DOKUZ EYLUL UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

SEPARATION AND PRECONCENTRATION OF LANTHANUM(III) ION FROM AQUEOUS SOLUTION USING *CYSTOSEIRA BARBATULA*

by
Deva KOCAİBİŞ

April, 2009
İZMİR

SEPARATION AND PRECONCENTRATION OF LANTHANUM(III) ION FROM AQUEOUS SOLUTION USING *CYSTOSEIRA BARBATULA*

A Thesis Submitted to the Graduate School of Natural and Applied Sciences of Dokuz Eylül University In Partial Fulfillment of the Requirements for the Degree of Master of Science in Chemistry

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M. Sc. THESIS EXAMINATION RESULT FORM

We have read the thesis entitled **"SEPARATION AND PRECONCENTRATION OF LANTHANUM(III) ION FROM AQUEOUS SOLUTION USING *CYSTOSEIRA BARBATULA*"** completed by **DEVA KOCAİBİŞ** under supervision of **PROF.DR. MELEK MERDİVAN** and we certify that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science.

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Deva KOCAİBİŞ

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ABSTRACT

The biosorption of lanthanum(III) on *Cystoseira barbatula* for preconcentration–separation has been investigated. The sorbed lanthanum on biosorbent was eluted by using 0.005 M etilen diamin tetra acetic acid (EDTA) and lanthanum was determined by complexing with Arsenazo-III following UV-visible absorption spectrometry. The influences of analytical parameters including amounts of biomass, initial pH of solution, contact time, desorption solution type and concentration, eluent volume, sample volume on the quantitative recoveries of lanthanum were investigated. The effects of alkaline and earth alkaline ions and some transition, lanthanide and actinide ions on the retention of lanthanum on *Cystoseira barbatula* were also examined. Separation and preconcentration lanthanum from water and soil samples was achieved quantitatively. 5 to 50 fold preconcentration was obtained for sample solution having volume from 100 to 1000 mL. The precision (relative standard deviation %) of the method was 2.86 % for lanthanum at a concentration of 1μg mL^{-1} for a series of 10 replicates. The recovery for lanthanum was equal or high 94 percent. The validation of the procedure was performed by the analysis of the certified standard reference soil material (SRM 2709). The presented method was applied to the determination of lanthanum in tap water and soil sample with successfully results. The maximum adsorption capacity is between 24.88-83.96 mg g^{-1} according to linearized Freundlich model.

Keywords: Solid phase extraction, preconcentration, biosorption, lanthanum.

CYSTOSEİRA BARBATULA KULLANARAK SULU ÇÖZELTİDEN LANTANYUM(III) İYONUNUN AYRILMASI VE ÖNDERİŞTİRİLMESİ

ÖZ

Önderiştirme-ayırma amaçlı *Cystoseira barbatula* üzerinde lantanın biotutunması araştırılmıştır. Bisorbent üzerinde tutulan lantan, 0,005 M EDTA ile elue edilmiş ve Arsenazo-III ile kompleksleştirme sonrası UV-görünür absorpsiyon spektrometresi ile tayin edilmiştir. Biyokütle miktarı, çözeltinin başlangıç pH'sı, etkileşme süresi, sıyırma çözeltisi, derişimi ve hacmi ve örnek hacmi gibi analitik parametrelerin etkileri lantanın nicel geri kazanımında araştırılmıştır. Alkali ve toprak alkali iyonları, bazı geçiş metalleri, lantanit ve aktinit iyonlarının lantanın *Cystoseira barbatula* üzerinde alıkonmasındaki etkileri incelenmiştir. Lantanın su ve toprak örneklerinden ayrılması ve önderiştirlmesi nicel olarak başarılmıştır. 100-1000 mL lik örnek hacimleri ile yapılan çalışmalarda 5 ile 50 kat önderiştirme sağlanmıştır.Yöntemin kesinliği (% bağıl standart sapma) 1 $\mu g\ mL^{-1}$ derişimdeki standart çözelti için 10 kez tekrarlanmış ve 2,86 % olarak bulunmuştur. Lantanın geri kazanımı yüzde 94 veya büyük olarak belirlenmiştir. Yöntemin validasyonu sertifikalı standart referans toprak maddesi ile (SRM 2709) gerçekleştirilmiştir. Önerilen yöntem, musluk suyu ve toprak örneğinde lantan tayinine başarı ile uygulanmıştır. Linearize edilmiş Freudlich modeline göre maksimum adsorpsiyon kapasitesi 24,88-83,96 $mg\ g^{-1}$'dir.

Anahtar sözcükler : Katı faz eksraksiyonu, önderiştirme, biosorbentler, lantanyum.

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CHAPTER ONE

INTRODUCTION

1.1 The Lanthanides

Lanthanides are f-electronic, chemically active, silvery-white metals with atomic numbers from 51 to 71. In nature, they never exist as pure elements, but only as sparsely distributed minerals, e.g., cerite, monazite and euxenite, or as admixtures in other ores. Lanthanides are definitely electropositive metals with the oxidation number of (III). Only cerium, terbium and praseodymium with an oxidation number of (IV) and samarium, europium and ytterbium with the oxidation number of (II) form stable compounds. The ionic radii of lanthanides range from 0.0848 nm (Lu) to 0.1034 nm (Ce), the values being relatively higher than those in other elements with the same oxidation number (Marczenko, 1986).

Cytophysiological effects of lanthanides appear to result from the similarity of their cationic radii to the size of Ca(II) ions. Trivalent lanthanide ions, especially La(III) and Gd(III), block different calcium channels in human and animal cells (Palasz & Czekaj, 2000).

Lanthanides, also called rare-earth elements, are an interesting group of 15 mainly trivalent metals. In fact, lanthanides are not as rare as the name implies, except for promethium, a radioactive artificial element not found in nature. The mean concentrations of lanthanides in the earth's crust are comparable to those of life-important elements like iodine, cobalt and selenium (Palasz & Czekaj, 2000).

1.1.1 Chemical Properties

Lanthanides are chemically similar to each other, to scandium and yttrium, and to the actinidies. The lanthanides are typically placed below the main body of the

periodic table in the manner of a footnote. The full-width version of the periodic table shows the position of the lanthanides more clearly (Winter, 2005).

Lanthanide compounds frequently have magnetic, catalytic and optic properties and therefore they are widely used in industry. Such industrial sources of lanthanides are potentially hazardous to human health and therefore there is a need to more closely investigate the effects of these elements on tissue and organ functioning. In recent years, new experimental methods have been developed, thanks to which we have obtained new data on the role of lanthanides in the biochemical processes operating in cellular membranes, organelles and cytoplasm (Palasz & Czekaj, 2000).

Rare-earth element hydroxides, $M(OH)_3$ precipitate from nitrate solution at pH values above 6.3-7.8 and reveal no amphoteric properties. The rare-earth elements yield acid-insoluble fluorides and oxalates, and soluble EDTA, tartrate, and citrate complexes. The lanthanides are all very reactive and electropositive. Despite the high charge, the large size of the Ln(III) ions results in low charge densities and their compounds are predominately ionic in character (Massart & Hoste,1963).

The ionic radii decrease smoothly across the series. This decrease in size is the famous ‘lanthanide contraction’. The lanthanide contraction is caused by the increase in effective nuclear charge across the series due to the poor shielding ability of 4f electrons. This is seen every period as a shell is filled (Winter, 2005). The Ln(III) ions are class-a or hard in character so they tend to bond to O, N and F-donors with F preferred to Cl>Br >I (Winter, 2005). The smooth decrease in the ionic radii results in a regular variation in chemical properties. As the Ln(III) radius decreases across the series, the salts become (somewhat) less ionic (Winter, 2005).

1.1.2 Lanthanum

Lanthanum is a chemical element with the symbol La and atomic number 57. Lanthanum is a silvery white metallic element that belongs to group 3 of the periodic

table and is a lanthanoid. Found in some rare-earth minerals, usually in combination with cerium and other rare earth elements. Lanthanum is malleable, ductile, and soft enough to be cut with a knife. It is one of the most reactive of the rare-earth metals. The metal reacts directly with elemental carbon, nitrogen, boron, selenium, silicon, phosphorus, sulfur, and with halogens. It oxidizes rapidly when exposed to air. Cold water attacks lanthanum slowly, while hot water attacks it much more rapidly (Winter, 2005).

1.1.2.1 Uses of Lanthanum

Carbon lighting applications, especially by the motion picture industry for studio lighting and projection. La_2O_3 improves the alkali resistance of glass, and is used in making special optical glasses, such as: Infrared absorbing glass, camera and telescope lenses, because of the high refractive index and low dispersion of rare-earth glasses.

Small amounts of lanthanum added to steel improves its malleability, resistance to impact and ductility. Small amounts of lanthanum added to iron helps to produce nodular cast iron. Small amounts of lanthanum added to molybdenum decreases the hardness of this metal and its sensitivity to temperature variations. Small amounts of lanthanum are present in many pool products to remove the phosphates that feed algae. Mischmetal, a pyrophoric alloy used in such things as lighter flints, contains 25% to 45% lanthanum.

Lanthanum oxide and the boride are used in electronic vacuum tubes as hot cathode materials with strong emissivity of electrons. Crystals of LaB_6 are used in high brightness, extended life, thermionic electron emission sources for scanning electron microscopes. in Gas tungsten arc welding electrodes, as a substitute for radioactive thorium.

Hydrogen sponge alloys can contain lanthanum. These alloys are capable of storing up to 400 times their own volume of hydrogen gas in a reversible adsorption

process. Petroleum cracking catalysts. Gas lantern mantles. Glass and lapidary polishing compound. La-Ba age dating of rocks and ores.

Lanthanum carbonate is used medically as a phosphate binder for the treatment of hyperphosphatemia. See details below under Biological Role. Lanthanum nitrate is mainly applied in specialty glass, water treatment and catalyst. Cerium activated Lanthanum bromide is the recent inorganic scintillator which has a combination of high light yield and the best energy resolution. Lanthanum fluoride is used with Europium flouride in the crystal membrane of Fluoride Ion-Selective Electrodes Lanthanum oxide is used as a grain growth additive during the liquid phase sintering of silicon nitride. Like horseradish peroxidase, lanthanum is used as an electron-dense tracer in molecular biology. Lanthanum is an intermetallic component of nickel-metal hydride batteries (Winter, 2005).

1.1.2.2 Biological Role of Lanthanum

Lanthanum has no known biological role. The element is not absorbed orally, and when injected its elimination is very slow. Lanthanum carbonate was approved as a medication (Fosrenol, Shire Pharmaceuticals) to absorb excess phosphate in cases of end-stage renal failure. Some rare-earth chlorides, such as lanthanum chloride ($LaCl_3$), are known to have anticoagulant properties.

While Lanthanum has pharmacological effects on several receptors and ion channels, its specificity for the GABA receptor is unique among divalent cations. Lanthanum acts at the same modulatory site on the GABAR as zinc- a known negative allosteric modulator. The Lanthanum cation La(III) is a positive allosteric modulator at native and recombinant GABA receptors, increasing open channel time and decreasing desensitization in a subunit configuration dependent manner (Winter, 2005).

