption of Pb, Cd and Ni by free and immobilized *Spirulina platensis* (immobilized into alginate) (Biosorbent amount: 10 mg, initial metal concentration: 250.0 mgL^{-1}, solution pH: 6, shaking time: 60 min.)

Sorbent	**% Pb Sorption**	**% Cd Sorption**	**% Ni Sorption**
Alginate	59 ± 1	29 ± 4	11 ± 6
0.1 g *Spirulina*-alginate	49 ± 1	21 ± 4	4 ± 1
0.2 g *Spirulina*-alginate	69 ± 6	16 ±2	6 ± 1
0.5 g *Spirulina*-alginate	35 ± 6	10 ± 3	10 ± 3
Spirulina	64 ± 1	25 ± 1	12 ± 5

According to the SEM images obtained, as the biosorbent amount was increased, there was also an increase in the biosorbent amount embedded within the surface. The possibility of functional groups being free also increased with the amount of *Spirulina platensis* used in the immobilization process. Generally, it was thought that according to this type of Ca-alginate immobilization procedure, *Spirulina platensis* was almost completely covered by the matrix. Hence, it was defined that this caused a decrease in the sorption properties of the functional groups belonging to the biosorbent or a delay in the sorption.

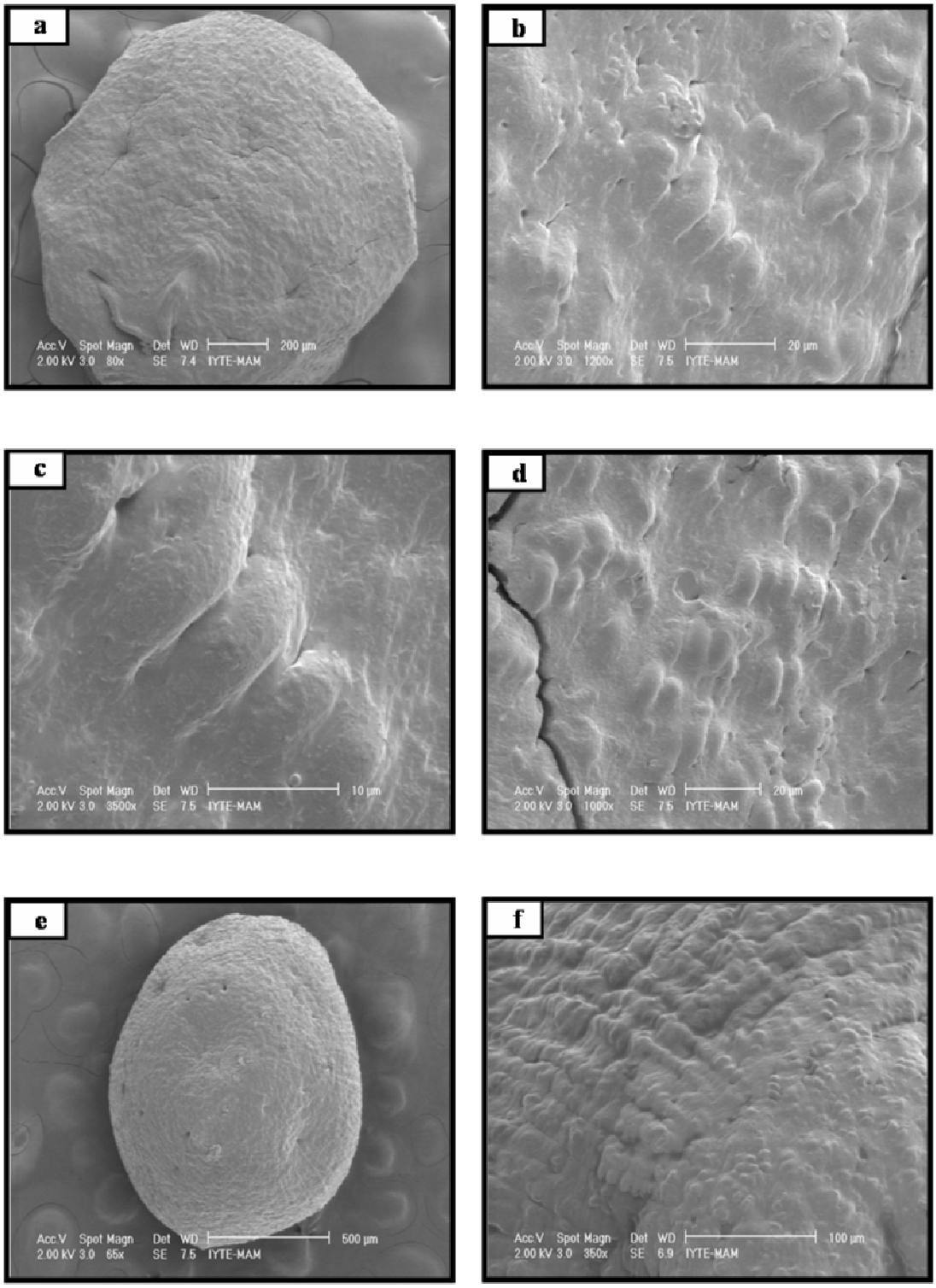


Figure 3.12. Immobilization of *Spirulina platensis* into alginate matrix II (a,b,c,d) SEM images of 0.05 g *Spirulina platensis* immobilized into alginate matrix (80x, 1200x, 1000x and 3500x) (e,f,g,h) SEM images of 0.1 g *Spirulina platensis* immobilized into alginate matrix (65x, 350, 2500x and 1000x) (i,j,k,l) SEM images of 0.5 g *Spirulina platensis* immobilized into alginate matrix (65x, 800x, 2000x and 5000x)

