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Escuela de Ciencias Químicas e Ingeniería

ENVIRONMENTAL WATER DEPOLLUTION BY USING HIERARCHICALLY TEXTURED HYBRIDS ALGAE-SILICA MONOLITHS

Trabajo de titulación presentado como requisito para la obtención del título de Petroquímico(a)

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Dedication

This work and all my efforts are always dedicated to my mother, who with her courage teaches me to give the best of me. I admire her unconditional love, her strength and her confidence. I love you, you are my treasure and my reason to improve myself.

To my grandfather, the exemplary man with the precise words. This works is dedicated to you and to your prayers.

To my teacher Alicia, this work would not be possible without your time, patience, knowledge and efforts.

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Abstract

Microalgae from a fresh natural source called Yahuarcocha's Lagoon located in Imbabura province in Ecuador were immobilized into silica monolith with hierarchical porosity by high internal phase emulsion (HIPE) procedure. The samples collected from Yahuarcocha's lagoon were characterized by UV-Vis spectroscopy, column chromatography and optical and fluorescence microscopy. The microorganisms analyzed were classified as blue-green microalgae because of the morphology seen and the presence of photosynthetic pigments characteristic of microalgae (mainly chlorophylls). The characterization of immobilized microalgae into the silica matrix was developed by diffuse reflectance UV-Vis spectroscopy and fluorescence microscopy, evidencing that the silica matrix is a good host for the preservation of microalgae even after several weeks (15 weeks) of encapsulation. Finally the hybrid monoliths as well as the free microalgae were used to perform heavy metal bioremoval. It turns out that better results on metal removal were achieved with the hybrids than with the free microorganisms, with a Copper and Nickel removal of 90.6% and 50.4% (using monolith of 3 months old), respectively of a total ions concentration of 3000 ppm.

Keywords: microalgae, immobilization, monolith, silica, bioremoval, metal removal, copper, nickel.

Resumen

Se inmovilizaron microalgas de una fuente natural llamada Laguna de Yahuarcocha ubicada en la provincia de Imbabura, Ecuador en monolitos de sílice con porosidad jerarquizada por procedimientos que involucran emulsiones de alta fase interna (HIPE por sus siglas en inglés). Las muestras recolectadas de la laguna de Yahuarcocha se caracterizaron por espectroscopia UV-Visible, cromatografía en columna y microscopía óptica y de fluorescencia. Con el uso de estas técnicas los microorganismos analizados fueron designados como cianobacterias (blue-green algae) por la morfología observada y la presencia de pigmentos fotosintéticos característicos de microalgas (principalmente clorofilas). La caracterización de microalgas inmovilizadas en la matriz de sílice fue desarrollada por espectroscopia de reflectancia difusa en UV-Vis y microscopía de fluorescencia, evidenciando que la matriz de sílice es un buen huésped para la conservación de las microalgas incluso por varias semanas (15 semanas). Finalmente, los monolitos híbridos, así como las microalgas libres, se utilizaron para realizar la bioextracción de metales pesados. Se lograron mejores resultados en la remoción de los metales con los híbridos que con los microorganismos libres, con una remoción de Cobre y Níquel de 90.6% y 50.4%, respectivamente, de una concentración total de iones de 3000 ppm.

Keywords: microalgas, inmovilización, monolito, sílice, bioremoción, remoción de metales, cobre, níquel.

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Introduction

Huge scale production of wastewater is an inexorable effect of the modern society. The wastewater are dangerous for human health and biodiversity of ecosystems, also they release large volumes of phosphorus and nitrogen which are the main agents responsible for eutrophication of rivers, lagoons, and sea. Furthermore, elevated concentrations of heavy metals are extremely toxic for the living organisms and represents another important worldwide problem but they can be treated by means of biosorption. Biosorption is the removal of pollutants using biological materials (Al-Rub et al., 2004). Many studies are focused on the biomass-related technologies for metal removal as an attractive and cheap method (Mehta and Gaur, 2001) (Al-Rub et. al., 2004). The microalgae and its capacity to the metal sorption make them a great source for bioremoval treatments. They can sequester toxic heavy metal ions by the formation of phytochelatins (Wilde and Benemann, 1993). Microorganisms immobilization is an attractive technique to keep the biomass into a friendly matrix and to protect them from the external conditions. Moreover, a porous matrix is advantageous to the sorption kinetics (Sommer-Marquez et. al., 2016).

The current worldwide eutrophication problem has been affected the Yahuarcocha's lagoon where the overpopulation of microalgae is evidenced in the greenish color of this Ecuadorian lagoon. In order to threat the pollution caused by heavy metals and the abundant blooms of microalgae into Yahuarcocha’s lagoon, the encapsulation of these microalgae into a silica matrix is a considerable way that would remediate both established problems. Silica was chosen as matrix because of their friendly characteristics and its capability to form hierarchical structures. The encapsulation of Yahuarcocha’s microalgae into a monolith structure was carried out by High Internal Phase Emulsion. In the present work is established the opportunity to remove Copper and Nickel ions by the use of hybrid silica-microalgae monoliths.

Chapter 1 established the main concepts of microalgae, their growth, uses and applicability for metal removal. The current worldwide situation about eutrophication and the Yahuarcocha scenario about this environmental problem are also topics described in this chapter. Moreover, in this chapter features about encapsulation, advantages and disadvantages are developed.

In Chapter 2 is described the methodology for Yahuarcocha's microalgae encapsulation into a silica matrix, also the characterization techniques for the collected samples from the lagoon and for the hybrid monoliths synthetized. The evolution of the encapsulated microalgae was evaluated in this part. Furthermore, this chapter contains the main objective experimentation that is metal depollution, with the use of metal solutions of Copper and Nickel in order to evaluate the metal sorption of the hybrid monoliths.

The obtained results are shown in the Chapter 3, showing the successful metal removal of Copper and Nickel ions, the evolution of the microalgae mass into the hybrid monolith and the characterization results gotten. Additionally, the discussion and comparison with other similar literature studies are developed in this chapter.

Problem statement

The eutrophication is a worldwide problem caused by the uncontrolled growth of algae that affects water bodies like lagoons and rivers and has a preoccupant impact in the human health, environmental wellness and in the touristic activity. This issue is evident in Yahuarcocha lagoon, Ecuador where the green color of the water is clearly notorious accompanied with a disagreeable smell. The high concentration of nutrients as nitrogen and phosphorous in the water increase the population rate of this microorganisms, this high concentration is due to the wastewater pour into the lagoon without previous treatment (de-Bashan & Bashan, 2010). The heavy metal removal is another environmental problem that have been treat with some mechanisms that would become very expensive or inefficient. In developing countries like Ecuador the conjunction of heavy metal pollution and eutrophication means a serious problem, threating against the environmental conservation of its emblematic water source and their biodiversity.

Nevertheless, a hopeful option can help in order to give an efficient Yahuarcocha´s microalgae use and reducing the metal ions concentration. The use of encapsulate microalgae in a hierarchical silica monolith is a very promising strategy in order to use pollution against pollution with low costs and good efficiency.

General and specific objectives:

- General objective:

  To encapsulate Yaguarchocha's microalgae into a hierarchical silica monolith by high internal phase emulsion and to evaluate the obtained material's performance as remover of metal from solutions with known concentration.

- Specific objectives:
  - To characterize the microalgae collected from Yahuarcocha's lagoon using UV-Vis Spectroscopy, Column Chromatography and Optical and Fluorescence Microscopy.
  - To encapsulate Yahuarcocha's microalgae collected *in situ* within a hierarchical porous silica matrix.