1.2 Separation Methods of Metals

These can be divided into four types: precipitation, ion-exchance, liquid-liquid extraction(LLP) and solid phase extraction(SPE).

1.2.1 Precipitation

Precipitation of metals has long been the primary method of treating metal-laden industrial wastewaters. As a result of the success of metals precipitation in such applications, the technology is being considered and selected for use in remediating ground water containing heavy metals, including their radioactive isotopes. In ground water treatment applications, the metal precipitation process is often used as a pretreatment for other treatment technologies (such as chemical oxidation or air stripping) where the presence of metals would interfere with the other treatment processes (NESSA, 1993).

Metals precipitation from contaminated water involves the conversion of soluble heavy metal salts to insoluble salts that will precipitate. The precipitate can then be removed from the treated water by physical methods such as clarification (settling) and/or filtration. The process usually uses pH adjustment, addition of a chemical precipitant, and flocculation. Typically, metals precipitate from the solution as hydroxides, sulfides, or carbonates. The solubilities of the specific metal contaminants and the required cleanup standards will dictate the process used. In some cases, process design will allow for the generation of sludges that can be sent to recyclers for metal recovery (NESSA, 1993).

1.2.2 Liquid Phase Extraction (LPE)

Solvent extraction is based on differences in the solubility of elements and their compounds in two immiscible liquid phases. Usually, the initial phase is an aqueous solution and the second phase is an organic solvent immiscible with water (Marczenko, 1986).

Rare-earth elements can be separated by extraction methods based on the thiocyanate, nitrate with tri-n-butyl phosphate and EDTA in the presence of capriquat (trioctylmethylammoniun chloride) complexes (Fischer, Bramekamp, Klinge & Pohlmann, 1964).

1.2.3 Ion-exchange

Ionic attraction is common for inorganic molecules and salts of organic molecules and involves the attraction of a negative atom for a positive atom. The ionic bond formed has a strong attractive force of 5 kcal $mole^{-1}$ or more and is least affected by temperature and distance (Lemke, 1992). This ionic bond is more difficult to break compared with van der Waals forces. The manipulation of solvent pH and ionic strength are necessary to promote conditions favoring retention and elution of analyte to the sorbent. Another important ionic chemical bond formed is the ion-dipole bond, seen when an organic salt is dissolved in water. This bond is formed by the association of either a cation or an anion with a dipole such as is found in water. A cation bonds to a region of high electron density (the oxygen atom in water) and an anion bonds to an electron deficient region (the hydrogen atom in water). The attraction between ion and dipole is strong and is insensitive to temperature and distance (Lemke, 1992).

Examples of this type of attraction include the formation of an ionic salt when a basic compound *(e.g.,* amine) is added to an aqueous acidic medium (pH below 7.0) or when an acidic compound *(e.g.,* carboxylic acid, phenol, unsubstituted sulfonamide or imide) is added to an aqueous basic medium (pH above 7.0).

A variety of cation and anion exchange sorbents is available, having both strong and weak affinities. The strong ion exchange sorbents are always ionized to promote elution; the charge on the analyte is eliminated through pH manipulation. The functional groups bonded to weak ion exchange sorbents can be made ionized or unionized through the manipulation of pH and analytes can be eluted without

modification of their own existing charge. Ion exchange sorbents are found in the polymer series and the bonded silica based series (Wells, 2003).

1.2.4 Solid Phase Extraction

1.2.4.1 Fundamental Principles

Solid-phase extraction (SPE) is a specific type of sample preparation in which an analyte, contained in a liquid phase, comes in contact with a solid phase (sorbent particles contained within a packed bed or a disk) and is selectively adsorbed onto the surface of that solid phase by chemical attraction. All other materials not adsorbed remain in the liquid phase and pass through the sorbent particle bed to waste. A wash solution is passed through the sorbent bed to ideally remove adsorbed endogenous contaminants from the sample matrix, yet retain the analyte of interest on the solid phase. A selective elution step is then performed in which the analytes partition away from the solid support and into another solvent in which there is a greater affinity than for the sorbent bed. The overall procedure for solid-phase extraction is illustrated in Figure 1.1

The key points to successful use of solid-phase extraction for sample preparation are:

1. Proper choice of sorbent phase chemistry to attract the analyte of interest
2. Efficient utilization of pH to manipulate the analyte into the desired ionic or neutral form
3. Solubility of analyte in the solutions used for the extraction method

Adjustments in pH are used to vary retention and selectivity of the solid-phase extraction process. The pH of the solution is very important during the adsorption step to promote analyte retention. Likewise, pH adjustrrients are often also made during the wash step for selective removal of interferences. During the elution step, pH can be used to either ionize the analyte or make it neutral; likewise, in some cases the solid sorbent particle to which the analyte is bound can be made ionized or

neutral to facilitate analyte release. Note that an SPE procedure that is not using pH control during at least one of the steps is not fully exploiting the utility of this powerful technique(Wells, 2003).

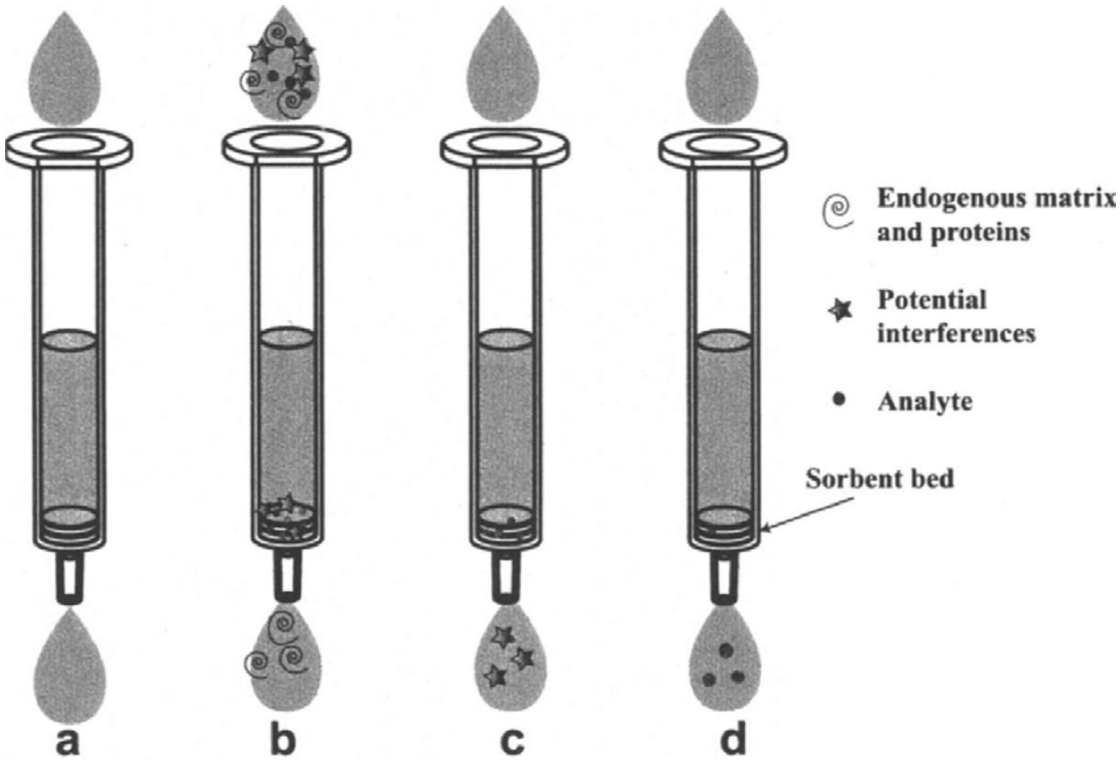

Figure 1.1 The basic steps for solid-phase extraction involve (a) conditioning the sorbent bed, (b) loading analytes, (c)washing away interferences and (d) selective elution for further workup and analysis. The SPE product format shown is for a disk in a cartridge or column although the procedure is generally similar regardless of format.

Three fundamental concepts are required in understanding and using SPE:

1.A strong chemical attraction must exist *(e.g.,* nonpolar, ion exchange or polar) between the analyte and the sorbent chosen so that the analyte preferentially adsorbs to the solid sorbent as it passes through the bed(Figure 1.2).

2.Adsorption requires that sufficient residence time is permitted for the interaction of analyte and sorbent to occur (Figure 1.3). The sorbent particles have a certain distance between them, and the analyte in solution flows through the sorbent bed at a

certain linear velocity. Traditional geometries of sorbents packed in columns have large diffusion distances, thus the flow rate of solution through the bed is performed at slow rates. The advent of particles packed more tightly into a disk format has reduced this need for slow flow rates during adsorption.

3.The elution solvent chosen *(e.g.,* an organic solvent such as methanol or acetonitrile, with or without pH modification) must be strong enough to disrupt the attraction between analyte and the solid phase, causing desorption, or elution from the particle (Figure1. 4) (Wells, 2003).

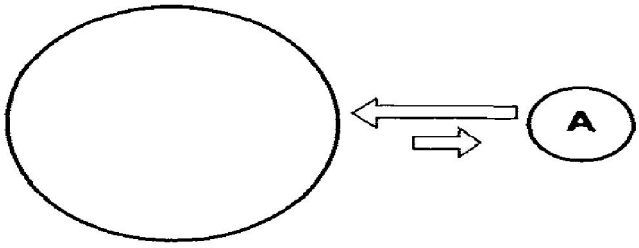

Figure 1.2 An analyte (A)is adsorbed onto the surface of a sorbent particle by a chemicalattraction.

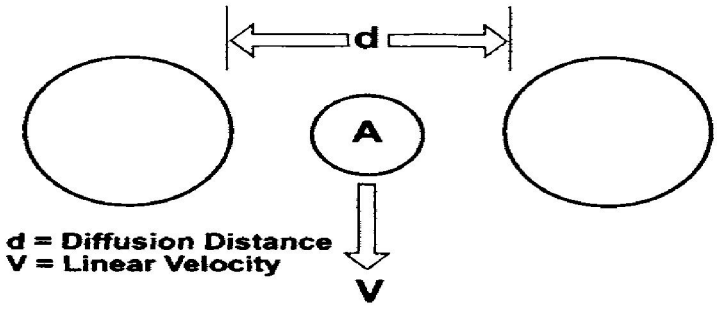

Figure 1.3 An analyte (A) passes through a sorbent particle bed at a certain linear velocity (V). The particles are separated within the bed at a certain distance (d).

The volume of elution solvent necessary is typically larger than is desirable as a result of the excess particle mass used in the SPE procedure. It is usually necessary to perform a solvent exchange before injection into a chromatographic system for analysis and detection.

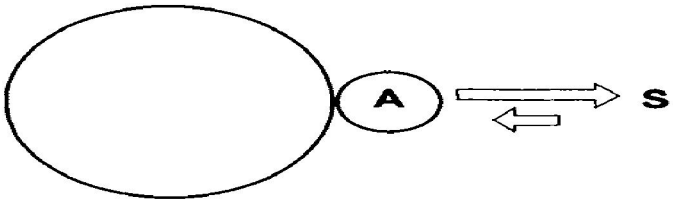

Figure 1.4 An analyte (A) is desorbed, or eluted, from a solid particle when it is attraction is disrupted by a particular solvent (S) that is passed through the particle bed. Note that a change in pH and/or ionic strength can also facilitate analyte release.