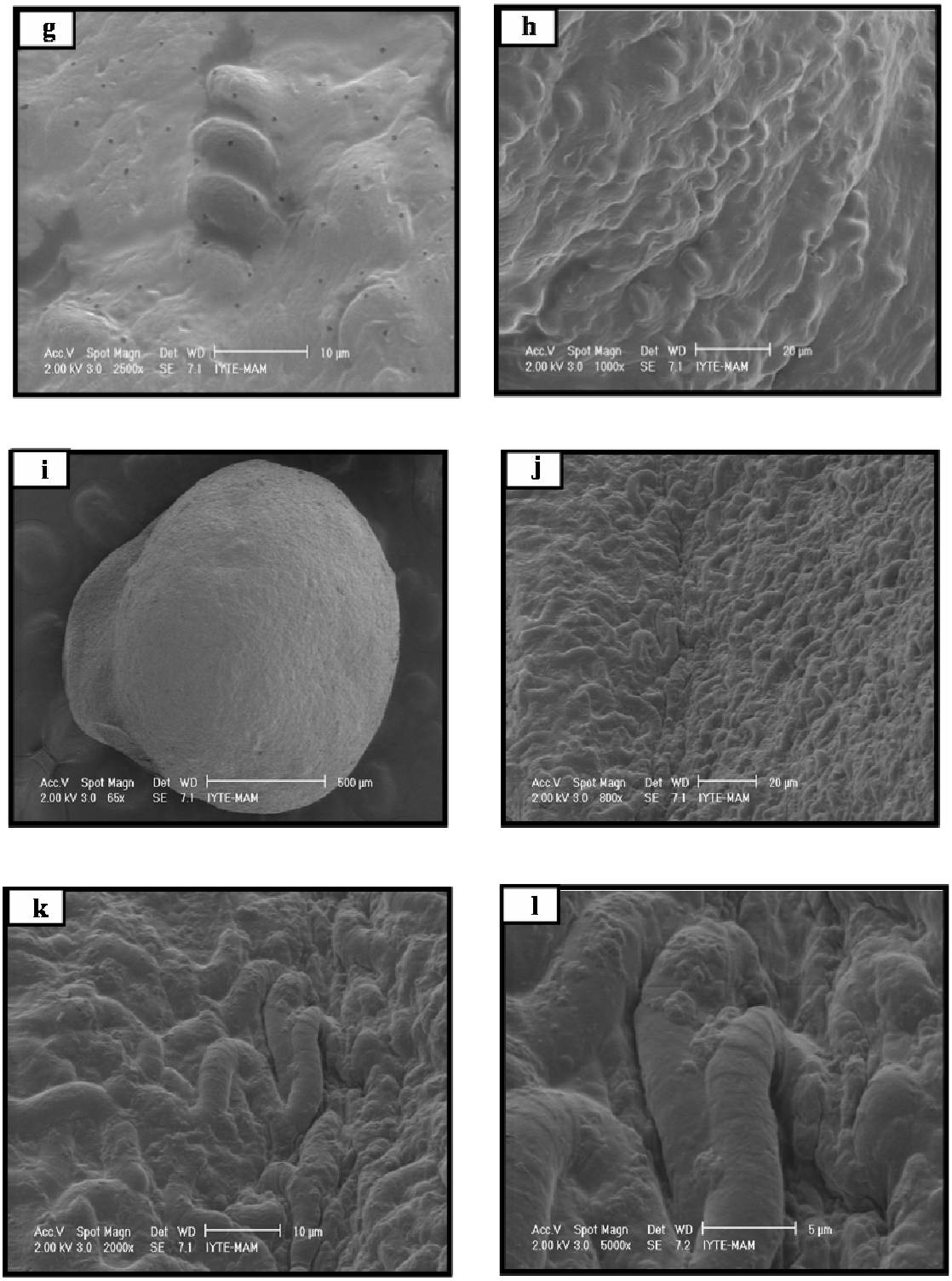


Figure 3.12. Immobilization of *Spirulina platensis* into alginate matrix II (a,b,c,d) SEM images of 0.05 g *Spirulina platensis* immobilized into alginate matrix (80x, 1200x, 1000x and 3500x) (e,f,g,h) SEM images of 0.1 g *Spirulina platensis* immobilized into alginate matrix (65x, 350, 2500x and 1000x) (i,j,k,l) SEM images of 0.5 g *Spirulina platensis* immobilized into alginate matrix (65x, 800x, 2000x and 5000x) (cont.)

3.9. Immobilization of *Spirulina platensis* into Sodium Silicate

Another inorganic synthetic polymer matrix often used to entrap the algal cells is silica gel. It was prepared by decreasing the pH of alkali silicate to less than 10. The solubility of silica was then reduced to form the gel. As silica began to gel, cells in silica were entrapped in porous gel in three-dimensional SiO_2 network. Immobilization experiments were also performed with silica gel and it was seen that the functional grous were available on the surface.

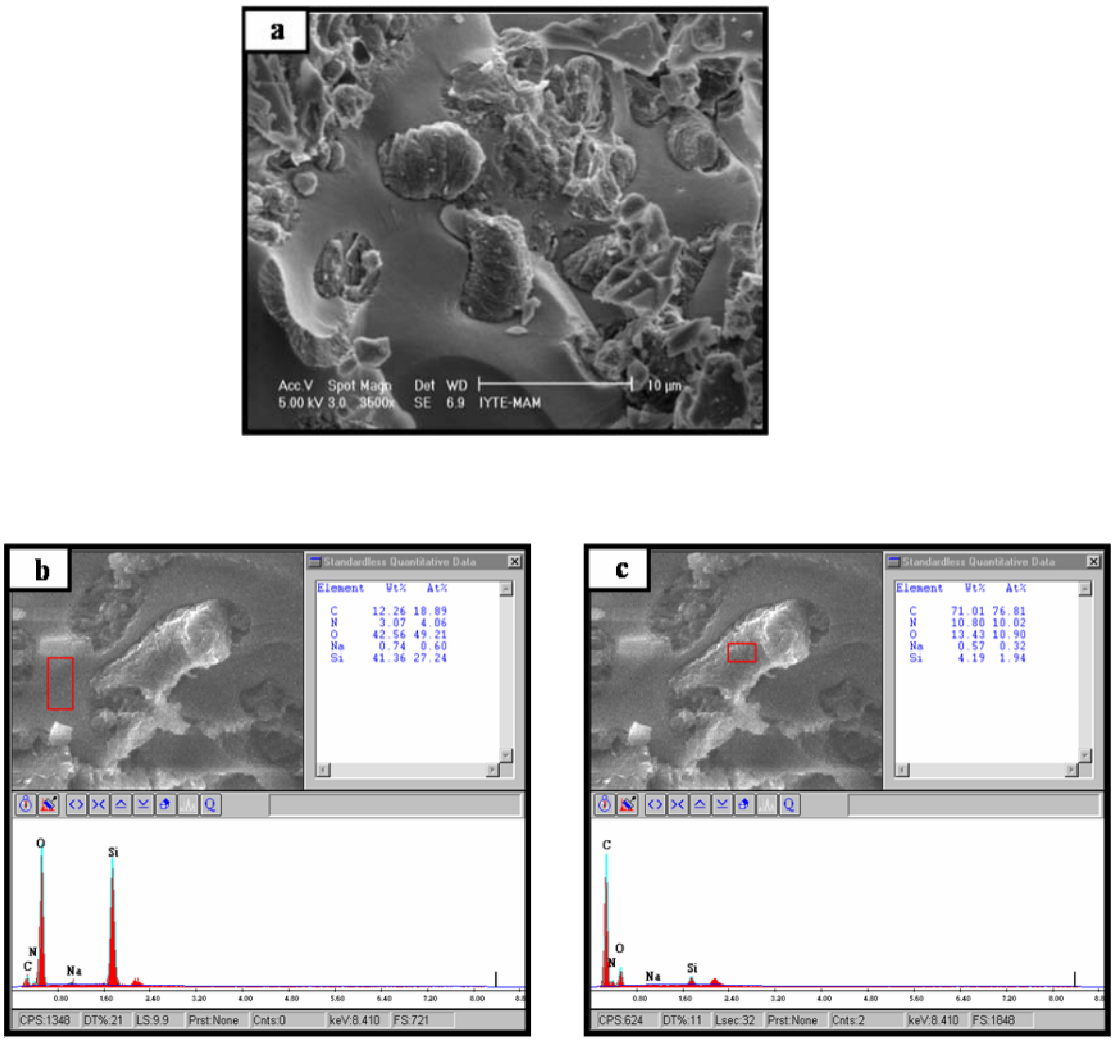


Figure 3.13. Immobilization of *Spirulina platensis* into silicate matrix I (a) SEM images of immobilized *Spirulina platensis* into silicate matrix (3500 x) (b) EDX spectrum taken from the silicate surface (c) EDX spectrum of immobilized *Spirulina platensis* into silicate surface.

Finally, the biosorbent amount immobilized was changed. SEM images showed that as the biosorbent amount was increased the immobilized *Spirulina platensis* in the

matrix was also increased (Figures 3.14 and 3.15). When the EDX spectra were observed it might be thought that the functional groups of the biosorbent were available (Figures 3.13 and 3.14 e, f, k, l). According to the results of metal sorption experiments, the silicate alone was a better sorbent for Pb^{2+} than the *Spirulina platensis* immobilized silica (Table 3.8).

Table 3.8. Percent sorption of Pb, Cd and Ni by free and immobilized *Spirulina platensis* (immobilized into silicate) (Biosorbent amount: 10 mg, initial metal concentrations: 250.0 mgL^{-1}, solution pH : 6, shaking time: 60 min.)