- To characterize the hybrid materials silica monolith/microalgae by Diffuse Reflectance UV-Vis spectroscopy and Fluorescence Microscopy.
- To evaluate the sorption of copper and nickel ions by hybrid silica monolith/microalgae and microalgae without encapsulation.
- To evaluate the resistance of microalgae into hybrid monoliths thought the time using Fluorescence Microscopy.

Chapter 1: Background

1. Microalgae

1.1 Generalities

The microalgae are unicellular and photosynthetic organisms which live individually or in colonies in large environments especially in freshwater and marine locations (Jankowska et al., 2017; Priyadarshani and Rath, 2012). The term microalgae refers to the small algaes that can be well seen with a microscope (Larsdotter, 2006).

The microalgae term includes eukaryotic microalgae and the prokaryotic cyanobacteria. The most important common feature of all eukaryotic microalgae and cyanobacteria is that they have oxygen-evolving photosynthesis and that they use inorganic nutrients and carbon (Larsdotter, 2006; Blankenship, 2014).

Chemically, they are composed by lipids, proteins and carbohydrates (Jankowska et al, 2017). A stoichiometric formula for the most common elements in an average algal cell is $C_{106}H_{181}O_{45}N_{16}P$, these are the necessary elements for their optimal growth (Larsdotter, 2006).

Morphological and physiological characteristics of microalgae are highly diverse, their size goes through 1 to 50 μm, and they do not have roots, stem nor leaves (Jankowska et.al, 2017; Osundeko et.al, 2019).

They are the dominant species in aquatic environments, and also live on land, including habitats as apparently improbable as the surface of snowfields and the hairs of polar bears (Blankenship, 2014).

1.2 Types of Microalgae

According to Jankowska et. al.(2017) and Correa (2017) there are approximately 100000 species of microalgae with a huge variation of their cells morphology (oval, cylindrical, rounded, fusiform and some of them have flagella or cilia). Microalgae are categorized in are blue-green algae (Cyanophyceae), green algae (Chlorophyceae), diatoms (Bacillariophyceae), and golden algae (Chrysophyceae), and they are classified by some features like cellular structure, pigmentation composition, storage profile of products, and life cycle (Osundeko et.al., 2019; Jankowska et.al., 2017). In their biomass, microalgae produce various valuable bioactive substances such as pigments, polyunsaturated fatty acids, antioxidants, essential amino acids or immunologically-

effective, virostatic and cytostatic compounds. Therefore, microalgae are commercially cultivated for biomass as food and feed additives, as a source for pharmacology and cosmetics, or, on a small-scale, for research of diagnostic products (Millie et.al., 2002). Their uses are better exemplified in section 1.3.

The main interest of this work is to use with green algae and cyanobacteria so in the next section is provided a brief description of them

1.2.1 Green microalgae and cyanobacteria characteristics

The green microalgae are the most studied specie, because their properties are the closest to higher plants. The diameter of green microalgae is between 3 to 8 µm, including strains. Moreover, they have a high temperature tolerance since some can grow between 15°C and 40°C (Millie et.al., 2002).They have *chlorophyll-a* and *chlorophyll-b*, carotenoids and bilins as photopigments (Blankenship, 2014). Cyanobacteria or also denominated blue green microalgae have sizes of 1 to 20 µm (Guamán and González, 2016) and lives in freshwater ecosystems. They do not present chloroplasts but they have *chlorophyll-a* and produce the phycobilin pigment, phycocyanin, which gives their color (Whitton and Potts, 2012). As it was mentioned before microalgae are involved in the photosynthesis process.

Photosynthesis

Microalgae are autotrophs, synthesizing organic molecules themselves from inorganic nutrients. They transform carbon dioxide into sugars in the presence of water and sunlight as energy source, also they produce oxygen. The overall stoichiometric formula for photosynthesis is: $6H_2O + 6CO_2 + \text{sunlight} \rightarrow C_6H_{12}O_6 + 6O_2$ (Larsdotter, 2006). In the **Figure 1.1** is shown the photosynthesis performed by microalgae.

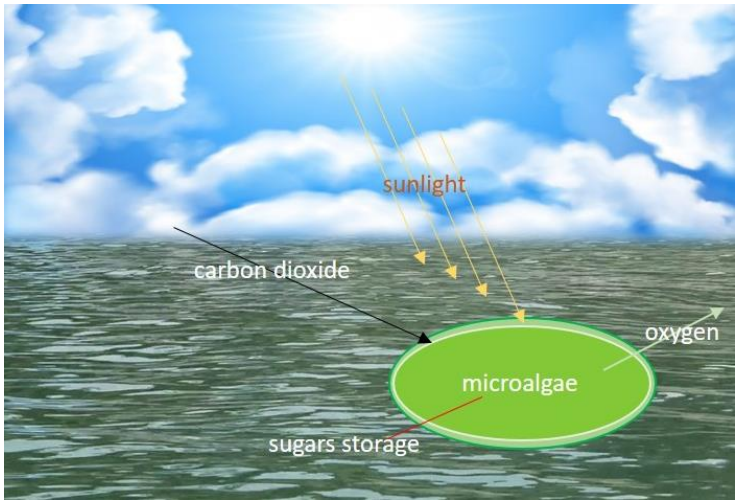

Figure 1.1 Microalgae photosynthesis. Adopted from Microalgae and biofuels by García, P, 2016, (https://www.eoi.es/blogs/merme/microalgas-y-biocombustibles/).

Their simple cellular structure makes them efficient to converter solar energy, to photoautotrophically grow and to approximately half of the global atmospheric oxygen while removing carbon dioxide. (Osundeko et al., 2019)

In eukaryotic photosynthetic cells, photosynthesis is carried on into the chloroplasts (Blankenship, 2014). The prokaryotic cyanobacteria have internal membranes called photosynthesizing lamellae (which makes them autotrophic) arranged in a multilayered complex homologous to the thylakoids of chloroplasts.

Photosynthetic pigments

The pigments are compounds that capture sunlight for photosynthesis, they absorb only determinant wavelength of the visible light which are described in **Table 1** (University of California Museum of Paleontology, s.f.). There are three basic classes of photosynthetic pigments: chlorophylls, carotenoids and billins. The green microalgae contains c*hlorophyll-a, chlorophyll-b* and carotenoids. The red and brown algae do not have *chlorophyll-b* but in the case of the brown algae they have *chlorophyll-c* (Blankenship, 2014).

Chlorophylls

Chlorophylls are greenish pigments located at the chloroplast (University of California Museum of Paleontology, n.d.). The molecular chemical formula for

chlorophyll- a is $C_{55}H_{72}N_{40}O_5Mg$. The chlorophylls are named *a* to *f* in order of their discovery (Blankenship, 2014).