1.2.4.2 Advantages

Numerous advantages to the solid-phase extraction technique for sample preparation include: very selective extracts (reduce the potential for ionization suppression from matrix materials); wide variety of sample matrices accepted; analytes can be concentrated; high recoveries with good reproducibilities; improved throughput via parallel processing; low solvent volumes; suitable for full automation; and no emulsion formation as seen with liquid-liquid extraction (LLE). In addition, so many formats and chemistry choices are available that nearly all requirements for sample preparation can be met. The technology has been improved in recent years with the introduction of more selective but also more generic solid sorbent chemistries, improved disk based SPE devices, smaller bed mass sorbent loading, improved plate formats for using smaller volumes, and faster and more efficient automation hardware (Wells, 2003).

1.2.4.3 Disadvantages

Many of the disadvantages commonly mentioned for SPE are directed toward the perceived difficulty to master its usage. The great selectivity and the many choices for manipulating pH and solvent conditions make it difficult to grasp the chemistry of the technique. As a result of this perceived complexity, generic sorbents and

methods have been developed to make it easier to perform SPE. The procedure itself has many sequential steps, which result in increased time requirements. However, efficient utilization of a lower sorbent mass product is the key to improved throughput in this regard. Occasionally issues arise related to slow or difficult flow of sample through the particle bed; precipitated fibrins, thrombins or other particles in the biological sample are often at fault. In order to avoid this potential problem, samples are usually centrifuged or filtered prior to extraction and this step is especially important with the use of automated methods. Sometimes a larger particle size packing *(e.g.,* 100µm instead of 40µm) is used to improve flow characteristics. The batch to batch variation of bonded silica sorbents had infrequently been a cause of concern for users, although in recent years manufacturers have improved the quality control of their products (Wells, 2003).

Also related to the time issue is the historical perception that the SPE method development process takes too much time. The cost of performing a solid-phase extraction procedure can be greater than that of other techniques, such as protein precipitation (PPT) and liquid-liquid extraction. In retum for its greater selectivity, which is of paramount importance in the bioanalysis of clinical samples which strive to reach the lowest quantifiable concentrations (pg mL^{-1}), the cost per sample may be greater. However, any estimate of cost should not include only the consumable items needed to perform the extraction but also must factor in the time savings that the approach offers. Some users perceive the off-line approach to SPE as a disadvantage because a batch of samples must be prepared independently of the analysis and then taken to the instrument, rather than performed in a connected (on-line) step (Wells, 2003).

1.2.4.4 Solid Sorbent

The nature and properties of the sorbent are of prime importance for effective retention of metallic species. Careful choice of the sorbent is thus crucial to the development of SPE methodology. In practice, the main requirements for a solid sorbent are:

1. The possibility to extract a large number of trace elements over a wide pH range (along with selectivity towards major ions);
2. The fast and quantitative sorption and elution;
3. High capacity;
4. Regenerability;
5. Accessibility.

Adsorption of trace elements on the solid sorbent is required for preconcentration. The mechanism of retention depends on the nature of the sorbent, and may include simple adsorption, chelation or ion-exchange. Also, for trace elements, ion-pair solid phase extraction may be used. Trace elements are usually adsorbed on solid phases through van der Waals forces or hydrophobic interactions. Hydrophobic interaction occur when the solid sorbent is highly non-polar (reversed phase). More recently, reversen polymeric phases have appeared especially the styrene-divinylbenzene copolymer that provides addition Π-Π interaction when Π-electrons are present in analyte (Camel, 2003).

While elution of trace elements from the sorbent the same kind of interactions usually occurs during the elution step. This time, the type of solvent must be correctly chosen to ensure stronger affinity of the trace element for the solvent, to ensure disruption of its interaction with the sorbent (Camel, 2003).

1.2.4.5 Sorbent Types

It is convenient to divide sorbents for SPE into three groups: inorganic oxides; low-specificity sorbents; and, compound-specific and class-specific sorbents (Poole, 2003).

The most important inorganic oxides adsorbents for SPE are silica gel, alumina, Florisil (synthetic magnesium silicate) and diatomaceous earth (Barker; 2000).

Adsorbent properties that increase retention are a larger surface area and a high activity. Hydrogen-bonding functional groups are strongly retained (e.g. sulfonic acid, carboxylic acid, phenol and hydroxyl). Coating inorganic oxides with a complexing agent (e.g. silver nitrate, and caffeine) or an acid or base (an acid can be used for the selective isolation of bases and vice versa) can be used to modify selectivity for the isolation of target compound (Thurman & Mills, 1998).

Low-specificity sorbents, which include chemically-bonded silicas, porous polymers and carbon, are commonly used for the isolation of contaminants from aqueous solution. Silica-based, siloxane-bonded sorbents can be prepared with a wide range of bonding density, pore size and functional group types. Silica gels of high surface area, 500-600 m^2 g^{-1}, are generally used to prepare sorbents for the isolation of small molecules. Siloxane-bonded sorbents with high surface areas, long alkyl chains and high phase loading maximize retention of small molecules from aqueous solution, while wide-pore materials with a low phase loading short alkyl chains are used to isolate macromolecules. Porous polymer sorbents are generally copolymers of styrene and divinylbenzene processed to enhance their properties for SPE. They are either similar in chemistry to porous polymers developed for HPLC and have moderate surface area (< 600 m^2 g^{-1}) or are biporous and highly crosslinked with surface areas of 700-1200 m^2 g^{-1}. The modern forms of carbon used for SPE are graphitized carbon blacks and porous graphitic carbon. Graphitized carbon blacks are (largely) non-porous with moderate surface area of 100-210 m^2 g^{-1} (Poole, 2003).

Various selective sorbents based on ion exchange, bioaffinity, molecular recognition, and restricted access material are used. Ion-exchange sorbents are usually classified as weak or strong, depending on the identity of the ionic group and whether its charge is independent of the sample pH (strong ion exchanger) or can be manipulated by changing pH (weak ion exchanger) (Poole, 2003).

Restricted access sorbents were initially developed for the isolation of low-molecular-mass drugs from biological fluids with minimum sample pretreatment and

now also find use in the isolation of herbicides from surface waters containing high levels of humic substance (Simpson, 2000).

Immunosorbents (or immunoaffinity sorbents) have long been used for sample pretreatment in medicine, biology and food science, but more general applications, such as to environmental samples. Immunosorbents are prepared by covalently bonding a suitable antibody to an appropriate sorbent (Delaunay, Pichon & Hennion, 2000).

Polymeric adsorbents, which are sub-groups of low-specificity sorbents, are highly porous structures whose internal surfaces can adsorb and then desorb a wide variety of different species depending on the environment in which they are used. For example, in polar solvents such as water, polymeric adsorbents exhibit non-polar or hydrophobic behaviour and so can adsorb organic species that are sparingly soluble. This hydrophobicity is most pronounced with the styrenic adsorbents. In non-polar solvents, such as hydrocarbons, etc. most adsorbents exhibit slightly polar or hydrophilic properties and so will adsorb species with some degree of polarity. This polarity is most pronounced with the acrylic adsorbents and the phenolic adsorbents.The efficiency of the polymeric sorbents depends on various physico-chemical parameters such as particle size, surface area, pore diameter, pore volume, degree of crosslinking and particle size distribution. The highly porous materials have higher real activity surface available which allows higher π- π interactions and so higher adsorption capacity. The band broadening with highly porous sorbents is only slightly greater than with other sorbents because porosity minimizes band dispersion. Band broadening is also minimized when working with sorbents with a narrower particle size distribution (Puig & Barceló, 1996).

1.2.4.5.1 Silica Sorbents. Raw, underivatized silica is an amorphous, porous solid that contains polysiloxane (Si-O-Si) and silanol groups (Si-OH). The presence of thesesilanol groups allows polar adsorption sites and makes the surface weakly acidic. Silica is covalently bonded to a functional group through a chemical process. These groups are commonly alkyl chains of varying length, such as octadecyl (C18),

octyl (C8), butyl (C4), ethyl (C2) and methyl (C1). The base silica used to produce a bonded sorbent has an impact on the performance of the finished product. An illustration of an alkyl chain bonded to silica is shown in (Figure 1.5)

The chemical bonding reaction with silica can result in a monomeric species or a polymeric species. The polymeric bonding process is most often used to achieve a greater degree of alkyl loading with increased stability toward hydrolysis. However, this process is less reproducible batch to batch and can result in a high degree of residual terminal silanol groups. One cause of this variability is that reagents in the synthesis tend to react with trace amounts of water and then polymerize in solution before bonding to the surface hydroxyls in ways that may or may not conform to the original surface(Golding, Barry & Burke 1987). The monomeric process provides better control of the reaction (better reproducibility) and higher silica activity.

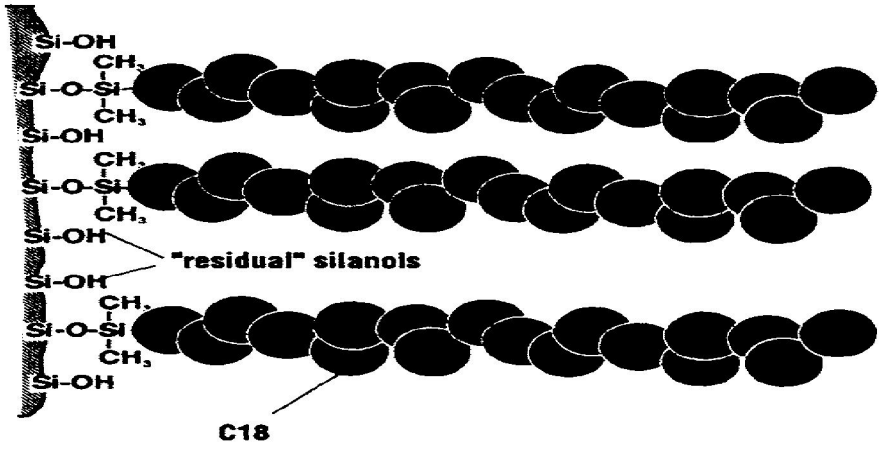

Figure 1.5 Illustration of the surface of silica bonded with an octadecyl (C18) alkyl chain. Note the presence of residual silanol groups on the silica surface Illustration reprinted with permission from LC Resources.

1.2.4.5.2 Polymer Sorbents. An alternative to the traditional bonded silica sorbent is a polymer sorbent. The first polymer sorbent for SPE was a cross-linked poly(styrene divinylbenzene) as shown in Figure 1.6. The advantages of a polymer sorbent include the following:

1. 100% Organic composition (chemically synthesized)
2. Stable across the entire pH range from 0-14
3. Predictable attraction (no silanols)
4. More consistent manufacturing process batch to batch
5. Greater capacity per gram than bonded silica

The sorbent poly (styrene divinylbenzene) is commonly abbreviated as SDB, SDVB or PSDB and is available from sorbent manufacturers under many different brand names. This polymer imparts a different selectivity than C18 bonded silica by nature of the aromatic rings and vinyl groups in its chemical structure. Generally, it is able to adsorb a wider range of analytes; some more polar analytes are able to be captured compared with traditional reversed phase bonded silica sorbents. A common misconception is that C18 groups are bonded onto polymers; while C18 bonded to a polymer is reported (Patel, Benson, Hometchko & Marshall, 1990), most polymer sorbents impart their selectivity through a different reversed phase attraction using an affinity for their aromatic tings and attached functional groups (Wells, 2003).