Sorbent	**% Pb Sorption**	**% Cd Sorption**	**% Ni Sorption**
Silicate	79 ± 3	12 ±1	9 2
0.1 g *Spirulina*-silicate	53 ± 2	17 ± 2	10 ± 3
0.2 g *Spirulina*-silicate	43 ± 2	19 ± 7	12 ± 4
0.5 g *Spirulina*-silicate	51 ± 4	13 ± 4	15 ± 1
1 g *Spirulina*-silicate	51 ± 2	21 ± 4	20 ± 3
Spirulina	64 ± 1	25 ± 1	12 ± 5

In line with these results, 1 g *Spirulina platensis* was used for the immobilization studies by changing the initial metal concentrations and it was observed that silicate was still a better sorbent for Pb^{2+} (Table 3.8). However, the situation was different for Cd^{2+} and Ni^{2+}. When the initial metal concentration of these two metals are 10.0 mgL^{-1}, silicate uptakes the metals as did the free *Spirulina platensis* (Table 3.10 and 3.11). As the initial metal concentrations were increased, the sorption characteristics of silicate decreased more compared to free and immobilized *Spirulina platensis*.

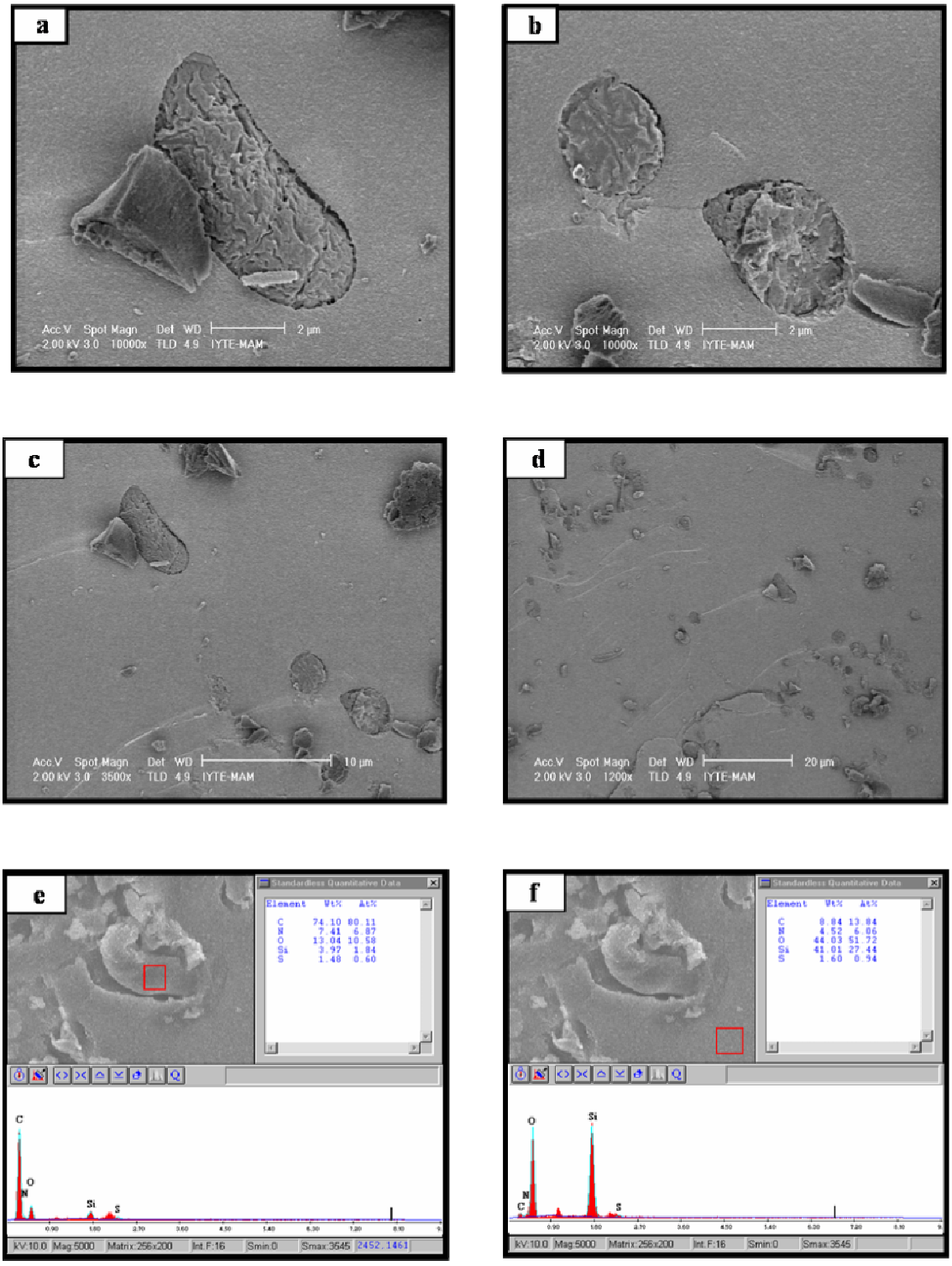


Figure 3.14. Immobilization of *Spirulina platensis* into silicate matrix II (a,b,c,d) SEM images of 0.1 g *Spirulina platensis* immobilized silicate matrix (10000x, 10000x, 3500x and 1200x) (e,f) EDX spectra of 0.1 g *Spirulina platensis* immobilized silicate matrix (g,h,i,j) SEM images of 0.2 g *Spirulina platensis* immobilized silicate matrix (1200x, 1200x, 3500x and 5000x) (k,l) EDX spectra of 0.2 g *Spirulina platensis* immobilized silicate matrix.