According to Blankenship (2014) and Ruiz et.al. (2019), *chlorophyll- a* is found in all known eukaryotic photosynthetic organisms, it adsorbs sunlight during photosynthesis on wavelength between 430-475 nm and 578-675nm and it is the major reaction center that carries out the electron transfer to produce photosynthesis. *Chlorophyll-b* absorbs the light on wavelengths of 644, 549 ad 455 nm and transfer the energy to *chlorophyll-a*. This change shifts the maximum absorption to shorter wavelengths; chlorophyll b is the major accessory light-absorbing pigment in light harvesting complexes in the majority of eukaryotic photosynthetic organisms, including plants and green algae, and is not found in reaction center complexes. *Chlorophyll-c* is perhaps the most unusual and found exclusively in various groups of marine algae, such as diatoms and dinoflagellates. *Chlorophyll-d* is only found in *cyanobacteria*, and that earlier reports of *chlorophyll-d* in red algae resulted from contamination of the algal surface by epiphytic cyanobacteria that contain *chlorophyll-d;* its absorption wavelength is on 697, 456 and 400 nm. *Chlorophyll-f* is the most recently discovered of all the chlorophyll-like pigments, it was found in cyanobacterial cultures isolated from microbial structures called stromatolites. The spectroscopic properties of chlorophylls are the principal tool for the study of plant and microalgae.

Carotenoids

Carotenoids are red, orange, or yellow pigments. They do not dissolve in water. They are denominated as accessory pigments because they cannot transfer sunlight energy directly to the photosynthetic pathway, but must pass their absorbed energy to a chlorophyll-type pigment (University of California Museum of Paleontology, n.d.). They usually exhibit an intense absorption band, typically in the 400– 500 nm range (Blankenship, 2014).

Bilins

Bilins absorb in the spectral region from 550 nm to 650 nm. Bilins are the only class of photosynthetic pigments that are covalently attached to proteins (Blankenship, 2014).

In the **Table *1.1*** are described the absorbance wavelength of the photosynthetic pigments that can be found in microalgae.

Table 1.1 Chlorophylls, carotenoids, and bilins spectroscopic properties of microalgae.

Pigment		Absorbance wavelength (nm)
Chlorophyll	a	662, 578 430
	b	644, 549, 455
	c	640, 593, 462
	d	697, 456, 400
	f	707, 440, 398
Carotenoids		400 to 500
Bilins		550 to 650

1.3 Uses of Microalgae

Algae are much demanded because they are great valuable source for different kind of uses. The microalgae species more used in industry are *Arthrospira (Spirulina), Chaetoceros, Chlorella, Dunaliella, and Spirulina platensis* (Rizwan, 2018). One of the most common and widely used specie is *Chlorella vulgaris* (*C. vulgaris*). Their shape is psheric with a diameter of 2.5–5 μm, and they do not have flagellum (Reimann, 2020).

According to Jankowska et. al. (2017), Osundeko et. al. (2019), Larsdotter (2006), Rizwan (2018), Chisti (2007), and Priyadarshani and Rath (2012) on the article Commercial and Industrial Applications of Microalgae, various microalgae are used in the following industries:

1.3.1 Human Health

Due to their wide content of vitamins, minerals, carotenoids, carbohydrates, protein, enzymes and fiber, *Chlorella vulgaris* and *Spirulina platensis* represent a rich food source with very promising benefits as antimicrobial, antibacterial, antiviral, on cholesterol and glucose regulation, and drugs for the heart and cancer.

1.3.2 Food Industry

The great amount of *Beta Carotene* into microalgae *C. Ellipsoidea* is used to color the food, providing yellow tonality which is used in margarine, fish flesh color, snacks, candy bars or gums, pastas, and drinks. The extracts have a huge applicability in, ravioli,

noodles, soups, and sweets as cookies, bread and rolls, ice cream fruit puddings and sauce production.

1.3.3 Cosmetic industry

In this scenery the *Arthrospira* and *Chlorella vulgaris* constituents are used as thickening agents, water-binding agents, and antioxidants, on anti-wrinkles creams, emollients, anti-irritant, revitalizing or hair, skin and sun care products.

1.3.4 Animal feeding

Arthrospira Chlorella vulgaris, Dunaliella sp., and *Spirulina* have a good benefits on animal feeding as supplement, more than 30% of the algae growth is for the animals feeding products.

1.3.5 Fertilizers

Cylindrospermum sp., Hapalosiphonfontinalis and *Nostocmuscorum* act as biofertilizer, soil conditioner, increasing residual soil nitrogen and carbon, and improving pH and electrical conductivity of the soil

1.3.6 Biofuel

Syngas, bio-oil, and charcoal, biodiesel and bioethanol can be obtained from *Haematococcus pluvialis* and *Nannochloropsis sp* o. Using for biofuel production, the net release of carbon dioxide can be zero because of its fixation through photosynthetic process compensates the power plants generation.

1.3.7 Environmental applications

Moreover that it use as fuel can remediate the carbon dioxide release. Some species of microalgae, for example *Spirulina platensis*, *Chlorella vulgaris* and *Scenedesmus obliquus*, had showed great advantages on wastewater treatment, removal of nitrogen and phosphate from industry and domestic wastewater, the immobilized species also present hopeful results on color removal from textiles dyes. Additionally, the promising benefit to absorb heavy metals would give a great solution for the current pollution level of wastewater especially in mining.

In the **Figure 1.2** is shown the main uses of microalgae and the components of interest in each area.

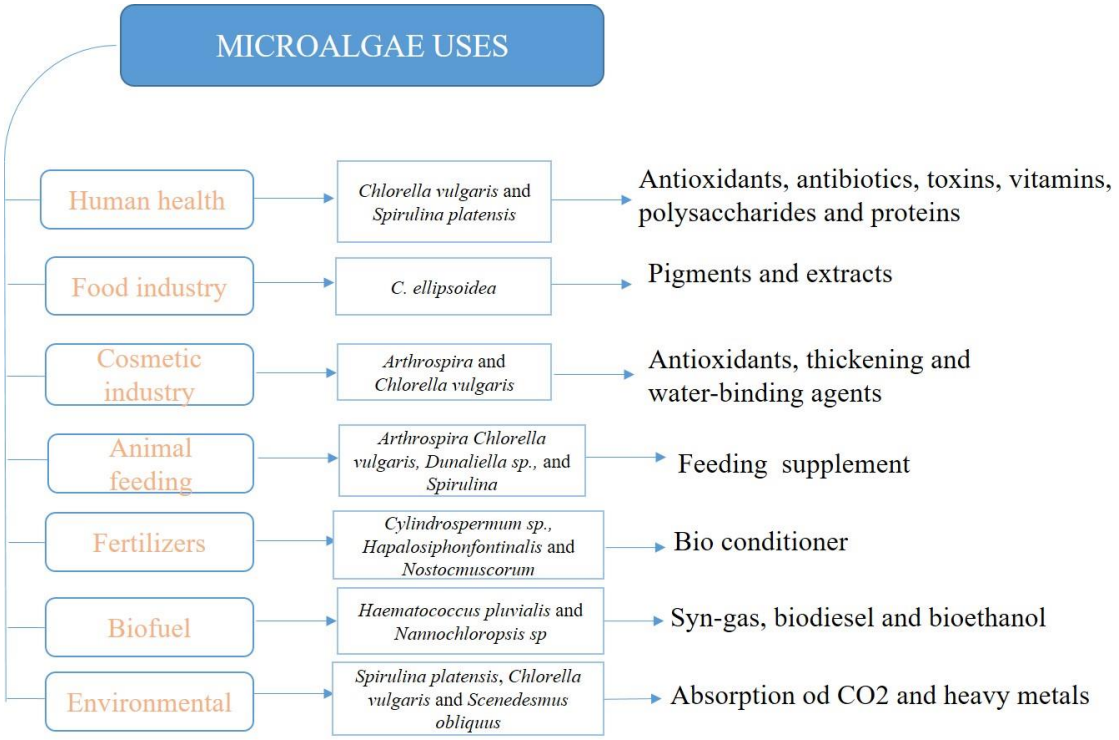

Figure 1.2 Microalgae uses.