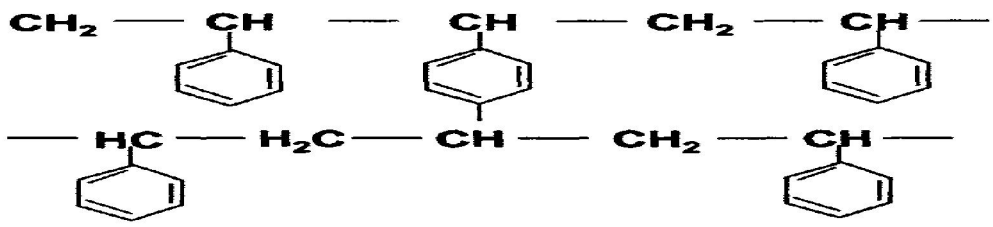

Figure 1.6 An original polymer sorbent useful for solid-phase extraction is poly-(styrene divinylbenzene).

While this sorbent can perform selective extractions when a method has been optimized for particular analytes, its great appeal is its ability to extract analytes with success using a generic methodology (condition with methanol then water, load sample, wash using 5% methanol in water, and elute with methanol). This common approach is especially appealing for drug discovery bioanalysis where time constraints are great. Summaries of the characterization of a hydrophilic lipophilic balanced SPE sorbent (Bouvier, Iraneta, Neue, McDonald, Phillips, Capparella &

Cheng, 1998). and useful discussion of this same polymer sorbent is contained within a review of sample preparation methods (Gilar, Bouvier & Compton 2001).

1.2.4.5.3 Biosorbents. The need for an effective and economical process to remove heavy metal ions and/or radionuclides from large volumes of diluted industrial wastewater streams has stimulated increasing interest in the metal binding capacities of various microorganisms (Blackwell, Singleton & Tobin, 1995; Kapoor & Viraraghavan, 1995; White, Wilkinson & Gadd, 1995).

Biosorption is known as a potential purification process for sequestering metallic cations from diluted aqueous solutions (Zouboulis, Matis & Hancock, 1997; Kratochvil & Volesky, 1998). Metal sorption on the cell surfaces can occur in non-living microorganisms (Delgado, 1998; Mameri, 1999) and inexpensive biological materials available in large quantities.Biomass can be supplied as a by-product of industrial fermentations (Bustard & McHale, 1998), from natural sources (activated sludges) or using inexpensive growth media.

Recently, the applicability of biosorption in continuous metal recovery processes has received an increasing attention from researchers (Hu & Reeves, 1997; Mutlu, 1997).

Biosorbents are also an important place on the solid phase extraction studies (Kuyucak & Volesky, 1990; Baytak & Talanta 2005; Godlewska & Zyłkiewicz 2004). Heavy metal ions at trace level could be quantitatively adsorbed on the organisms including mosses, bacteria, algae (Kuyucak & Volesky, 1990; Baytak & Turker, 2005; Godlewska & Zyłkiewicz, 2004). Biosorption is responsible for metal concentration by non-living biomass owing to the absence of metabolic activity necessary for intracellular metal accumulation (Godlewska & Zyłkiewicz, 2004; Bag, Turker, Lale & Tunceli, 2000). The biosorption system for enrichment of heavy metals is based on biosorption of heavy metals on the organisms and desorption of adsorbed metals from the organisms. An important part of the studies on biosorption of heavy metals is based on the immobilization of the organisms on various

adsorbents (Krishna, Chandrasekaran, Rao, Karunasagar & Arunachalam, 2005; Godlewska, Zyłkiewicz, & Kozłowska 2005; Bag, Lale, Turker & Fresen, 1999; Meneg´ario, Smichowski & Polla, 2005) Microorganisms loaded natural and synthetic adsorbents have been used for separation and preconcentration of heavy metals at trace levels. A preconcentration procedure for some metal ions prior to their atomic absorption spectrometric determinations has been proposed by Baytak and Turker (Baytak & Turker 2005).

Microalgal biomass has been successfully used as sorbing agent. Because microalgae use light as an energy source, facilitating the maintenance of metabolism in the absence of organic carbon sources, and electron acceptor required by bacteria or fungi. Thus the use of metabolically active microalgal systems may be more readily achieved. Also, microalgae cultures can be cultivated in open ponds or in large-scale laboratory culture, providing a reliable and consistent supply of biomass for such studies and eventual scale-up work (Aksu, 1998; Wilde & Benemann 1993).

In recent years, biosorption has emerged as a cost-effective and efficient alternative for the removal of heavy metals from low strength wastewaters. Biosorption is the uptake of heavy metal ions and radionuclides from aqueous solutions by biological materials. Microorganisms, including algae, bacteria, yeast, fungi, plant leaves and root tissues can be used as biosorbents for detoxification and recovery of toxic or valuable metals from industrial discharges. One of the most promising biosorbents is ‘‘algae’’ (Veglio & Beolchini, 1997). In fact, the uses of algae in the sorption of heavy metals were dated back to 1986 when reported that there was an sorption of cadmium on the cell of the green microalga *Stichococcus bacillaris* (Skowron´ski, 1986). Following this work, there were a number of reports on the sorption of heavy metals using microorganisms such as single cell algae and macroalgae species (Volesky, 1990; Wase & Foster, 1997).

Generally the sorption of heavy metals on the biosorbents could be described as a two-step process where the metal was initially uptaken onto the surface of the cell followed by the bioaccumulation inside the cell due to the metal uptake metabolisms.

Different species often had different sorption characteristics, and external factors such as pH, metal ion concentration, temperature, other metal ions, etc., were always found to influence the sorption (Kojima & Lee, 2001). *Caulerpa lentillifera* is a marine green macroalga cultivated as food for animals and humans, and also commonly used to treat wastewater from shrimp farms. Due to its rapid growth rate, farmers often have trouble with the over populated biomass. Turning excess C. lentillifera into biosorbent could be a viable answer to this problem. This work focused on the investigation of the application of *C. lentillifera* to the sorption of copper, cadmium, lead and zinc ions in concocted metal solutions.(Pavasant, Apiratikul, Sungkhum, Suthiparinyanont, Wattanachira & Marhaba , 2005)

The biosorption process represents an effective passive sequestration of organic or inorganic substances by certain types of non-living biomass. For practical application considerations, it is important to select biomass types that are either naturally abundant, such as seaweeds, or industrial biomass wastes especially from fermentation or seaweed processing plants (Volesky, 2003a).

Biosorption has mainly been considered for heavy metal removal in order to detoxify metal-bearing effluents. The process features a high heavymetal selectivity and cost-effectiveness for low metal concentrations as compared with ion exchange resins. There is also no hazardous sludge by-product generation in both processes and biosorbents can also be easily regenerated for multiple reuse (Volesky, 2001).

Considerable attention has been paid especially to batch equilibrium studies and to modeling of the isotherms. While ion exchange is considered to be the main mechanism involved in the biosorption process (Davis, Mucci & Volesky, 2003; Volesky, 2003a), most of the isotherms derived under constant pH are usually conveniently modeled using Langmuir and Freundlich equations. However, none of these two models reflects the ion exchange mechanism involved in which ions are released from the biosorbent while others become bound. During this process, the total normality of the solution, i.e., the total concentration of cations in terms of the number of equivalents, remains constant, the electroneutrality of the solution thus

being maintained. Correspondingly, with no pH adjustment it would vary naturally according to the exchange of ions during the process. This approach is also used in order to follow the behavior of a fixed-bed flow-through sorption column where the pH cannot be conveniently controlled inside the column and will vary with the ion exchange and speciation of the ions present in solution, especially if protons are the exchanged species. The same total normality should be used to feed the column. Modeling the batch equilibrium isotherm could then be used as a basis for simulation and for predicting the fixed-bed sorption column behavior. Equilibrium equations should be simple enough in order to be used in the column combined model in a direct way with no need for often troublesome iterations. Other equilibrium isotherm models for simulating the biosorption process have been used for simulating single- and multi-component systems such as multi-component Langmuir, combination Langmuir–Freundlich, BET (Texier, Andres & Le Cloirec , 1997), the ideal adsorbed solution theory (Radke & Prausnitz, 1972) and the surface complexationmodel among others (Jeon & Holl, 2004).

However, many of these largely empirical models have serious limitations and more often do not represent the ion exchange mechanism occurring during the process. Other models reflecting sorbate speciation, pH and electrostatic attraction have also been suggested (Schiewer & Volesky, 1996, 1997) and their advantages and disadvantages are summarized in the literature (Volesky, 2003a). Moreover, ion exchange systems can also be modeled by using the separation factor concept between two elements, which represents the ratio of the distribution coefficients between them. While a constantseparation- factor approach is frequently applied to systems involving ions with the same valence, this concept may also be applied to heterovalent systems with good approximation (Tondeur & Klein, 1967).

Ion exchange reactions occur between an electrolyte in solution and an insoluble electrolyte with which the solution is contacted. Early applications of ion exchangers were limited to water-softening problems. In 1935, synthetic materials were introduced such as insoluble polymeric resins containing sulfonic, carboxylic or

phenolic groups, while different cations would have a different affinity to the resin (Treybal, 1987).

The rate of ion exchange depends on the following processes:

(a) diffusion of ions from the bulk of the liquid to the external surface of the exchanger particle or sorbent in this case;
(b) intra-particle diffusion of ions through the solid to the binding sites;
(c) exchange of the ions;
(d) diffusion of the released ions to the surface of the solid; and
(e) diffusion of the released ions fromthe surface to the liquid bulk (Treybal, 1987).

In the biosorption process using *Sargassum* sp. as a sorbent, intra-particle mass transfer resistance appears to be dominant (Kratochvil, Volesky & Demopoulos, 1997; Volesky, 2003a).

The application of flow-through fixed-bed sorption columns constitutes the most preferable process device for biosorption operations due to the most effective use of the concentration gradient that can drive the process even at low levels of metal concentrations encountered. This leads to the maximization of the (bio)sorbent uptake capacity even though the pH cannot feasibly be controlled inside the column. During the ion exchange mechanism, the ions are naturally exchanged and the total normality of the solution is supposed to remain constant; however, the pH will likely vary when protons are released into the solution. The equilibrium batch system should be able to represent such a system whereby the total normality of the solution is constant. Therefore, an ion exchange equilibrium isotherm should be derived based on the same normality with varying final equivalent fractions of the elements present in both liquid and solid phases. This model can then be used for predicting the behavior of a breakthrough curve with the column being fed a solution under the

same total normality as used in the batch equilibrium experiments (Diniz, Weber, Volesky & Naja , 2007).