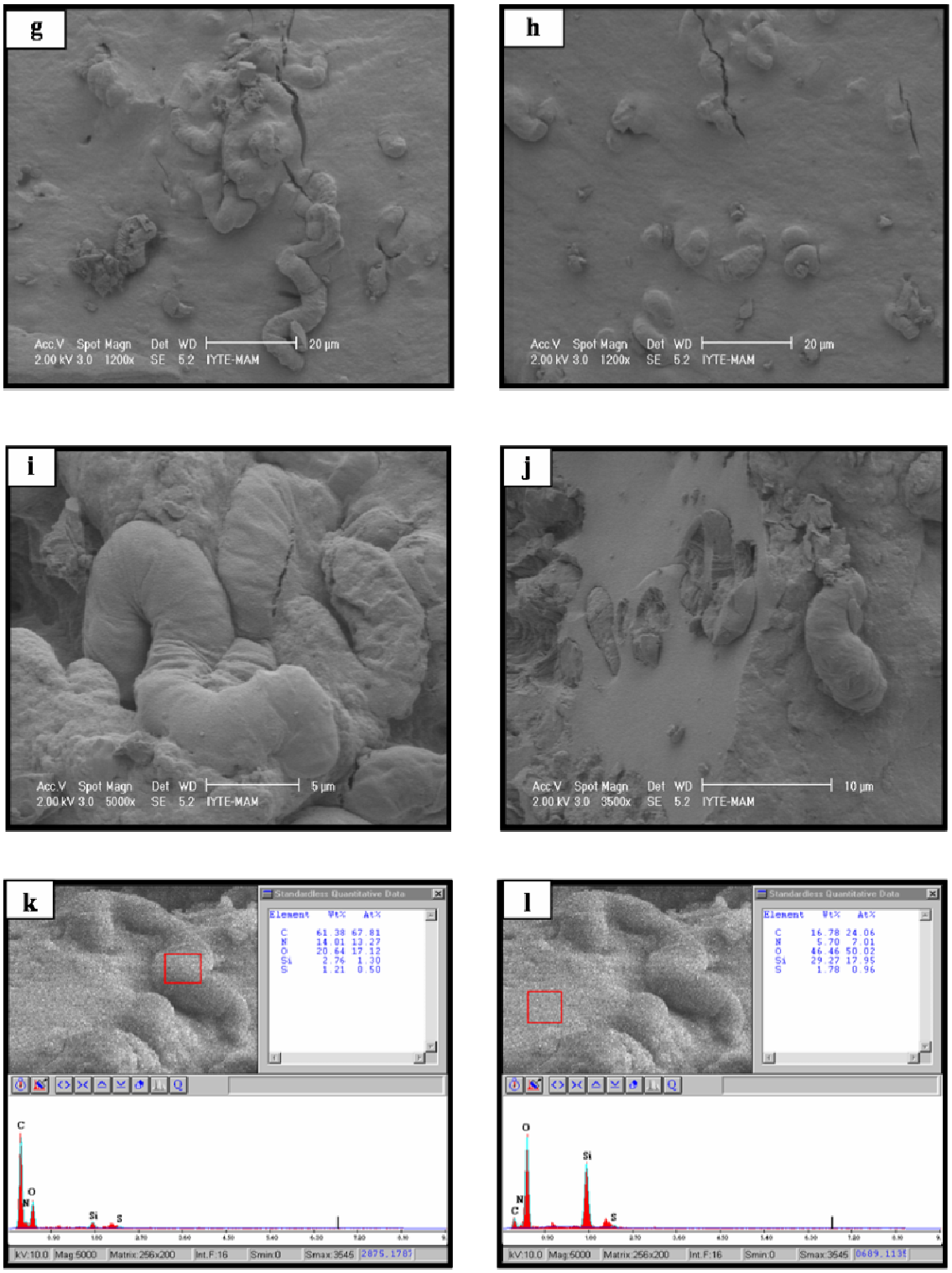


Figure 3.14. Immobilization of *Spirulina platensis* into silicate matrix II (a,b,c,d) SEM images of 0.1 g *Spirulina platensis* immobilized silicate matrix (10000x, 10000x, 3500x and 1200x) (e,f) EDX spectra of 0.1 g *Spirulina platensis* immobilized silicate matrix (g,h,i,j) SEM images of 0.2 g *Spirulina platensis* immobilized silicate matrix (1200x, 1200x, 3500x and 5000x) (k,l) EDX spectra of 0.2 g *Spirulina platensis* immobilized silicate matrix (cont.)

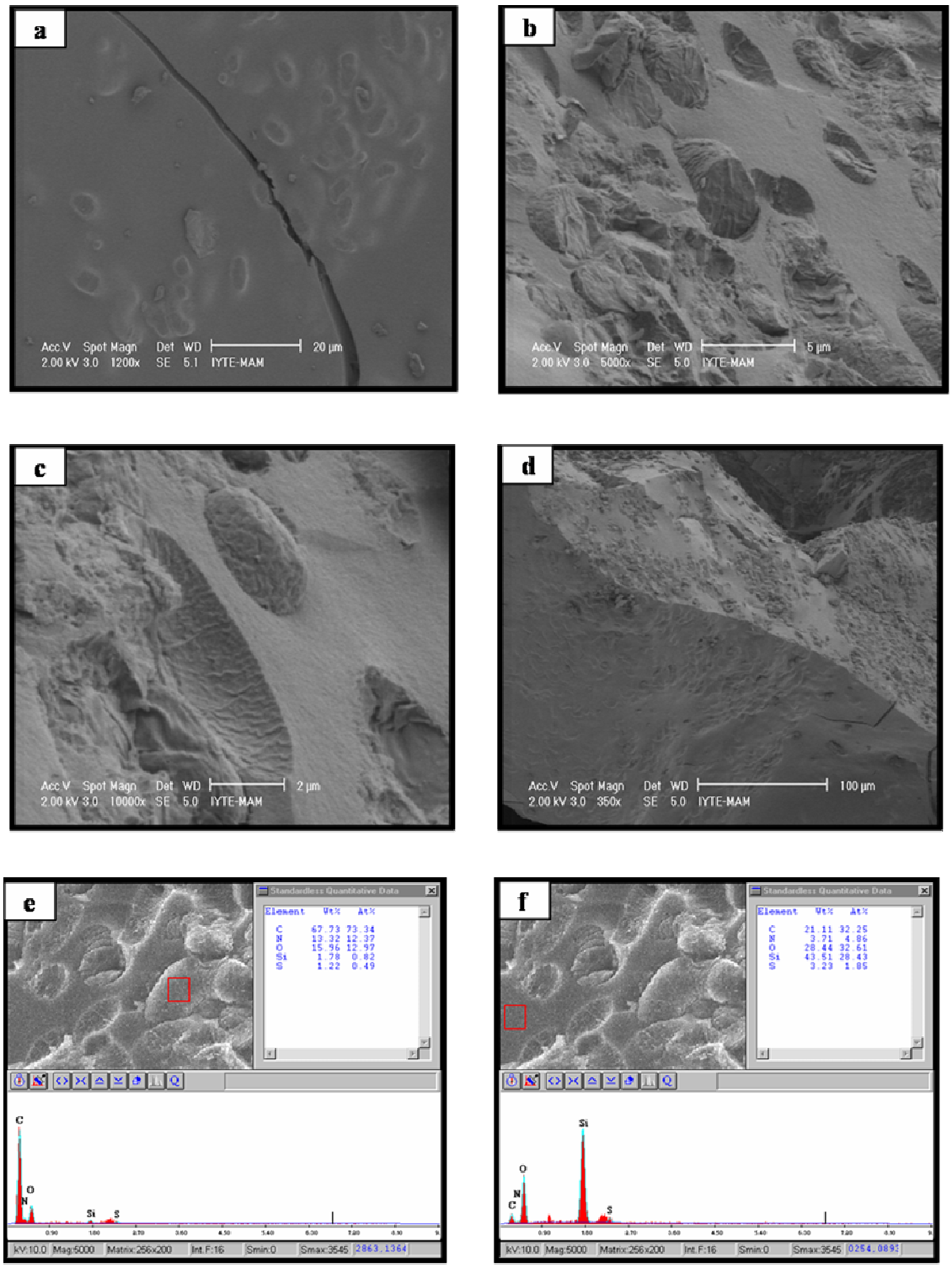


Figure 3.15. Immobilization of *Spirulina platensis* into silicate matrix III (a,b,c,d) SEM images of 0.1 g *Spirulina platensis* immobilized into silicate matrix (1200x, 5000x, 10000x and 350x) (e,f) EDX spectra of 0.5 g *Spirulina platensis* immobilized into silicate matrix (g,h,i,j) SEM images of 1 g *Spirulina platensis* immobilized into silicate matrix (1200x, 5000x, 6500x and 12000x) (k,l) EDX spectra of 0.5 g *Spirulina platensis* immobilized into silicate matrix.