Toxic heavy metal contamination of the environment is a significant world-wide phenomenon (Wilde & Benemann, 1993). The traditional techniques to remove heavy metals do not have efficient results and are very expensive. This traditional methods are the precipitation of the metals using chemical like sulfide, the ion exchange where resins are used to bind the metal ions to the substrate, the reverse osmosis and electrodialysis (Wilde and Benemann, 1993). The bioremoval is a new and promising option in order to decrease the metal concentration at low costs and giving a good disposal of the pollutants- Bioremoval is the use of biological systems to remove metals from wastewaters with high efficiency and cheaper costs than the conventional treatments (Wilde and Benemann, 1993). This process shows advantages when the pH and concentrations vary and when another metal or suspended solids are present (Wilde and Benemann, 1993). Microalgae used have the potential to offer a significant improvement in worldwide depollution of water from heavy metals (Balasubramani et. al., 2016) (de-Bashan and Bashan, 2010). Early research has focused on the use of microalgae to remove contaminants before discharge into rivers in order to reduce the eutrophication (Osundeko et. al., 2019).

Some of these conditions are very suitable for algae and microalgae growth.

1.4 Microalgae growth

For their growth, they use water, sunlight and carbon dioxide from the atmophere and the soluble carbonates as well as some mineral salts. Depending of the microalgae specie, the concentration of carbon dioxide varies (Jankowska et. al., 2017). Lipids are obtained from the photosynthesis process where carbon dioxide is used for this purpose (Jankowska et. al., 2017).

Microalgae can grow in wide varieties of wastewater such as municipal, industrial, artificial, and agricultural types (Osundeko et. al., 2019). Microalgae are known to be very adaptive and can be grown in salt water, freshwater, or even on contaminated industrial effluents (known as wastewater) without any extra requirements of nutrients or agricultural land (Rawat et al. 2016). On top of these advantages, microalgae can grow much better when fed with greenhouse gas (carbon dioxide) and waste nutrients like sewage (Osundeko et. al., 2019).

The main nutrient for this objective is the inorganic carbon, also the nitrogen have an important role as ammonium and the phosphorous as phosphates are important for fast growth of microalgae (Jankowska et. al., 2017). On the green microalgae, the concentration up to 50% do not affect their growth. The *Chlorella* specie is not affected by the minor addition of gases like NOx and SOx (Jankowska, 2017). An excess of dissolved oxygen can produce photooxidative damage on the cells affecting the algae metabolic development (Jankowska et. al., 2017; Millie et. al., 2002).

High concentration of some compounds can be lethal for microalgae growth, these are herbicides, detergents substances, skin creams, pesticides and heavy metal removal (Larsdotter, 2006).

Microalgae harness nature’s energy using photosynthesis and can double their biomass in few hours during their exponential growth period (Osundeko et.al., 2019).

Successful cultivation requires continuous monitoring of physico-chemical parameters, i.e. irradiance, pH, temperature, dissolved oxygen concentration, and nutrient status. The basic biological method used is a microscopic examination to detect morphological changes and contamination by other microorganisms (Millie et.al., 2002).

1.4.1 Open and Closed systems in microalgae growth

For microalgae cultivation there are closed and open systems. **Figure 1.3** Are shows the diagram of production of these two systems. In the open systems the culture has contact with the air and other external factors, but closed systems have more control on the growth conditions.

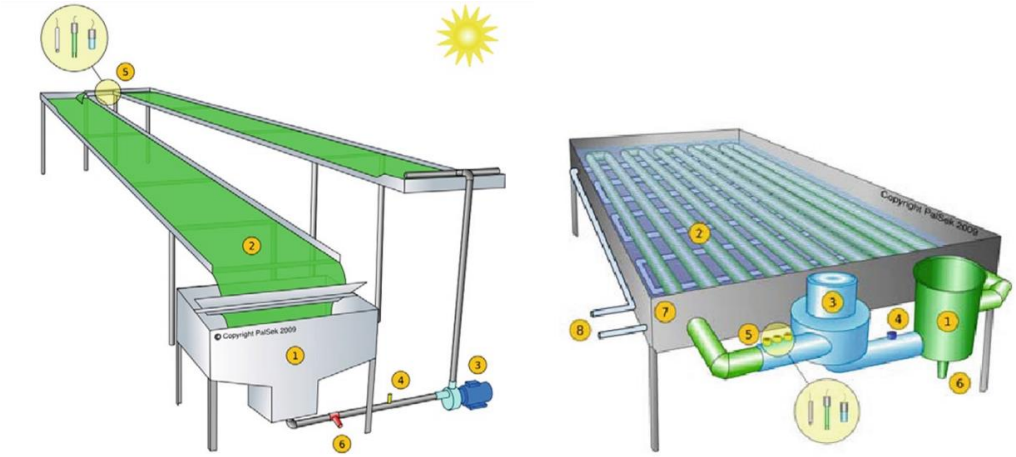

Figure 1.3 Schematic diagram of open (left) and closed (right) system for microalgae culture (Masojídek et.al., 2010)

The closed systems improve the cell density and reduce the evaporation that occurs in open systems. Another advantage and difference from open systems is the monoculture is easily to attach (Osundeko et.al., 2019).

The open systems suffer the variation of the environment conditions as light, temperature; closed systems have established cycles of light and obscurity (Millie et.al., 2002).

Economically, the open systems represents lowest costs for assembly and operation (Larsdotter, 2006). Nevertheless, the viability of cultivation of microalgae by the mentioned closed systems in an industrial scale is difficult to calculate because of the parameters that have to be kept to the correct microalgae growth (Osundeko et.al., 2019).

Nowadays, open ponds are used for the autotrophic production of *Chlorella*, semi-closed tubular photobioreactors, or inclined cascades, since its high growth rate prevents contamination by other microalgae. The yearly amount of *Chlorella* and *Arthrospira* production isof 3,000 t and 4,000 t, respectively (Millie et. al., 2002).

Microalgae can perform a dual role for remediation of nutrient pollutants and biomass production when grown in wastewater (Osundeko et.al., 2019).

Microalgae need metals as trace nutrients, since they are part of the active sites of essential enzymes. Thus, they have evolved highly efficient mechanisms for recovering, often from very low concentrations, specific metal ions, including As, Co, Cr, Cu, Fe, Mo, Ni, Se, Sn, V, Zn, etc (Abdel-Raouf et.al., 2012; Wilde and Benemann, 1993). Microalgae can produce peptides with the capacity to join to metals, forming organometallic complexes in partitions in the vacuoles improving the adequate control of the heavy metal ions concentration in the cytoplasm, preventing their toxic effect (Cerón et.al., 2015; de-Bashan and Bashan, 2010). Microalgae can catch heavy metal ions by the same adsorption and absorption mechanisms as other microbial biomass as well as by the formation of phytochelatins which they synthesize in response to toxic heavy metal stress (Cobbett, 2000; Wilde and Benemann, 1993). Microalgae use metals for growth and metabolically ignore the non-essential heavy metals. Microalgae have an affinity for polyvalent metals, leading to their application as cleaning agents in water and wastewater containing dissolved metallic ions (de-Bashan and Bashan, 2010).