Recently, biosorption in fixed-bed columns and its modeling has been receiving more attention. As it is virtually useless to carry out column experimentation without an appropriate understanding and interpretation of the results, empirical and mechanistic models have been used to describe the breakthrough curves obtained especially for single-metal systems, but also for a few multi-metal systems (Hatzikioseyian, Tsezos & Mavituna, 2001). Most of the multi-component ion exchange studies used to consider local equilibrium theory whereby local equilibriumis assumed at all points at any time (Klein, Tondeur & Vermeulen, 1967; Helfferich, 1967). Because this approach neglected mass transfer it was just barely useful for assessing the feasibility of ion exchange processes. Better models assumed either intra-particle mass transfer resistance and/or liquid film resistance (Weber & Crittenden, 1975;Weber & Liu, 1980) or even combined liquid and solid mass transfer resistances as the rate-controlling step (Tan & Spinner, 1994).

Many of the modeling studies have concluded that axial dispersion effects could be neglected (Weber & Liu, 1980; Da Silva, Cossich, Tavares, Filho, & Guirardello, 2002). For dilute solutions, however, liquid phase rate controlling is usually assumed (Tan & Spinner, 1994). Even neural networks have also been used to model the breakthrough curves (Texier, Andrès, Faur-Brasquet & Le Cloirec, 2002). More or less successful attempts have been made to model (and predict) the breakthrough curves resulting from biosorption column processes (Volesky, 2003; Klein, Tondeur & Vermeulen, 1967). Other types of reactors such as membrane reactors have also been studied (Beolchini, Pagnanelli, Toro & Veglio, 2005). Although many simulation models have been proposed, there is still a lack of suitable and reliable process simulation tools that would be efficient, realistic and user-friendly, especially for multicomponent systems (Volesky, 2003b).

1.3 Determination of Lanthanum

Determination of lanthanides is important from the point of view of their technological applications especially in metalurgy, ceramic, nuclear industry, geological materials and soils and sediments. Their determination in geological materials is helpful in mineral exploration program. Knowledge of geochemistry of these elements is also necessary for understanding different geological processes such as chemical fractionation, terrestial evolution, etc. For all these purpose, the development of analytical methodologies for the seperation and determination of these elements becomes essential (Raut, Jaison & Aggarwal, 2004).

The lanthanides in the (III) oxidation state have only very slight chromophoric properties, but, more sensitive spectrophotometric methods utilize colored many organic reagents. Arsenazo III method is the most important commonly used, but ArsenazoI and Xylenol Orange methods are also important (Eremin & Bondarenko, 1972)

The absorbance of free Arsenazo III (λ_{max}=520-530 nm) at the absorption maxima of the metal complexes (λ=655-665 nm) is very slight. The large difference ($\Delta\lambda$) between the wavelengths of the absorption maxima of the complexes and free reagent is important (Marczenko, 1986).

Arsenazo III forms 1:1 complexes with bivalent or tervalent cation (e.g.UO_2^{2+} , Pb^{2+} , La^{3+} , and Ce^{3+}) and 1:1 or 1:2 complexes with quadrivalent cation (e.g. Th^{4+} and U^{4+}), depending on the pH and the excess of the reagent (Marczenko, 1986).

One half of the symmetrical Arsenazo III molecule complexes with a metal ion. The metal ion bonds to the nitrogen atom of the azo group, the oxygen atom of the hydroxyl group. The distortion of the symmetric of the reagent molecule gives rise to two neighbouring absorption maxima in the visible spectra of the Arsenazo III metal complexes (Marczenko, 1986).

In weakly acidic media the lanthanides react with Arsenazo III to colored complexes which are the basis of this sensitive method. In weakly acidic solution, the reagent is violet, whereas its complexes with the rare-earth elements are green. The method has the advantage that the reagent does not absorb at the wavelenght of the absorption maximum of the complex (650 nm) (Marczenko, 1986).

Chloride, sulphate, and phosphate do not interfere in the detemination of rare-earth elements with Arsenazo III. Neither do small amounts(less than 1 mg per 50 ml of solution) of titanyum, aluminum, calcium, and iron [reduced to Fe(II) with ascorbic acid], but larger quantities of these metals and certain other metals (Th, Zr, U, Bi, Cu) should be removed. Many interfering metals can be masked with EDTA. (Vdovenko & Lisichenok, 1967).

1.4 Related Studies

Nostoc-based biosorbents (AlgaSORBs) useful as chromatographic column-packing materials were prepared by immobilizing cyanobacteria onto solid support in three different fashions:

(i) cyanobacterial biofilm (Nostoc- dimethylformamide slurry) over polymer-modified silica gel,
(ii) cyanobacterial biofilm over bare silica gel, and
(iii) cyanobacteria as such onto polymer-modified silica gel.

The materials were characterized for their stabilities and metal sorption/elution conditions under static and dynamic equilibrations. Preconcentrated metals from a test sample were detected following ‘standard addition’ method using a diferential pulse anodic stripping voltammetric technique. All sorbents showed 100% affinity for Cd^{2+} ion in a multielemental sample at pH 6.9 and a flow rate of 0.5 ml min^{-1} with a preconcentration factor varying between 28 and 75 fold. The first type of AlgaSORB was also found to be selective for Cu^{2+} ion in multielemental analysis at pH 5.2 and a flow rate of 1.0 ml min^{-1} with a preconcentration factor of 75. The low

capacity and favourable kinetics of these sorbents for $Cu^{2+,}$ Cd^{2+}, Zn^{2+} and Pb^{2+} ions reflect the suitability of AlgaSORB columns for satisfactory performance in single column ion-chromatography. The polymer spacer between cyanobacterial biofilm and silica gel plays a vital role in holding the immobilized biofilm resulting in better endurance and recyclability for the first type of biosorbent (Prasad & Pandey, 2000).

The sorption of $Cu^{2+,}$ Cd^{2+}, Pb^{2+}, and Zn^{2+} by a dried green macroalga *Caulerpa lentillifera* was investigated. The removal efficiency increased with pH. The analysis with FT-IR indicated that possible functional groups involved in metal sorption by this alga were O–H bending, N–H bending, N–H stretching, C–N stretching, C–O, S=O stretching, and S–O stretching. The sorption of all metal ions rapidly reached equilibrium within 20 min. The sorption kinetics of these metals were governed by external mass transfer and intraparticle diffusion processes. The sorption isotherm followed the Langmuir isotherm where the maximum sorption capacities was Pb^{2+} > Cu^{2+} > Cd^{2+} > Zn^{2+} (Pavasant, Apiratikul, Sungkhum, Suthiparinyanont, Wattanachira & Marhaba, 2005).

Ulva rigida is a widespread green seaweed found on the Mediterranean coast that is proposed as a biosorbent for Au, Hg and Ag. The uptake of Au, Ag and Hg is 0.46, 0.81 and 0.75 mmol g^{-1}, respectively. The influence of pH, time of contact and concentration were studied. The authors propose an electroanalytical method to study the properties of the sorbent by including the sorbent in the composition of a carbon paste electrode. The result is a satisfactory reduction of the amount of reagents and sorbent required for all the experiments and a substantial reduction of the required time to perform the experiments(Cepria, Irigoyen & Castillo, 2006).

The brown alga *Pilayella littoralis* was used as a new biosorbent in an on-line metal preconcentration procedure in a flow-injection system. Al, Co, Cu and Fe were determined in lake water samples by inductively coupled plasma optical emission spectrometry (ICP-OES) after preconcentration in a silica-immobilized alga column. Like other algae, *P. littoralis* exhibited strong affinity for these metals proving to be an effective accumulation medium. Metals were bound at pH 5.5 and were displaced

at pH< 2 with diluted HCl. The enrichment factors for Cu(II), Fe(III), Al(III) and Co(II) were 13, 7, 16 and 11, respectively. Metal sorption efficiency ranged from 86 to 90%. The method accuracy was assessed by using drinking water certified reference material and graphite furnace atomic absorption spectrometry (GFAAS) as a comparison technique. The column procedure allowed a less time consuming, easy regeneration of the biomaterial and rigidity of the alga provided by its immobilization on silica gel (Carrilho, No´brega & Gilbert, 2003).

Biosorption of Cu^{2+} and Pb^{2+} by *Cladophora fascicularis* was investigated as a function of initial pH, initial heavy metal concentrations, temperature and other co-existing ions. Adsorption equilibriums were well described by Langmuir and Freundlich isotherm models.The maximum adsorption capacities were 1.61 mmol g^{-1} for Cu^{2+} and 0.96 mmol g^{-1} for Pb^{2+} at 298K and pH 5.0. The adsorption processes were endothermic and biosorption heats calculated by the Langmuir constant b were 39.0 and 29.6 kJ mol^{-1} for Cu^{2+} and Pb^{2+}, respectively. The biosorption kinetics followed the pseudo-second order model. No significant effect on the uptake of Cu^{2+} and Pb^{2+} by co-existing cations and anions was observed, except EDTA. Desorption experiments indicated that Na_2EDTA was an efficient desorbent for the recovery of Cu^{2+} and Pb^{2+} from biomass. The results showed that *Cladophora fascicularis* was an effective and economical biosorbent material for the removal and recovery of heavy metal ions from wastewater(Deng, Y. Su, H. Su, Wang & Zhu, 2006).

The biosorption of copper(II), lead(II), iron(III) and cobalt(II) on *Bacillus sphaericus*-loaded Diaion SP-850 resin for preconcentration–separation of them have been investigated. The sorbed analytes on biosorbent were eluted by using 1 mol L^{-1} HCl and analytes were determined by flame atomic absorption spectrometry. The influences of analytical parameters including amounts of pH, *B. sphaericus*, sample volume etc. on the quantitative recoveries of analytes were investigated. The effects of alkaline, earth alkaline ions and some metal ions on the retentions of the analytes on the biosorbent were also examined. Separation and preconcentration of Cu, Pb, Fe and Co ions from real samples was achieved quantitatively. The detection limits by 3 sigma for analyte ions were in the range of 0.20–0.75 μgL^{-1} for aqueous samples and

in the range of 2.5–9.4 ng g^{-1} for solid samples. The validation of the procedure was performed by the analysis of the certified standard reference materials (NRCC SLRS 4 Riverine Water, SRM 2711 Montana soil and GBW 07605 Tea). The presented method was applied to the determination of analyte ions in green tea, black tea, cultivated mushroom, boiled wheat, rice and soil samples with successfully results (Tuzen, Uluozlu, Usta & Soylak, 2006).