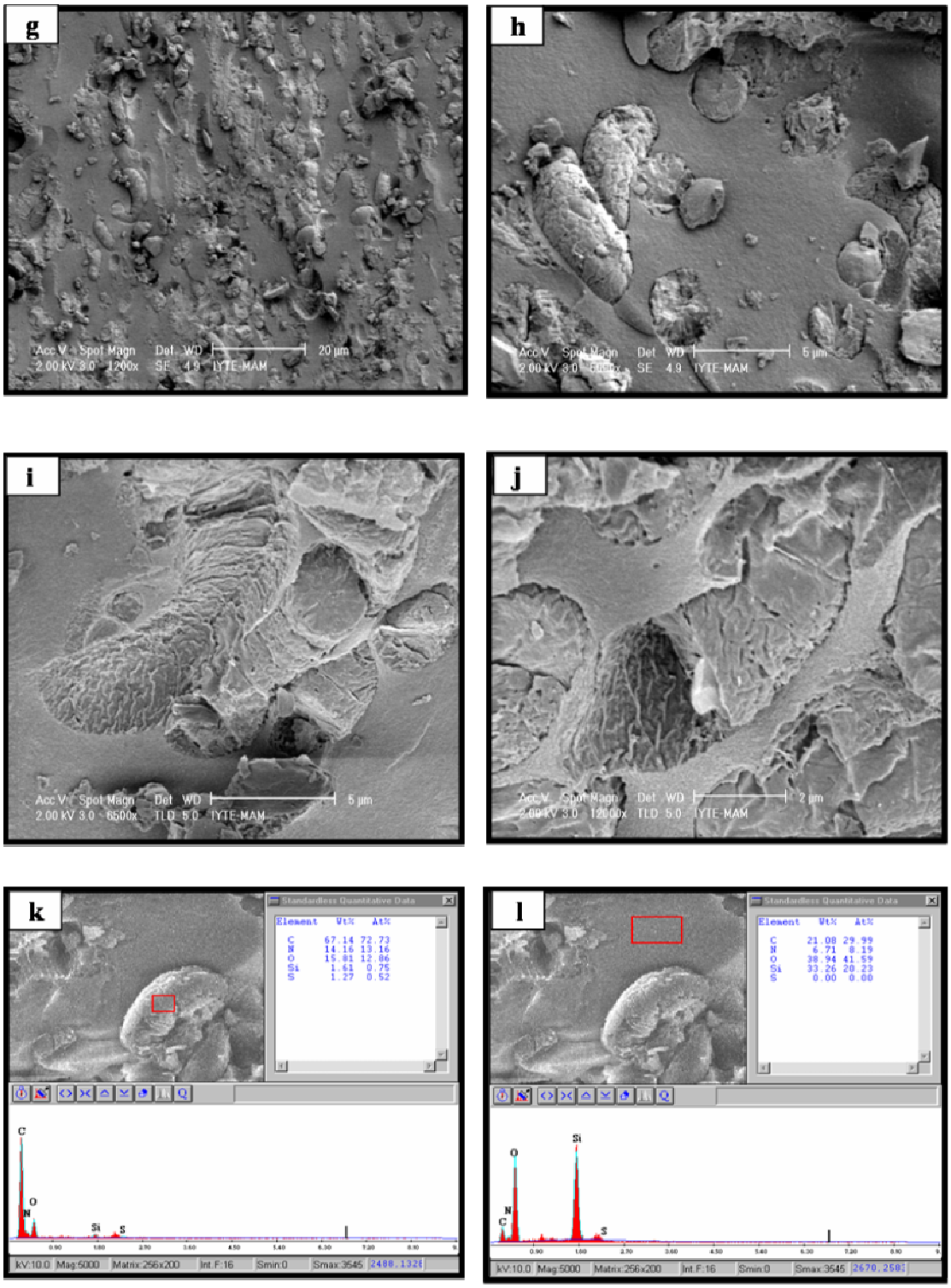


Figure 3.15. Immobilization of *Spirulina platensis* into silicate matrix III (a,b,c,d) SEM images of 0.1 g *Spirulina platensis* immobilized into silicate matrix (1200x, 5000x, 10000x and 350x) (e,f) EDX spectra of 0.5 g *Spirulina platensis* immobilized into silicate matrix (g,h,i,j) SEM images of 1 g *Spirulina platensis* immobilized into silicate matrix (1200x, 5000x, 6500x and 12000x) (k,l) EDX spectra of 0.5 g *Spirulina platensis* immobilized into silicate matrix (cont).

Table 3.9. Percent sorption of Pb^{2+} by free and immobilized *Spirulina platensis* (immobilized into silicate)

Sorbents	10 mgL^{-1}	50 mgL^{-1}	100 mgL^{-1}	250 mgL^{-1}
Spirulina	99 ± 1	99 ±0	75 ± 3	64 ± 1
Silicate	100 ± 0	95 ± 2	80 ± 5	82 ± 3
***Spirulina*-silicate**	95 ± 0	97 ± 3	55 ± 2	61 ± 3

Table 3.10. Percent sorption Cd^{2+} by free and immobilized *Spirulina platensis* (immobilized into silicate)

Sorbents	10 mgL^{-1}	50 mgL^{-1}	100 mgL^{-1}	250 mgL^{-1}
Spirulina	99 ± 1	82 ± 2	54 ± 0	25 ± 1
Silicate	98 ± 2	52 ± 2	28 ± 2	9 ± 2
***Spirulina*-silicate**	98 ± 2	65 ± 2	46 ± 2	21 ± 3

Table 3.11. Percent sorption of Ni^{2+} by free and immobilized *Spirulina platensis* (immobilized into silicate)

Sorbents	10 mgL^{-1}	50 mgL^{-1}	100 mgL^{-1}	250 mgL^{-1}
Spirulina	95 ± 0	64 ± 3	31 ± 6	12 ± 5
Silicate	85 ± 3	40 ± 3	11 ± 4	4 ± 3
***Spirulina*-silicate**	87 ± 3	50 ± 2	30 ± 3	20 ± 1

3.10. Immobilization of *Spirulina platensis* into Carboxymethylcellulose

The immobilization of *Spirulina platensis* in carboxymethylcellulose (CMC) seemed to be more successful compared to the alginate immobilization (Figure 3.16). The spectra taken from the free CMC (Figure 3.16b) and *Spirulina platensis* immobilized into CMC surfaces (Figure 3.16c) showed that there was an important difference between the N percents (5.32 and 12.89, respectively). The functional groups were available. If the results are observed the sorption capacity of algae immobilized into CMC seemed to be higher than the sorption capacity of CMC. However, free *Spirulina platensis* was able to uptake metals in higher amounts (Table 3.8). Although

the biosorbent amount was increased there could not be obtained a significant change in the sorption capacity. For this reason, it was deduced that CMC was not suitable for the immobilization of *Spirulina platensis.*

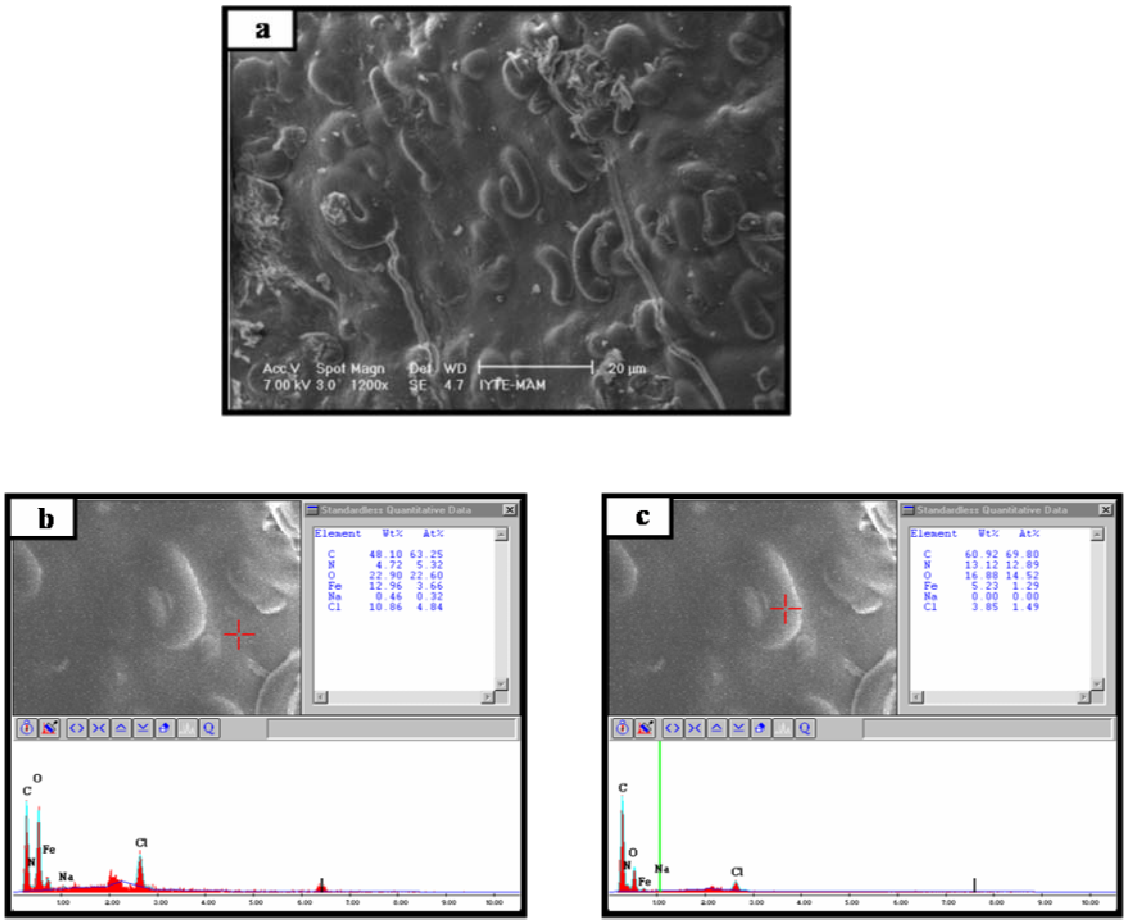


Figure 3.16. Immobilization of *Spirulina platensis* into carboxymethylcellulose I (CMC) (a) SEM image of *Spirulina platensis* immobilized into CMC (1200x) (b,c) EDX spectra of *Spirulina platensis* immobilized into CMC.