1.4.2 Wastewater for microalgae growth

Wastewater provides a cheap source of growing microalgae; however, it comes with some challenges in terms of contamination and difficulty in maintaining a monoculture system. Using a natural strain that is indigenous to the cultivation pond might bring a solution to this. Fungal, viral, and bacterial pathogenic activities can outcompete microalgae and disrupt the culture stability in large-scale cultivation (Cerón et al., 2015).

High Rate Algae Ponds (HRAP)

This high-rate algae system is used in water treatment, biofuel production, leachate treatment, elimination of drug residues, cultivation and production of algae biomass, cultivation of algae with wastewater (Cerón et al., 2015).

The HRAP operation is based mainly on the oxidation of organic matter due to photosynthetic oxygenation of microalgae in the system, which are caused by solar energy and the CO_2 dissolved in water, derived mainly from the mineralization of matter causing the organic carried out by the bacterial community present. Then, the algae

release oxygen through photosynthesis process, which is used by bacteria that also mineralize nutrients such as nitrogen and phosphorus, although the microalgae biomass is also assimilated, thereby achieving a good nutrient recycling process. (Cerón et al., 2015). HRAPs are mixed by gentle stirring in an aerobic throughout its volume; the velocities goes through of 5–20$cm\ s^{-1}$, too high turbulence can be destructive to the culture (Larsdotter, 2006).

Cerón et al (2015), Millie et al (2002) and Larsdotter (2006) describe the HRAPs specific conditions for the growth of algae that can be used in wastewater treatment. The most important is the content of nitrogen, phosphorous and ammonia. These ponds have great resistance to seasonal conditions and diurnal in outdoor conditions, especially in changes in temperature and solar radiation. The most advantageous temperature for microalgae cultures are between 20 to 24 °C, temperature below than 16 °C delay growth, while temperatures above 35 °C are lethal for some species; overheating of algal cultures is a problem especially in humid climates where evaporation occurs. In summer, the morning temperature of the culture in open ponds can be as much as 10°C below the optimum value, causing a decrease of the photosynthetic capacity of the microalgal culture for a few morning hours.

The organic carbon sources can be assimilated either chem- or photoheterotrophically. The quantity of carbon dioxide dissolved in water varies with pH, and addition of CO_2 results in a pH decrease. At higher pH values, most of the inorganic carbon is in form of carbonate which cannot be assimilated by the algae micronutrients. Likewise, small quantities of manganese, molybdenum, copper, iron, zinc, boron, chloride and nickel, sodium, silicon, cobalt, iodine, and selenium are necessary for certain algal species (Larsdotter, 2006).

1.5 Algae Autotrophic Growth and its Contamination Problem

Eutrophication is the pollution of water bodies as the result of the excessive growth of phytoplankton produced by nutrient enrichment especially phosphorous and nitrogen (Maridueña et al., 2011; Yang et al., 2008; Saelens, 2015). Other influencing factors on water eutrophication are hydrodynamics, environmental factors such as temperature, salinity, carbon dioxide, element balance, and microbial and biodiversity (Yang et al., 2008).

Water eutrophication is caused by the autotrophic algae blooming in this ambient, which composes its bioplasm by sunlight energy and inorganic substances through photosynthesis. This process of eutrophication is described with the following equation:

$$106CO_2 + 16NO_3^- + HPO_4^{2-} + 122H_2 + 18H^+ \xrightarrow{\text{energy+microelement}} + C_{106}H_{263}O_{110}N_{16}P(\text{algae bioplasm}) + 130_2$$

In lagoons, the nitrogen is on organic and ammonia forms and phosphorous is the limiting nutrient (Maridueña et al., 2011).

The increase of this algae population causes the reduction of water transparency turning into a green color, and less sunlight pass (Smith and Schindler, 2009). Consequently, little sunlight can penetrate water body and plants under the water will not be able to make photosynthesis (Yang et al., 2008). This fact triggers in oxygen depletion leads to intrinsic disequilibrium of the aquatic ecosystem and damage of the water ecosystem (Maridueña et al., 2011). Moreover, the decay of algae blooms causes in an increment of concentration of dead organic matter, it consumes dissolved oxygen in the water, resulting in hypoxic conditions, which can also have a bad smell (Saelens, 2015). The modification of structural population, also the proliferation of determinant species, and the fast and gradual disappearance of the entire initial population are important effect of eutrophication on water bodies (Maridueña et al., 2011).

Eutrophication causes the lack supply of drinking water source due to the poor water quality. Furthermore, the blooming algae die, they can produce lots of algae's toxin which is harmful to human health (Yang et al., 2008), the tourism industry is affected by economic loses and the inhabitants are affected by the odor (Smith and Schindler, 2009).

The main reason for the eutrophication problem is the discharge of wastewater from human consumption, then the industry, farming, agriculture, touristic are also responsible of the contamination of water bodies (Yang et al., 2008; de-Bashan and Bashan, 2010). Lakes enriched by human activities are denominated as culturally eutrophic (Smith and Schindler, 2009). The aquatic environment receive wastewater from domestic use without any treatment, affecting the living organisms in water, increasing the suspended solids, increasing the nutrients in the water body. The lagoons usually receive raw sewage as effluents (Maridueña et al., 2011).

Worldwide situation

Nowadays water eutrophication has become a significant worldwide environmental problem (Yang et al., 2008). Lagoons around the world have been impacted by dangerous inputs of nutrients from human-related practices of the land. This fact outcomes in change from oligotrophic (poorly nourished) water bodies to mesotrophic (moderately nourished), eutrophic (well nourished) and lastly to hypertrophic (over nourished) systems (Saelens, 2015).

Shallow lakes are an important natural, social and economic element, they benefits with fishing, tourism, animal watching, swimming, water drinking source, irrigation, production of food and transportation (Saelens, 2015).

Water eutrophication is one of the most challenging environmental problems in the world. The increasing severity of water eutrophication has been brought to the attention of both the governments and the public in recent years; about 30-40% of the water bodies have been affected more or less by water eutrophication in the world (Yang et al., 2008).

Water eutrophication problem is widespread around the world and its severity is increasing, especially in the developing countries like China; in Europe initiatives are implemented to reduce the wastewaters discharged in waters, in UK exists regulations to remediate eutrophication induced by the level on nutrients in water bodies (Mainstone and Parr, 2002; Yang et al, 2008). In North to South America the eutrophication problem has several results because there is incompetent or absent wastewater treatment (González and Roldán, 2019).

The total economic impacts around the world because of harmful algal blooms have not been wholly calculated, but the lost costs of eutrophication to the society on fishing, water treatment and human health is billonary per year (Smith and Schindler, 2009; Saelens, 2015).

A huge number of investigations turn on removing contaminants from the wastewater that are discharged into lagoons, river and seas in order to prevent the eutrophication problem (Osundeko et al, 2019).

Eutrophication in Ecuador

Several studies shown that in Ecuador water bodies exist diversity of microalgae. (Guamán and González, 2016). One of the most recognized lagoon is Yahuarcocha in Imbabura province, which has dangerous ecological problems produced by eutrophication.

Yahuarcocha Eutrophication problem

In Ecuador the Yahuarcocha lagoon is an important touristic and economic lagoon. It is located at 2192 m near to Ibarra, Imbabura at 2200 m above sea with high exposure to ultraviolet (UV) and solar radiation, containing 12.7 million m^3 of water (Saelens, 2015; Maridueña et al., 2011; Jacome et al., 2018). Its shape is elongated, with surface area is approximately 2.41 km^2 with a maximum depth of 6.9 m and an average depth of 4.9 m **Figure 1.4** The average temperature is 15.9 °C, and there are two distinct seasons which correspond to wet and dry periods (Jacome et al., 2018).