Amicroorganism *Agrobacterium* tumefacients as an immobilized cell on a solid supportwas presented as a newbiosorbent for the enrichment of Fe(III), Co(II), Mn(II) and Cr(III) prior to flame atomic absorption spectrometric analysis. Amberlite XAD-4 was used as a support material for column preconcentration. Various parameters such as pH, amount of adsorbent, eluent type and volume, flow rate of sample solution, volume of sample solution and matrix interference effect on the retention of the metal ions have been studied. The optimum pH for the sorption of above mentioned metal ions were about 6, 8, 8 and 6, respectively. The loading capacity of adsorbent for Co(II) and Mn(II) were found to be 29 and 22μmol g^{-1}, respectively. The recoveries of Fe(III), Co(II), Mn(II) and Cr(III), under the optimum conditions were found to be 99 ± 3, 99 ± 2, 98 ± 3 and 98 ± 3%, respectively, at the 95% confidence level. The limit of detection was 3.6, 3.0, 2.8 and 3.6 ng ml^{-1} for Fe(III), Co(II), Mn(II) and Cr (III), respectively, by applying a preconcentration factor of 25. The proposed enrichment method was applied for metal ion determination from water samples, alloy samples, infant foods and certified samples such as whey powder (IAEA-155) and aluminum alloy (NBS SRM 85b). The analytes were determined with a relative error lower than 10% in all samples (Baytak & Türker, 2004)

A solid phase extraction (SPE) preconcentration system, coupled to a flame atomic absorption spectrometer (FAAS), was developed for the determination of copper(II), cadmium(II), lead(II), manganese(II), iron(III), nickel(II) and cobalt(II) ions at the μg L^{-1} levels on *Penicillium italicum* – loaded on Sepabeads SP 70. The analytes were adsorbed on biosorbent at the pH range of 8.5–9.5. The adsorbed metals were eluted with 1 mol L^{-1} HCl. The influences of the various analytical parameters including pH of the aqueous solutions, sample volume, flow rates were

investigated for the retentions of the analyte ions. The recovery values are ranged from 95–102 %. The influences of alkaline, earth alkaline and some transition metal ions were also discussed. Under the optimized conditions, the detection limits (3 s, n = 21) for analytes were in the range of 0.41 μg L^{-1} (cadmium) and 1.60 μg L^{-1} (iron). The standard reference materials (IAEA 336 Lichen, NIST SRM 1573a Tomato leaves) were analyzed to verify the proposed method. The method was successfully applied for the determinations of analytes in natural water, cultivated mushroom, lichen (Bryum capilare Hedw), moss (Homalothecium sericeum) and refined table salt samples (Mendil, Tuzen & Soylak, 2007).

Pseudomonas aeruginosa immobilized multiwalled carbon nanotubes has been used as biosorbent for the solid phase extraction of some heavy metal ions in environmental samples. Cobalt(II), cadmium(II), lead(II), manganese(II), chromium(III) and nickel(II) ions have been selected as analytes for the presented study, due to their important negative and positive roles in human life. In order to investigate quantitative biosorption conditions of the analytes, the influences of pH of the aqueous solution, eluent type, eluent volume, samples volume, etc. were examined. The effects of alkaline, earth alkaline and some transitions metals on the biosorption of analyte ions on *P. aeruginosa* immobilized multiwalled carbon nanotubes were also investigated. The presented biosorption procedure was applied to the determination of analytes in tomato leaves, bovine liver, boiled wheat, canned fish, black tea, lichen and natural water samples.(Tuzen, Saygi, Usta & Soylak, 2007)

A fixed-bed study was carried out by using cells of *Pseudomonas aeruginosa* immobilized in polyacrylamide gel as a biosorbent for the removal of lanthanide (La, Eu, Yb) ions from aqueous solutions.The effects of superficial liquid velocity based on empty column, particle size, influent concentration and bed depth on the lanthanum breakthrough curves were investigated.Immobilized biomass effectively removed lanthanum from a 6 mM solution with a maximum adsorption capacity of 342 μmolg^{-1} (±10%) corresponding closely to that observed in earlier batch studies with free bacterial cells. The Bohart and Adams sorption model was employed to

determine characteristic parameters useful for process design. Results indicated that the immobilized cells of *P. aeruginosa* enable removal of lanthanum, europium and ytterbium ions from aqueous effluents with significant and similar maximum adsorption capacities. Experiments with a mixed cation solution showed that the sequence of preferential biosorption was Eu(III)≥Yb(III) > La(III)Around 96 ±4% of the bound lanthanum was desorbed from the column and concentrated by eluting with a 0.1 M EDTA solution.The feasibility of regenerating and reusing the biomass through three adsorption/desorption cycles was suggested. Neural networks were used to model breakthrough curves performed in the dynamic process. The ability of this statistical tool to predict the breakthrough times was discussed.(Texier, Andres, Faur-Brasquet & Cloirec, 2001)

Batch and column biosorption of La(III) (lanthanum) and Eu(III) (europium) was studied using protonated *Sargassum polycystum* biomass. The ion exchange sorption mechanism was confirmed by the proportional release of protons and by the total normality of the solution, which remained constant during the process. Equilibrium isotherms were determined for the binary systems, La/H and Eu/H for a total normality of 3meqg^{-1}, which produced separation factors of 2.7 and 4.7, respectively, demonstrating a higher affinity of the biomass towards europium. Column runs with a single metal feed were used to estimate the intra-particle mass transfer coefficients for La and Eu (6.0×10^{-4} and 3.7×10^{-4} min^{-1}, respectively). Modeling batch and column binary systems with proton as the common ion was able to predict reasonably well the behavior of a ternary system containing protons. The software FEMLAB was used for solving the set of coupled partial differential equations. Moreover, a series of consecutive sorption/desorption runs demonstrated that the metal could be recovered and the biomass reused in multiple cycles by using 0.1 N HCl with no apparent loss in the biosorbent metal uptake capacity.(Diniz, Weber, Volesky & Naja, 2007)

1.5 Aim of This Study

Determination of lanthanides is important from the point of view of their technological applications and industrial applications, especially in metallurgy, glass, lighting, ceramic and nuclear industry. So, the development of analytical methodologies for the separation and determination of these elements becomes essential.

In the proposed work, a biosorption procedure was presented for sorption, enrichment of lanthanum(III) ion on *Cystoseira barbatula* prior to the spectrophotometric determination using Arsenazo III. Several optimum conditions; initial pH, initial metal concentration, temperature, reaction time, saturation capacity, type of desorption solution, flow rate and co-existing ions were investigated. The equilibrium and kinetics were obtained from batch experiments. Also, the proposed method was applied to real samples.

CHAPTER TWO

METHODS AND MATERIALS

2.1 Apparatus

Signals were detected with an UV-1601 model Shimadzu spectrophotometer with background corrector. IR spectra were obtained with a Perkin-Elmer Spectrum BX-II Model FTIR spectrophotometer using KBr discs in the range 4000–700 cm^{-1}, 30 co-added interferograms were scanned at 2 cm^{-1} resolution. An isothermal water-bath circulator (Memmert) and centrifuge (Nüve NF 1215) were used for batch sorption experiments. A peristaltic pump (Watson Marlow SCİ 323) was used for loading of lanthanum solutions. All pH measurement were performed on a Ino Lab WTW model digital pH meter. Metal sorption studies on column were performed using a Varian cartridge (polyethylene, 1.0 cm x 6.0 cm) equipped with 20 μm polypropylene frits. In experiments, the water used throughout the work was distilled water performed on GFL 2001/4 apparatus.

2.2 Reagents

All reagents used were of analytical grade. Standard stock solutions(1000 μg m L^{-1}) of lanthanum was prepared by dissolving the required amount of $La(NO_3)_3$. $6H_2O$, (Aldrich) in distilled water. Calibration standard solutions of La(III) from 1 to 3 μg $mL^{-1}$was prepared. 0.1% (w/v) Arsenazo III solution from Aldrich {3,6-bis[(2-arsonophenyl)-azo]-4,5-dihydroxy-2,7-nphthtalenedisulphonicacid}(Figure2.1) was prepared. KCl/HCl buffer solution having pH 2 was prepared by mixing 8.1 mL of 0.2 mol L^{-1} HCl and 41.9 mL of 0.2 mol L^{-1} KCl solutions and diluting to 100 mL with distilled water. A 0.01 M hydrochloric acid (HCl) and 0.01 M sodium hydroxide (NaOH) solutions were prepared for pH adjustment.

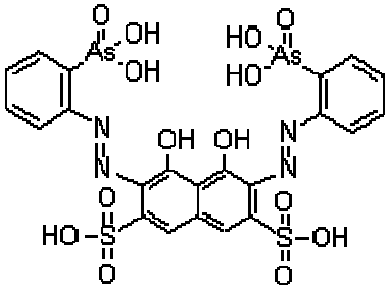

Figure2.1 Arsenazo III

2.3 Preparation of Biomass

The non-live algae, *Cystoseira barbatula Kützing,* was colllected on the beach of Turkish Aegean coast, Seferihisar-İzmir in winter season. The algae was washed twice with 0.12 M HCl to remove adsorbed metals and washed several times with distilled water until no chloride detected in the washing water. Subsequently it was dried in an oven at 70^0 C for 24 h. Then they were cut into small segments and then ground in agate mortar to obtain a fine powder.

2.4 Procedure for Batch Sorption Experiments

Batch sorption experiments were carried out by shaking known amounts of biomass with certain volume of La(III) containing solution in the range of 10-100 μg La mL^{-1} in a series of 50 mL reagent polyethylene flasks. The suspensions were kept under a constant speed of 200 rpm in a mechanical shaker for 30 min at sorption temperature. Then the suspensions were centrifuged at 5000 rpm for 15 min and supernatant solution was taken for analysis. All studies were carried out at pH 5.

2.5 Procedure for Flow-through Sorption Experiments

Column was purified with 0.1 M HCl solution after elimination of chlorides by washing with distilled water and known amounts of biomass ready for use were added. The column was prepared by aspirating water slurry of dried alg into polyethylene column. It was conditioned by passing 15-20 mL of buffer solution, then it was used for loading ion. After each use, the column was washed by passing 15-20

mL of buffer solution for regeneration of the biosorbent. The flow rates of the solutions were controlled by using peristaltic pump.

2.6 Procedure for the Determination of Lanthanum Using Arsenazo (III)

Calibration solutions and sample solutions containing 1 ml of standard/sample solution + 0.2 ml of Arsenazo III solution + 1 ml of pH 2 buffer solution were diluted to 5 ml with distilled water and measured at 655 nm for La(III).

Calibration curves of La(III) was linear and the correlation coefficients were 0.9989. At each measurement, fresh calibration curve was used. Reagent blank solution containing Arsenazo III and buffer solution was freshly prepared at each measurement.

2.7 Analysis of Synthetic Solutions and Samples

To check the applicability of the proposed method for sorption, preconcentration and determination of lanthanum, the biomass, *C. barbatula,* was subjected to various synthetic solutions and water and soil samples.

2.7.1 Preparation of Synthetic Solution

50 ml of 4 different synthetic aqueous solutions (Table 2.1) were prepared.

Table 2.1 Compositions of synthetic aqueous solutions

Composition of synthetic sample (µg mL^{-1})	**Added La(III) (µg mL^{-1})**
U(5), Co(5)	2
Ce(5), Y(5)	5
Cu(20), Zn(20)	7.5
U(10), Th(10)	10

2.7.2 Tap Water Samples

Tapwater samples were taken from Tire, İzmir. They were used without storing.