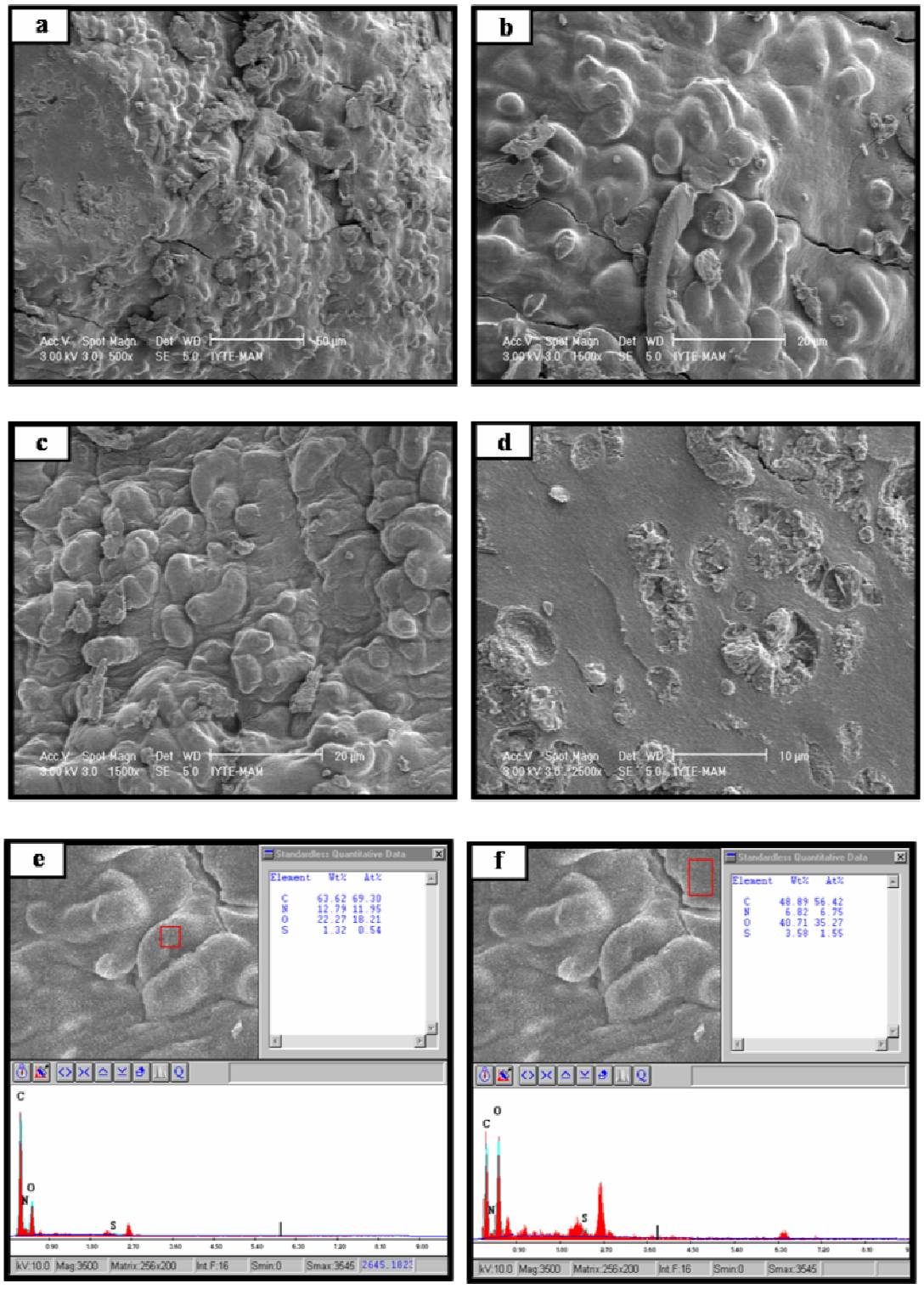


Figure 3.17. Immobilization of *Spirulina platensis* into carboxymethylcellulose II (CMC) (a,b,c,d) SEM images of 0.05 g *Spirulina platensis* immobilized CMC (500x, 1500x, 1500x and 2500x) (e,f) EDX spectra of 0.05 g *Spirulina platensis* immobilized CMC (g,h,i,j) SEM images of 0.1 g *Spirulina platensis* immobilized CMC (1000x, 2000x, 2500x and 1500x) (k,l) EDX spectra of 0.1 g *Spirulina platensis* immobilized CMC (m,n,o,p) SEM images of 0.5 g *Spirulina platensis* immobilized CMC (500x, 1500x, 2500x and 1500x) (r,s) EDX spectra of 0.5 g *Spirulina platensis* immobilized CMC.