Figure 1.4 Yahuarcocha lagoon's location.

Based on total nutrients and chlorophyll-*a* concentrations, Yahuarcocha can be considered to be a eutrophic lagoon (Saelens, 2015). Several studies indicate that the current state of the lagoon is on a critical polluted level, the water quality unfit human consumption, the water level has been reduced and the shores are degraded (Maridueña et al., 2011).

Fish species like tilapia were introduced in the lagoon two decades ago. This fact cause that many habitants make of this activity their commercial income (Maridueña, et al., 2011). Moreover, the mild temperature of Yahuarcocha makes it the most visited

lagoon in the province where 20 000 tourists weekly visit Yahuarcocha of which 85% passes during the weekend (Saelens, 2015).

Yaguarcocha lagoon is naturally fed by the surface water coming from runoff of the precipitations of three main entrances the Quebradas of Manzanahuayci-Santo Domingo, Polo Golo and San Antonio, with an intermittent water regime; it is also fed artificially by an irrigation ditch or canal from the Tahuando River. This income flows are mainly from domestic wastewaters (Saelens, 2015; Maridueña et al., 2011). The wastewater treatment plant that operate next to the lagoon, discharge an outflow directly to Yahuarcocha being the most contributing agent (Jacome et al., 2018). Furthermore, constant human activities on the lagoon shores and surrounding areas produces water pollution due to detergents, fertilizers, herbicides, sewage, and animal waste (Jacome et al., 2018).

The great incoming nutrients level is the key to understand the eutrophication process of this lagoon (Saelens, 2015). The grease levels are over the regulation due to the fats discharged by sewage and local restaurants (Jacome et al, 2018). This fact is producing harmful exposure to the habitats and it is deteriorating the touristic image of this emblematic place, its water appearance and odor decrease the tourist visits.

One preoccupant fact happened between 2003 and 2005, where a massive death of fishes occurred because of the decrease of dissolved oxygen, high concentration of organic matter and presence of polluting species (Jacome et al., 2018).

The favoring conditions of the phytoplankton blooms are the pH that is 8.55 at 17.6°C in the lagoon, also the shallow depth help this species to grow. The water of this lagoon is denominate as hard alkaline and eutrophic and it is established that Yahuarcocha lagoon has low diversity (Jacome et al., 2018; Maridueña et al., 2011).

Phytoplankton diversity is decreasing in Yahuarcocha. Forty years ago it represented biodiversity index of 3.5 and currently it is between 0.67 to 2.28, this reduction is due to the majority presence of some organisms and the displacement of older species (Maridueña et al, 2011).

According to Saelens (2015) the average of *Chlorella sp.* is 3.5 % into the lagoon. In the **Table *1.2*** is shown the principal algae species found in Yahuarcocha lagoon:

Table 1.2 Algae composition in Yahuarcocha lagoon (González and Roldán, 2019; Guamán and González, 2016):

	BACILLARYOPHYTA	CHLOROPHYTA
Yahuarcocha lake	*Navicula sp.*, *Epithemia sp.*, *Diatoma sp.*, *Gomphonema sp.*, *Synedra sp.*, *Nitzschia sp.* *Melosira sp.*	*Chlorella sp.* *Desmodesmus sp.* *Scenedesmus sp.* *Leptolyngbya.*

The solution to recuperate the Yahuarcocha lagoon is like first instance reduce the inflows with high content of nitrogen and phosphorus; then, the implementation of stronger legislation and environmental rules in order to remediate the damage that is producing the discharge of domestic wastewater and touristic activities into the lagoon. One may look for hopeful strategies against the environmental damage caused for the polluting microalgae population.

Lately, the encapsulation of biomolecules is an interesting topic of investigation, representing a big challenge is to develop a biocompatible matrix and a friendly synthesis procedure. A biocompatible material is referred to one without cyto- and geno-toxicity itself and its eventual degradation products (Meunier et al., 2010). A huge number of consideration interfere to the matrix selection, some of them are the chemical composition and stability, mechanical strength, the morphology of the surface, porosity to allow diffusion, and phototransparency if photosynthetic cells are encapsulated. Moreover, the conditions needed in order to have a biocompatible matrix are shown in the **Figure 1.5.**

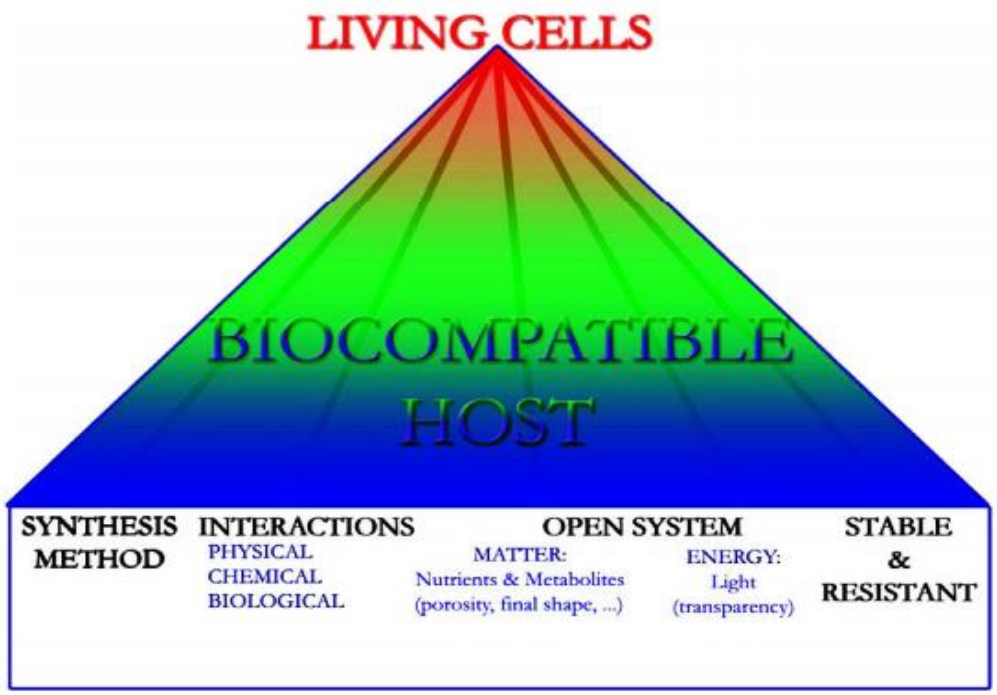

Figure 1.5 Requirements for a biocompatible encapsulation matrix (Meunier et al., 2010).

The main objective to encapsulate microalgae in a porous material is to protect them from the adverse conditions and the mechanical stress (Zhang et al., 2016). Contrary where the algae is dried and powdered or cultivated in a separate process, algae immobilization is done by physical or chemical procedures (Wilde and Benemann, 1993). Immobilization can be classified into passive and active, in passive the microorganisms themselves attach to surfaces and in active chemical attachment and flocculant agents are necessary (de-Bashan and Bashan, 2010).

Advantages

The encapsulate microorganism is protected by the external factor, making longer its living time, and reducing synthesis and conservation costs.

This revolutionary and green process is very promising for environmental objectives, like wastewater treatment from domestic use or from the industry. The main advantage of immobilized algae use is the high cell densities and column operations that can be performed (Wilde and Benemann, 1993).