2.7.3 Standard Reference Material

Standard Reference Materials as NIST SRM 2709, San Joaquin Soil was prepared; 0.200 g of the sample was treated in a PTFE beaker with 15 mL of 48 % hydrofluoric acid, and the mixture was allowed to stay for about 1 h at room temperature. After heating the mixture in a sand bath (150^0C) until dryness and an aliquot of (15 mL) of the mixture of concentrated nitric acit (HNO_3) and perchloric acids 3:1 (v/v) was added and heated again until dryness. The residue was then treated with 2.5 mL of concentrated hydrochloric acid, water (2 mL) and four drops of 30% hydrogen peroxide under gentle warming to achieve complete solubility (Boaventura, Hirson & Santelli; 1995). The volume was made up to 50 mL with distilled water.

CHAPTER THREE

RESULTS

3.1 Structural Characterization by IR bands

The possible interaction between the functional groups of *C. barbatula* biosorbent and lanhtanum(III) ion has been characterized using FTIR spectroscopy method. The FTIR spectra of water-washed biosorbent, acid-washed biosorbent and La(III)-acid washed biosorbent were shown in Figure 3.1. All the spectra give bands of a cellulose type substrate as it is expected for algae. The stretching vibration of hydrogen bonded –OH and -NH_2 were seen as strong band between 3422–3433 cm^{-1}. The saturated -C–H groups were seen around 2918-2924 cm^{-1}. The stretching vibration of carbonyl group, –C=O, was observed around 1626–1641 cm^{-1}. The band seen at 1032–1038 cm^{-1} belongs to stretching vibration of the groups of alcohol and carboxylic acid. The band at 1420 cm^{-1} in the spectra of untreated algae sample is attributed to the combination of absorption of O-H, C-H and C=O bonds. Sulfite and sulfate groups show bands at 875 and 601 cm^{-1}. The acid-washed and metal loaded biomass exhibits small shift to red region.

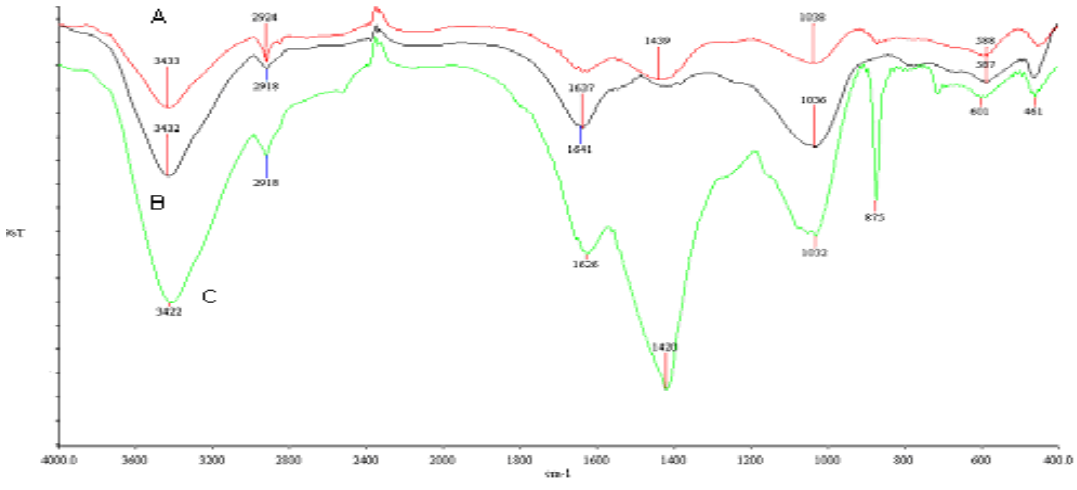

Figure 3.1 FTIR spectra of water-washed algae (C), HCl-washed algae (B), acid-washed algae loaded with lanthanum (A)

The stretching vibration bands at 3422, 1626, and 1032 cm^{-1} were shifted to 3433, 1641, and 1038 cm^{-1}, after biosorption of La(III), respectively. The results indicated that the biosorption could be carried out by ion-exchange between the La(III) ion and the hydrogen atoms of –OH, $–NH_2$ and –COOH groups of the biosorbent. The similar mechanism was reported for the biosorption of metal ions (Grimm, Zanzi, Bjornborn & Cukierman, 2008 ; Cepria, Irigouen & Castillo, 2006)

3.2 Effect of pH

The pH of solution has been one of the optimized parameters on sorption of metal ion. It is known that pH affects the protonation of the functional groups on the biomass. The effect of pH on sorption of La(III) was investigated in the pH range of 2-7 by using model solutions containing 10 mg La(III) L^{-1} and the results were given in Figure 3.2. Lanthanum(III) ion was quantitatively sorbed at the pH range 4-6. The maximum biosorption was found as 90% around pH 4-7. At the pH values lower than 4, the sorption decreased sharply due to positive charges on the surface of biosorbent. All further Works were carried out at pH 5.

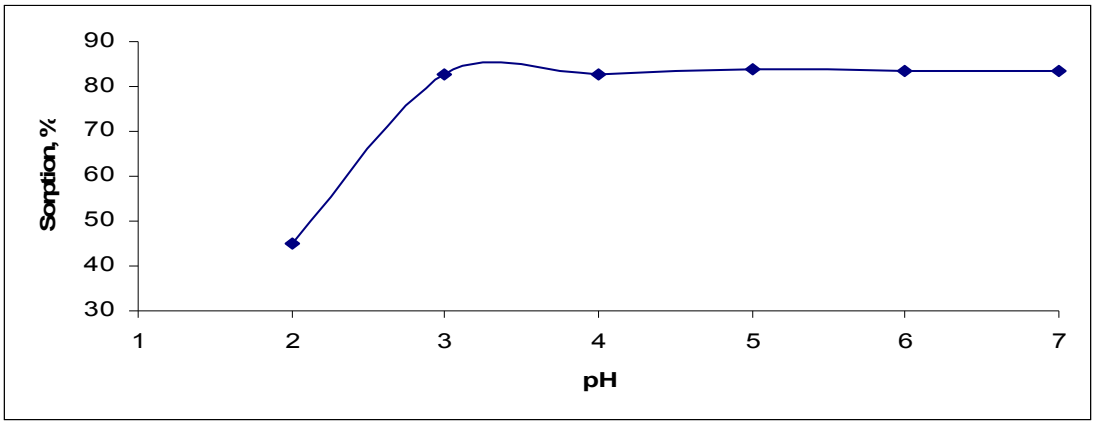

Figure 3. 2 Effect of pH on La(III) uptake (c_{int}= 10 mg L^{-1}, m= 500 mg, 25^0 C)

3.3 Effect of Amounts of *Biomass*

The retention of lanthanum(III) ion was examined in relation to the amount of adsorbent, which was varied from 100 to 1250 mg. The loading percentage of lanthanum was increased with the increased amount of *C. barbatula*. It was found that the adsorption of La(III) was gradually increased up to 500 mg of the adsorbent. Therefore, 500 mg of the adsorbent was used for La(III) in subsequent experiments.

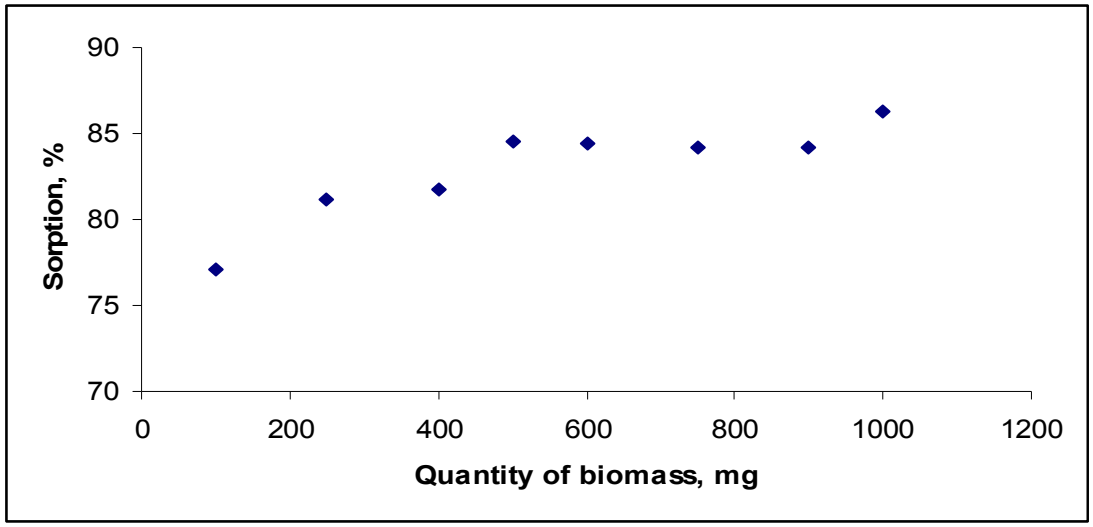

Figure 3. 3 Effect of quantity of biomass on La(III) uptake (c_0 = 10 mg L^{-1}, pH 5, 25^0 C)

3.4 Desorption Studies

The desorption of lanthanum(III) ion on *C.barbatula* was tested by model solution containing analyte ion at pH 5. The results ae given in Table 3.1. Quantitative recoveries as >95% were obtained for La(III) with 0.005 mol L^{-1} EDTA. The volume of eluent is important for high preconcentration factor. This was examined by varying of 0.005 mol L^{-1} EDTA volume from 5 to 20 mL. The smallest volume for quantitative elution was found to be as 10 mL.

Table 3.1 Effect of desorbent on the recovery of La(III) (n = 3, V= 20 mL)

Desorbent type	Desorbent conc. (M)	Recovery (%)
HCl	0.005	63
HCl	0.05	56
HCl	0.1	71
HCl	0.5	60
HCl	1	51
HCl	2	30
HNO_3	0.5	6
HNO_3	1	11
HNO_3	1.5	7
NaOH	0.05	7
EDTA	0.0025	67
EDTA	0.005	94
EDTA	0.01	85
EDTA	0.025	52
EDTA	0.05	38
EDTA	0.1	11

3.5 Effect of Contact Time

To determine the rate of loading of La(III) on the algae, batch experiments were carried out under the following conditions: 0.5 g of acid-washed algae was stirred with 25 mL of lanthanum solution at 25^0 C for tested each time intervals. The concentration of metal ion in the supernatant solution was determined and the amount of metal ion loaded on the algae was calculated by mass balance as mg g^{-1}. The amount of loaded metal ion became stable and made a plateau after 2 h. So, contact time for loading of La (III) was used as 2 h in subsequent experiments.