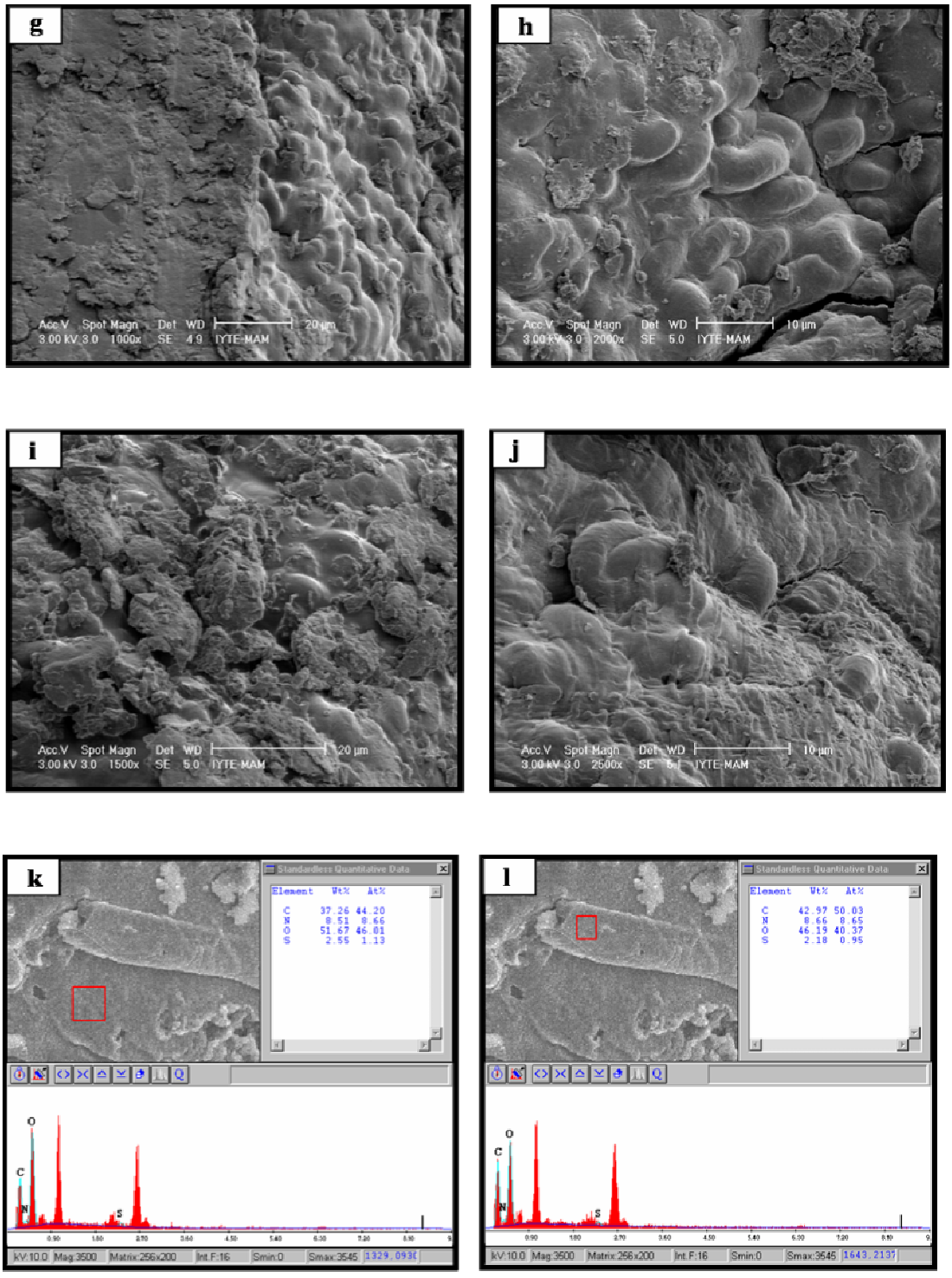


Figure 3.17. Immobilization of *Spirulina platensis* into carboxymethylcellulose II (CMC) (a,b,c,d) SEM images of 0.05 g *Spirulina platensis* immobilized CMC (500x, 1500x, 1500x and 2500x) (e,f) EDX spectra of 0.05 g *Spirulina platensis* immobilized CMC (g,h,i,j) SEM images of 0.1 g *Spirulina platensis* immobilized CMC (1000x, 2000x, 2500x and 1500x) (k,l) EDX spectra of 0.1 g *Spirulina platensis* immobilized CMC (m,n,o,p) SEM images of 0.5 g *Spirulina platensis* immobilized CMC (500x, 1500x, 2500x and 1500x) (r,s) EDX spectra of 0.5 g *Spirulina platensis* immobilized CMC (cont.)

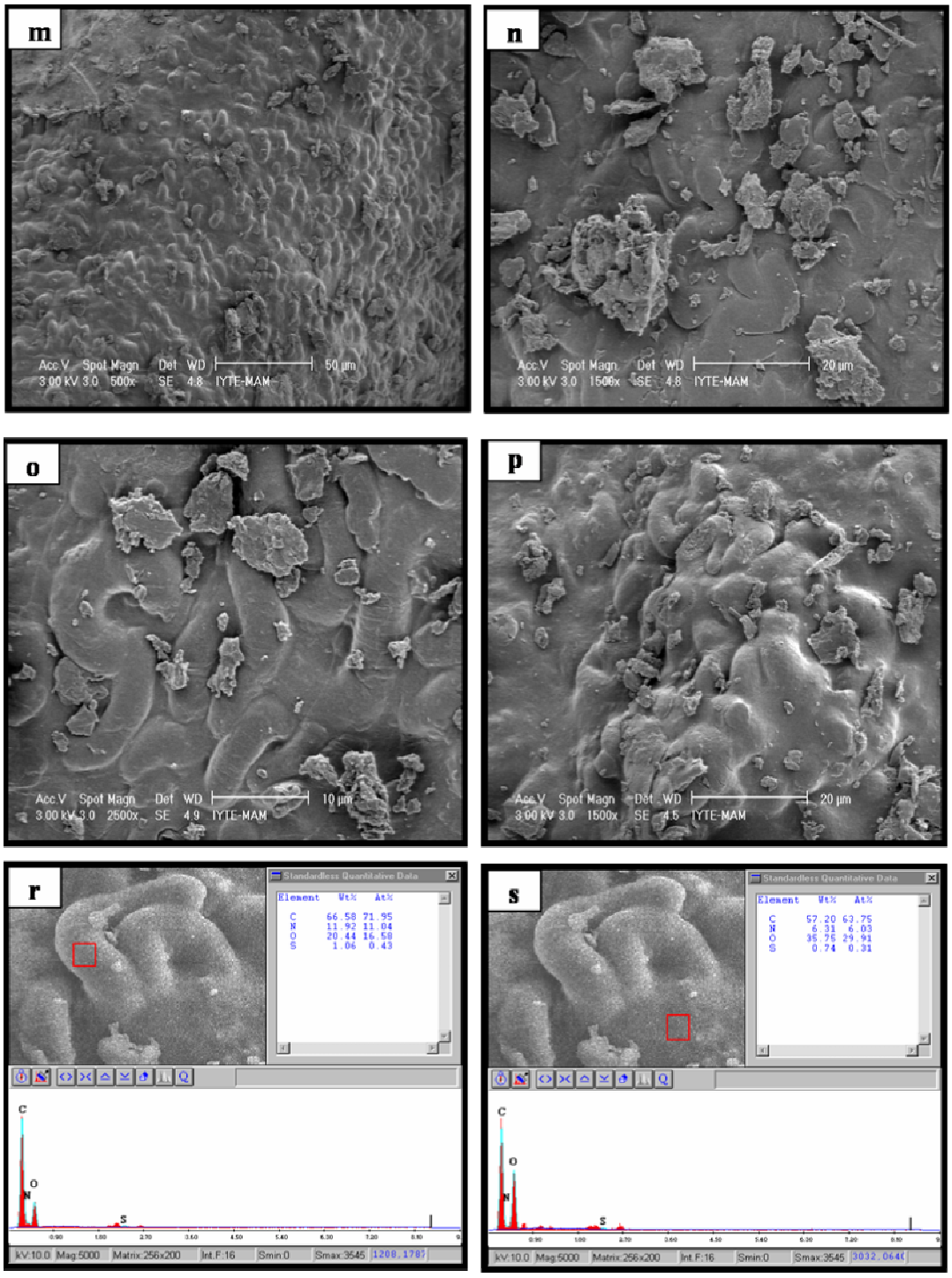


Figure 3.17. Immobilization of *Spirulina platensis* into carboxymethylcellulose II (CMC) (a,b,c,d) SEM images of 0.05 g *Spirulina platensis* immobilized CMC (500x, 1500x, 1500x and 2500x) (e,f) EDX spectra of 0.05 g *Spirulina platensis* immobilized CMC (g,h,i,j) SEM images of 0.1 g *Spirulina platensis* immobilized CMC (1000x, 2000x, 2500x and 1500x) (k,l) EDX spectra of 0.1 g *Spirulina platensis* immobilized CMC (m,n,o,p) SEM images of 0.5 g *Spirulina platensis* immobilized CMC (500x, 1500x, 2500x and 1500x) (r,s) EDX spectra of 0.5 g *Spirulina platensis* immobilized CMC (cont.).

Table 3.12. Percent sorption of Pb, Cd and Ni by free and immobilized *Spirulina platensis* (immobilized into CMC) (Biosorbent amount: 10 mg, initial metal concentrations: 250.0 mgL^{-1}, solution pH: 6, shaking time: 60 min.)