The most of the used matrixes are friendly with the environment, do not produce secondary pollution, the products can be used for other objectives and are not dangerous for the human life (de-Bashan and Bashan, 2010).

Disadvantages

The immobilization means stress on the encapsulated organisms due to the chemical interactions between the cell wall and the matrix, also reduce the metabolism of the immobilized cell (Wilde and Benemann, 1993). Immobilization or encapsulation of microorganisms in polymers exerts a significant stress on the microorganisms because of chemical forces and interactions between the immobilization matrix and the cell wall. Confinement in a limited space also affects the metabolism of the microorganisms (de-Bashan and Bashan, 2010)

Six different immobilization types have been defined: covalent coupling, affinity immobilization, adsorption, confinement in liquid–liquid emulsion, capture behind semi-permeable membrane, and entrapment in polymers (de-Bashan and Bashan, 2010).

The mostly used matrix for immobilization are sodium or calcium alginate, synthetic polymers and silica (Moreno-Garrido, 2008; Larsdotter, 2006; Wilde and Benemann, 1993). The synthetic material are acrylamide, polyurethane, polyvinyl, resins, the natural polymer derivate of algal polysaccharides as alginate, carrageenan, agar, agarose, also the chitosan (de-Bashan and Bashan, 2010; Maridueña et al., 2011). They are a good option in a small scale systems because they reduce the diffusion limitations (Wilde and Benemann, 1993). Natural polymers are less stable in wastewater, are more biodegradation vulnerable but they are less dangerous to prepare, and can dissolve in highly contaminated wastewater than the synthetic ones (de-Bashan and Bashan, 2010).

Alginates are widely used polymers obtained from brown algae, they are not toxic, are easy to handle, very available and inexpensive, and the microorganisms do not suffer considerable physical or chemical disturbances in the synthesis process (de-Bashan and Bashan, 2010).

Usually, the matrixes used for N and P removal are alginate, polyvinyl, polyurethane and polyvinyl foam, carrageenan, chitosan and the microalga employed for are *C. vulgaris* and *Anabaena doliolum* (Maridueña et al., 2011).

Newly, an immobilization technique in porous silica matrices was developed to avoid generation of byproducts that are detrimental to the microalgae when entrapped in silica (de-Bashan and Bashan, 2010).

Silica matrix

Silicate monoliths give an efficient strategy that restricts movement of the recognition molecule but allows free flow of analytes (Dave et al., 1994). These monoliths can be suitable vessels to take place a huge variations of chemical operations (Nakanishi, 2011). Silica is very commonly used as encapsulation matrix due to its good hierarchical structures and superior mechanical properties (Yang et al., 2009; Xu et al., 2003). Moreover, the advantages with the silica are its chemical stability, its improved mechanical strength, silica does not swell in aqueous or organic solvents avoiding leaching of entrapped biomolecules, it is not a nutriment source for microorganisms, is not toxic, it is biologically inert and is optically transparent letting pass the light radiation to the photosynthetic organisms entrapped. (Livage et al., 2001) (Nassif et al., 2002). This qualities make silica an ideal matrix for optically active organism, with a huge applicability in different industry aspects like biosynthesis (Meunier et al., 2010), medicines (Pérez-Esteve et al., n.d.) or catalysts (Qi et al., 2007).

Sol-gel encapsulation method

Sol–gel encapsulation in silica offers advantages in mechanical strength, chemical stability and negligible swelling (Nassif et al., 2002), this method is very used to encapsulate organisms into silica matrix.

This method involves hydrolysis of a monomeric silica precursor, usually the precursor is an *alkoxide* $Si(OR)_4$ being R an alkyl group (Dave et al.,1994). For the formation of network a two/step reaction is performed, in the first step silicic acid is obtained and in the second step silica particles grow gradually as condensation takes place. In the last step, the formation of colloidal solutions “sols” and gel which can be partially dried at room temperature giving porous network of hydrated silica ($SiO_2 \cdot nH_2O$) with porosity under micro- and meso scale (Livage et al., 2001).

Molecules of the corresponding alcohol are liberated in the process, this by-product is not cytocompatible (Nassif et al., 2002), being harmful for cells of higher organisms (Perullini et al., 2008). One strategy to modify this process in order to obtain a specific porosity in the network is the use of a structure directing agent or template giving the formation of meso and macropores (Roucher et al.). Taking control of the silica

porosity one can obtain a hierarchical porous silica monolith. The templates can be block polymer surfactants, solids and emulsion, often they can be used in conjunction. For the present microalgae encapsulation in silica matrix were used an emulsion (HIPE) to realize the macroporosity and a block copolymer to give the mesoporosity.

Another new method which is very promising and totally soft chemistry involved is the immobilization into hierarchical silica monoliths by high internal phase emulsion-copolymer double template procedure. This technique was already proven in "Algae Encapsulation into Silica Monoliths Synthesized by High Internal Phase Emulsions (HIPE)" by López, N. (2020) and "Immobilization of chloroplasts from grass within a silica matrix synthetized by HIPE method" by Vaca et al. (2020). It was demonstrated that the living organisms (*chlorella vulgaris* and chloroplasts) live long time into the matrix without any extra cares and no feeding compared with other encapsulation methods and matrices. The obtained hybrids can be used for air and water depollution using pollution to create a solution. Encapsulated microalgae can be used as well in heavy metals adsorption to decontaminate and at the same time they uptake CO_2.

Examples of encapsulated microalgae used in bioremoval

According Wilde and Benemann (1993) in the article "Bioremoval of heavy metals by the use of microalgae" explain that microalgae are huge used in different matrixes for bioremoval. The polysulfone beads denominated Bio-Fix was used for microalgae encapsulation, this material was improved in the properties of the surface in order to decrease the diffusion restrictions. Calcium alginate is used for gold recovery using *Chlorella and Spirullna*. Using silica as matrix, AlgaSORB TM was produced by Biorecovery Inc. of New Mexico and is developed with algal cell giving very promising results for metal removal from industry pollution waters (de-Bashan and Bashan, 2010; Wilde and Benemann, 1993).

Larsdotter (2006) and Moreno (2008) reported that encapsulated algae was used for wastewater treatment removing nitrogen, phosphorous and metals showing similar results in comparison with free cells. The living microalgae are mainly used for nutrient removal like nitrogen, and the dead cell for adsorption of metals.

De-Bashan & Bashan (2010) indicate that *Chlorella vulgaris* in carregenan and alginate matrix is used for domestic wastewater with good removal results of nitrogen and posporous compared with free cells, and solving the suspened biomass of microalgae suspended in the water. Moreover, Immobilized *Anabaena doliolum* and *C. vulgaris* into alginate have efficient nitrate and ammonium removal. Using a natural loofa Sponge and immobilized *C. sorokiniana* it is shown good results removing lead in aqueous solutions.

Often it is necessary to use large amount of microalgae population because they can survive against toxic effects of the matrix. This is the case of *Botryococcus braunii* in polyurethane, which was toxic for small microalgae populations (de-Bashan & Bashan, 2010).

The hybrid materials should have efficient results removing metals, and have cheapest costs of the materials and maintenance (de-Bashan and Bashan, 2010). The hybrid materials used for adsorption are recollected after the use for future catchment of metals (de-Bashan and Bashan, 2010).

The advantages of bioremoval are the great efficiency of metal ions reduction, cheapest costs, the use of a natural and available source, the capacity to treat a huge amount of volumes, the capacity to remove not only one metal, the extensive range of operational conditions, recovery of the metals removed, and less amount of dangerous waste (Wilde and Benemann, 1993).