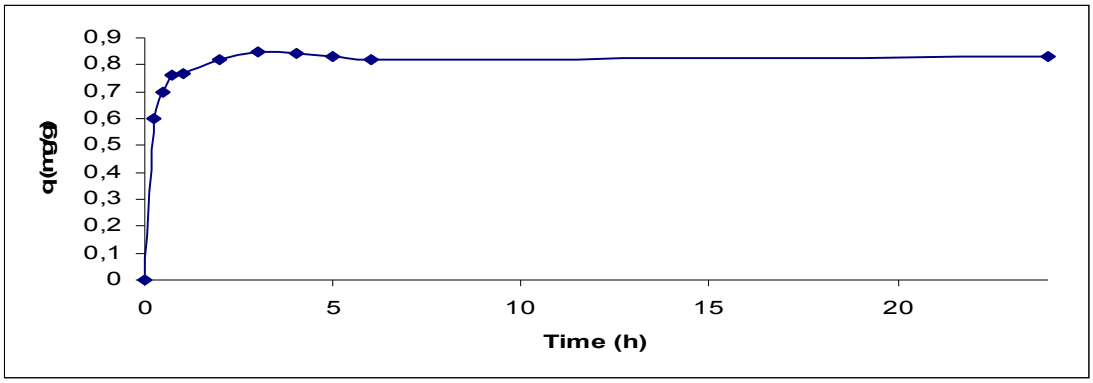

Figure 3.4 Kinetics of La 10 mg L^{-1} adsorption for algae at 25^0 C

3.6 Adsorption Equilibrium

Capacity of an adsorbent is described by its equilibrium sorption isotherm, which is characterized by certain constants whose values express the surface properties and affinity of the adsorbent. The most widely applied adsorption isotherm is Langmuir isotherm. By this model, adsoption takes place at specific homogeneous sites within the adsorbent. The isotherm is represented as:

$$q = \frac{q_m b C_e}{1 + b C_e}$$

where q_m is the maximum adsoption capacity (mmol/g) and b is an affinity constant related to the enegy of adsorption (L/mmol). The value of b indicates the strength or affinity of the sorbate for the solute (Holan, Volesky, Prasetyo, 1993). Freundlich isotherm is an emprical isotherm that can be used for non-ideal adsorption and expressed as follows:

$$q = K_F C_e^{1/n}$$

where K_F and n are Freundlich constants related to the adsorption capacity and adsorption intensity of the adsorbent, respectively. Freundlich model gives no information about the monolayer adsorption capacity as in Langmuir model.

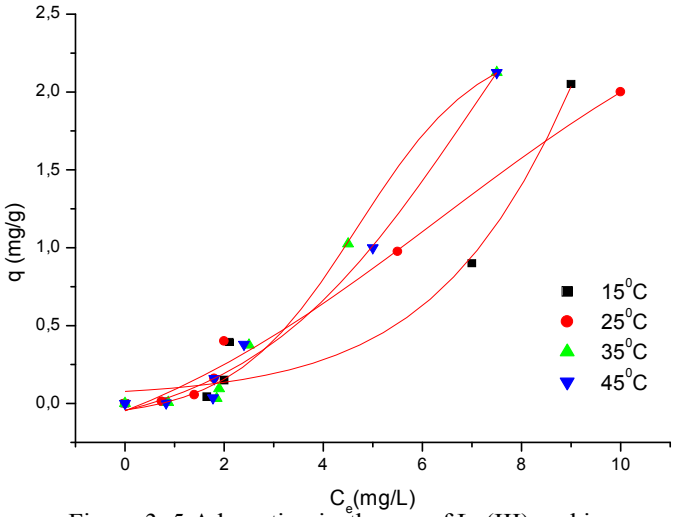

Figure 3. 5 Adsorption isotherms of La(III) on biomass

Adsorption isotherms were performed for initial La(III) concentrations ranging from 1 to 50 mg L^{-1}. Adsorption isotherms of La(III) by *C. barbatula* at different temperatures were given in Figure 3.5. The values of K_F and n showed high adsorption capacity and easy uptake. The isotherm parameters given in Table 3.2 showed that adsorption capacity was increased by increasing temperature. Since the values of n were higher than1.0, the strength of metal adsorption by biosorbent was quite intense (Özer, Aksu, Kutsal & Caglar, 1994). The maximum adsorption capacities obtained from the Langmuir isotherms increased with the increasing temperature, and the values of q_m were 24.5454-34.7970 $mg.g^{-1}$ for La(III) at 288 - 318 K and pH 5. The linearized Freundlich isotherms are indicating a physical adsorption for adsorbent. The maximum adsorption capacity is between 24.88-83.96 mg g^{-1} according to linearized Freundlich model. Freundlich model is more suitable than Langmuir model according to R^2 values.

Table 3. 2 Adsorption constants obtained from Langmuir and Freundlich adsorption isotherms at different temperature

T (K)	Langmuir			Freundlich		
	$L(L.g^{-1})$	q_m $(mg.g^{-1})$	R^2	n	K_F $(mg.g^{-1})$	R^2
288	-67.55	24.5454	0.3886	1.9641	34.60	0.8884
298	-53.59	26.1429	0.3038	1.8708	24.88	0.9049
308	-143.83	32.1993	0.4012	2.8027	83.96	0.9294
318	-267.91	34.7970	0.295	2.8113	81.60	0.8960

3.7 Effect of Flow Rate

The mass transfer form the solution of metal ion to the binding sites of biosorbent can be affected by the flow rate of sample solution. So, the dependence of metal ion sorption at various flow rates was studied at optimum pH The sample solution was passed through the column with the flow rates in the range of 15-60 rpm. No remarkable change was observed within studied flow rates as shown in Figure 3.6. In subsequent experiments, 30 rpm was generally used as the flow rate.

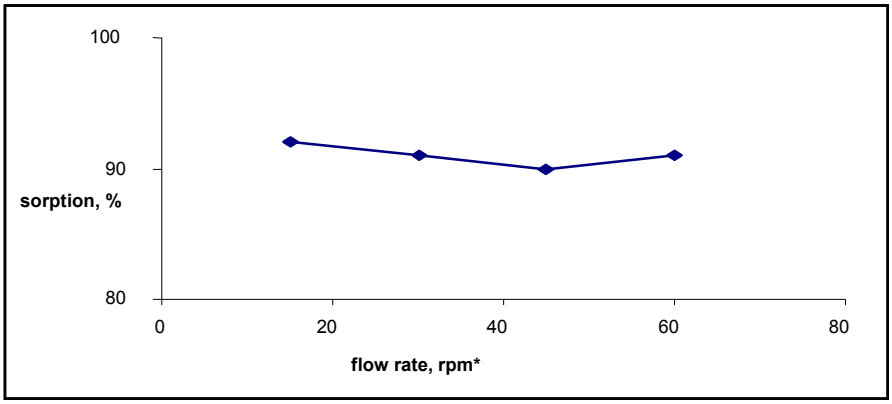

Figure 3.6 Effect of flow rate of sample solution on sorption of La(III), V:25 mL, 10 μg mL^{-1}
*15 rpm=1 ml min^{-1}

3.8 Effect of Volume of Sample Solution

In the analysis of trace metals in analytical samples, the applicable volume of sample solution will be determined. The effect of sample volume on the sorption of lanthanum on *C. barbatula* was investigated by varying the sample volume from 100 to 1000 mL containing 0.1 and 1 μg mL^{-1} of La(III). Quantitative desorption of lanthanum was possible from solution having 0.1 μg mL^{-1} and 1 μg mL^{-1} with the volume of 1000 mL with the recovery up to 94-96%. The preconcentration factors were summarized in Table 3.3. Because of 20 mL of elution volume, 5 to 50-fold preconcentration was obtained for sample solution having volume from 100 to 1000 mL.

Table 3. 3 Preconcentration factor for La(III)

Concentration (ng mL^{-1})	**Feed Solution (mL)**	**Stripping Solution (mL)**	**Recovery (%)**	**Preconcentration Factor**
100	100	20	96	5
	250	20	94	12.5
	500	20	95	25
	1000	20	96	50
1000	100	20	94	5
	250	20	95	12.5
	500	20	94	25
	1000	20	95	50

3.9 Effect of Interfering Ions

The effect of alkaline and alkaline earth elements and the main components of alloys were added to model solution containing La(III) ion. The tolerance limit is the maximum ratio of the concentration of interfering ion to that La(III). Several binary model solutions having 10 μg La mL^{-1} were tested. The presence of transition, actinide and lanthanide elements caused competition between analyte and diverse ions. The results were summarized in Table 3. 4. Effect of 1 M NaCl and KCl was controlled and sorption was possible up to 95%. However, Ca(II) and Mg(II) ions interfered when they existed above 0.15 M. The tolerance limits was in the range of 30 to 45 μg mL^{-1} for transition elements, 2 to 40 μg mL^{-1} for lanthanide elements and around 9 μg mL^{-1} for actinide elements in the analysis of La(III).

Table 3. 4 Tolerance limits of interfering ions on the sorption of La(III) on biomass [a]

Cation	**Tolerance limit (μg mL^{-1})**
Cu(II)	42.5
Co(II)	37
Mn(II)	31.5
Zn(II)	35
Ce(III)	2
Y(III)	40
U(VI)	9
Th(IV)	8.5

[a] c_{La} = 10 μg mL^{-1}

3. 10 Effect of Reuse

To test the biomass stability, it was subjected to several loading and elution column operations. A 100 mL of solution having a concentration as 1 μg La(III) mL^{-1} was passed through the column filled with 500 mg of biomass and desorbed with 10 mL of EDTA solution for 10 cycles. The results from these tests were agreed within 2-5 % error for La(III) up to 5 cycles of repeated experiments.

3. 11 Applications

To check the applicability of the proposed method for preconcentration and determination of lanthanum, the biosorbent was subjected to several model solutions, water and soil samples. Tap water in Tire, Izmir and standard reference soil material were used. The results were given in Table 3. 5. The lanthanum was determined with a relative error lower than 10% in all samples and model solutions.

Table 3.5 Determination of La(III) in model solutions and samples

Composition of synthetic sample (µg mL^{-1})	**Concentration of La(III) (µg mL^{-1})**	
	Added	**Found**
U(5), Co(5)	2	1.85±0.20
Ce(5), Y(5)	5	4.24±0.54
Cu(20), Zn(20)	7.5	6.89±0.86
U(10), Th(10)	10	9.15±0.75
Samples		
Tap water	1	0.95±0.24
	10	9.25±0.85
Soil (SRM 2709)	23*	21.85±0.87

* noncertified values

3. 12 Analytical Performance

The precision (relative standard deviation) of the method was 2.86% for lanthanum at a concentration of 1 µg mL^{-1} for a series of 10 replicates. In order to check the accuracy of the proposed method, the recovery for 1 and 10 µg mL^{-1} lanthanum added to 50 mL of water was measured. The recovery for lanthanum was $\geq$ 94%.

CHAPTER FOUR

CONCLUSIONS

Biosorption has become a significant area of environmental research with the investigation of a wide range of substrates such as bacteria, algae, plant tissue and yeast. This study offers a potential for evaluating the use of biomaterial in metal sorption and preconcentration.

The results presented in this study shows the usability of a new biosorbent, *C. barbatula*, for preconcentration of trace amount of lanthanum(III) ion. The ability of *C. barbatula* for selective biosorption of lanthanum from aqueous solution was confirmed.

The biosorption process was pH dependent. The equilibrium data fitted to linearized Freundlich isotherm model and the maximum adsorption capacity is between 24.88-83.96 mg g^{-1}. The linearized Freundlich isotherms are indicating a physical adsorption for adsorbent.

The proposed method provides a simple, reliable, accurate, sensitive and accurate technique for preconcentration and determination of lanthanum(III) ion. Quantitative recovery was achieved by using EDTA as a stripping solvent. The recovery of La(III) was nearly quantitative (>94%). The preconcentration factor was found between 5 and 50 by increasing volume of sample. The analyte could be directly enriched by using the proposed method. No immobilization process was used for biosorbent.

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