Sorbents	**% Pb Sorption**	**% Cd Sorption**	**% Ni Sorption**
CMC	28 ± 3	10 ± 0	8 ± 3
0.05 g *Spirulina*- CMC	25 ± 3	7 ± 4	8 ± 1
0.1 g *Spirulina*- CMC	38 ± 6	14 ± 6	8 ± 6
0.2 g *Spirulina*- CMC	41 ± 2	12 ± 4	10 ± 4
Spirulina	64 ± 1	25 ± 1	22 ± 3

3.11. Immobilization of *Spirulina platensis* into Polysulfone

The immobilization experiments were also performed with the polysulfone matrix. According to the results, it was seen that polysulfone beads in micro size formed (Figure 3.18a and b). The functional groups in *Spirulina platensis* seemed to be available as seen from the EDX spectra (Figure 3.18c and d). However, since the size of *Spirulina platensis* was much greater than these beads, it was observed that the immobilization of *Spirulina platensis* could not be possible with this procedure applied. Therefore sorption studies were not performed with this sorbent.

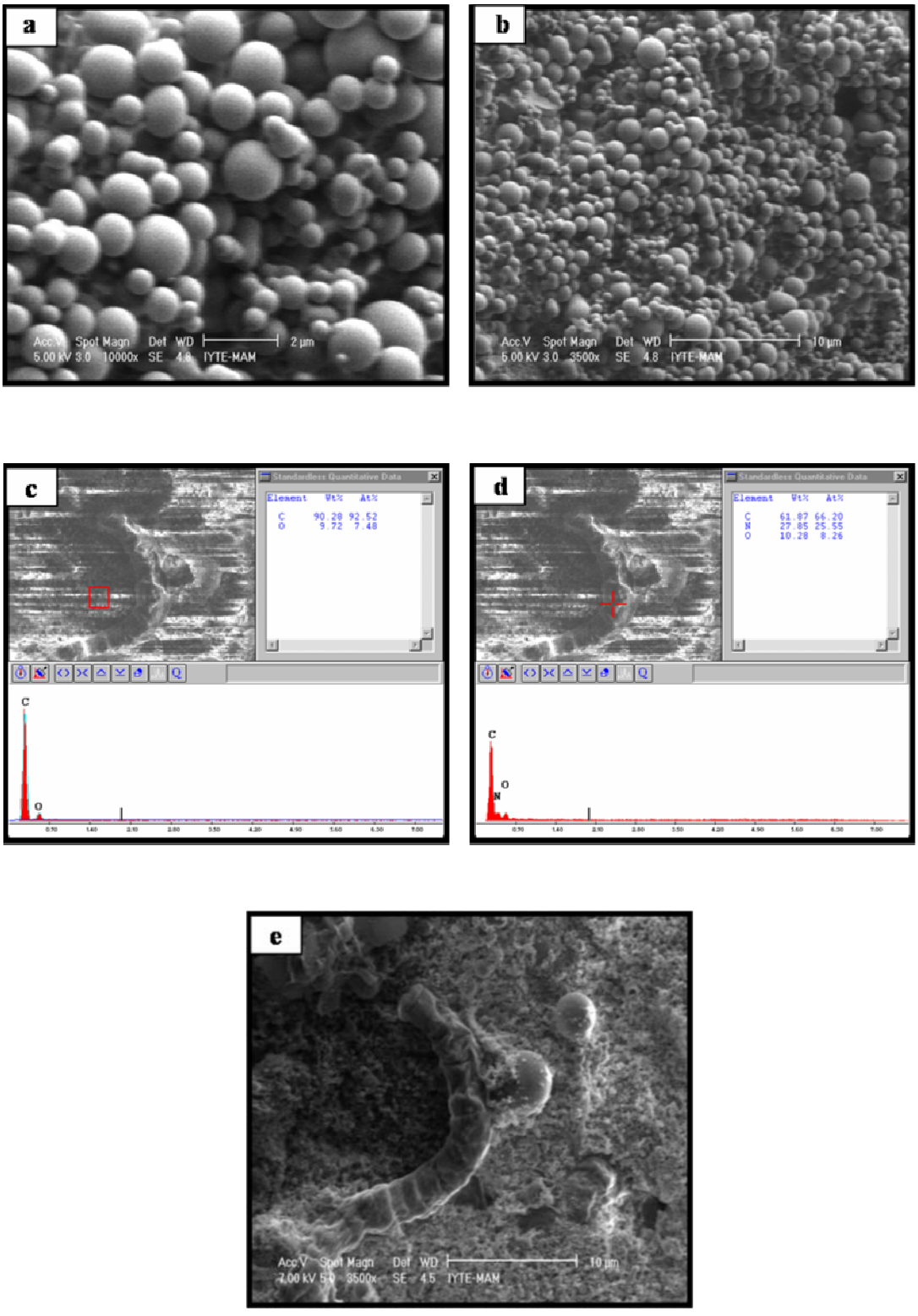


Figure 3.18. Immobilization of *Spirulina platensis* into polysulfone matrix (a,b) Polysulfone beads (10000x and 3500x respectively (c) EDX spectrum taken from polysulfone surface (d) EDX spectrum of *Spirulina platensis* immobilized polysulfone (e) SEM images of *Spirulina platensis* immobilized polysulfone (3500 x)

CHAPTER 4

CONCLUSION

The present study demonstrated that *Spirulina platensis* was a suitable biosorbent for the adsorption of Pb^{2+}, $Cd^{2+}$and Ni^{2+} ions from aqueous solution. The uptake of metal ions by *Spirulina platensis* seemed to be quite rapid and the experimental data obeyed well the pseudo-second-order model. Based on kinetic modeling, the apparent activation energy, E_a, was calculated for Pb^{2+}, $Cd^{2+}$and Ni^{2+}. The biosorption of metal ions by biosorbent was found to be endothermic. The equilibrium data fitted well to Freundlich, Dubinin-Radushkevich and Temkin isotherm models in the studied concentration range. Freundlich isotherm model was then used to predict the amount of *Spirulina platensis* required to remove desired amount of Pb^{2+}, $Cd^{2+}$and Ni^{2+} ions from solutions of initial concentration of 100.0 mg L^{-1}. Competitive biosorption experiments showed that *Spirulina platensis* had a relative selectivity towards Pb^{2+} ions. The reusability of the biosorbent was investigated for three metal ions for several trials. A little decrease in the sorption of Cd^{2+} and Ni^{2+} ions was achieved in comparison to Pb^{2+} ions indicating that the biosorbent could still be suitable for the removal of metal ions even after five successive runs.

Based on the results of these studies, the specific conclusions were deduced:

SEM images, EDX, IR spectra and elemental analysis results showed that *Spirulina platensis* have complex structures of a heterogeneous nature.

It was concluded that, the ability of *Spirulina platensis* to remove Pb^{2+}, $Cd^{2+}$and Ni^{2+} ions was found to be good enough and it could be used for the uptake of heavy metals in environmental samples.

The pseudo-second-order model was more suitable for the kinetic description of the sorption process for biosorption of three metals of interest.

The activation energies, E_a, for the biosorption of Pb^{2+}, $Cd^{2+}$and Ni^{2+} ions used were calculated to be 44 $kJmol^{-1}$, -16 $kJmol^{-1}$ and 54 $kJmol^{-1}$, respectively.

Positive entropy change was obtained at high initial concentrations. This indicates that at larger concentrations, more disorderness is associated with the sorption process.

Actually, none of the new sorbents generated by the immobilization of *Spirulina platensis* gave expected results. The results showed that there was no significant change in the sorption capacities of the immobilized biosorbents. Furthermore, it was deduced that they were not suitable for the micro-column flow injection systems as expansion and swelling of the new biosorbent occurred. In addition, the micro-columns prepared for flow injection systems were clogged.

Unfortunately, the mechanism of biosorption is still little understood. There is as yet no complete knowledge about the functional groups responsible for metal uptake and about the fundamental mechanisms by which metals are bound to the materials. Such understanding would allow their classification based on chemical interactions and extend their applications.

To sum up, biosorption phenomenon is a new developing area of which further studies are required to understand its full aspects.

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