One of the major problems in bioremoval research is the difficulty in developing generic technologies. Regarding this challenge, a lot of variables should to be considered like selecting the biomass, processing methods, environments, and chemical composition objective (Wilde and Benemann, 1993). It is important to understand that the success of a method should be measured in actual conditions not just in the laboratory conditions **Appendix A**.

In that sense this work proposes to encapsulate microalgae found in Yahuarcocha Lagoon into hierarchical silica monoliths by HIPE method and use the samples in the copper or nickel adsorption from water.

As it was already said the HIPE method looks very promising and in this work the next step was to use those hybrids for copper and nickel removal.

Chapter 2: Methodology

1. Microalgae handling

1. 1. Collection and processing of microalgae

The microalgae were harvest from the shore of Yaguarcocha lagoon located in Ibarra, Ecuador. The sampling was carried out in an area where the dark green color on the water surface was visible. The samples were kept into hermetic glass recipient at room temperature.

In the laboratory the samples were filtered under vacuum, weighted, and characterized by UV-Vis and Column Chromatography for its respective use in the immobilization of them into hierarchical silica monolith.

2. Synthesis of hybrid microalgae silica monolith by high internal phase emulsion

2.1. Materials and reagents

Cyclohexane (99.9%) was purchased from Fischer Chemical. Tri-block copolymer Pluronic® P123 ($EO_{20}PO_{70}EO_{20}$, Mn ~5800) was purchased from Merck Millipore, Tetraethyl orthosilicate (TEOS, 98%), sodium fluoride (NaF, >99%), Nickel (II) chloride hexahydrate ($NiCl_2$• 6 H_2O, Reagent Plus®), Copper (II) nitrate trihydrate ($Cu(NO_3)_2$ • $3H_2O$, > 98%), all of them were purchased from Sigma Aldrich. The solvent cyclohexane (99.9) was purchased from Fischer Chemical. On the other hand, the materials used for the monoliths synthesis are: 50mL and 100 mL beakers, 10 mL graduated cylinder, magnetic stirrer, hot plate magnetic stirrer, 1000 µL - 100 µL micropipette, glass desiccator and tubular plastic molds.

2.2. Synthesis procedure

The encapsulation was performed according to the established procedure founded in Vaca-Oviedo et. al (2020).

Hydrolysis of silica precursor (TEOS) was performed by its addition in an acidic solution with pH=2 of 20 % (w/w) of P123 at room temperature and continuous stirring of 400 rpm. Completed the hydrolysis after 30 minutes, NaF (8 g/L) was aadded. This improves the silica precursor polymerization and the regulation of the pH. A fixed quantity of microalgae were previously mixed with the oil phase (cyclohexane, 99%). This mixture was slowly dropped to the aqueous phase in order to allow the emulsification. The oil-in-water emulsion was poured into plastics molds and let into a

desiccator for aging at room temperature during 7 days. For reproducibility all samples were performed in triplicate. In the **Figure 2.1** is shown the procedure carried out in the laboratory:

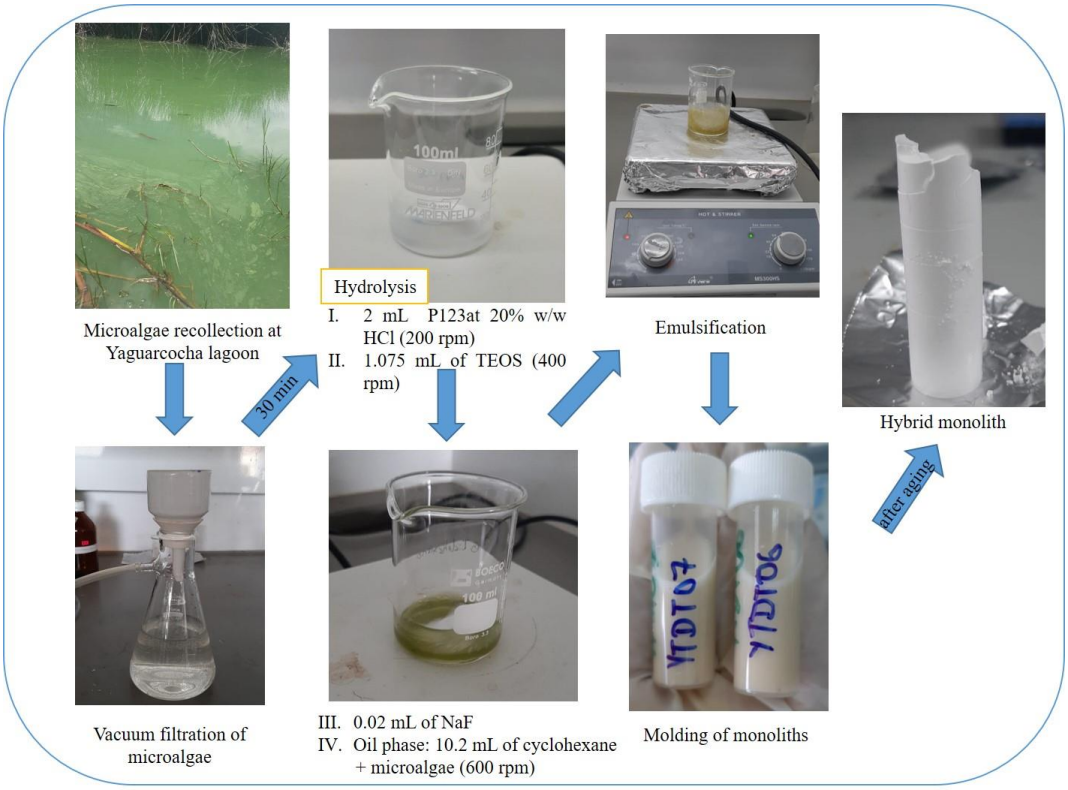

Figure 2.1 Synthesis procedure of microalgae encapsulation on hybrid monolith.

A reference sample it means without microalgae was also synthesized by the same procedure.

In the **Table 2.1** are described the synthesis conditions for the different samples and the codes of identification.

Table 2.1 Identification code of the monoliths and their synthesis conditions.

Monolith	Amouth of microalgae used (mg)	Monolith weight (mg)
reference sample	0	4134.4
monolith_ma(170)	170	3865.5
monolith_ma(70)	70	2427.1
monolith_ma(40)	40	2721.2
monolith_ma(4)	4	2425.2
monolith_ma(2.7)	2.7	2423.9
monolith_ma(1)	1	2422.2

3. Characterization

3.1 Microalgae from Yahuarcocha lagoon

Once the sample was filtrated a part of it was used to perform Optical Microscopy, Optical Fluorescence Microscopy, UltraViolet-Visible Spectroscopy and Column Chromatography to characterize them in a basis of chlorophyll content.

3.1.1 Optical Microscopy

Optical microscopy was performed with a LEICA® DM4000 microscope to observe the morphology and size of the microalgae. The objective used was 10X for the analysis.

3.1.2 Fluorescence Optical Microscopy

Fluorescence is a molecular phenomenon where a substance absorbs light of a color and almost instantaneously emits light of another color, one of lower energy and thus longer wavelength. This process is denominated excitation and emission (Reichman, 2010).

Fluorescence microscopy is a great instrument to control cell physiology. Fluorescent parts of the sample fluoresce against a dark background with sufficient contrast to allow detection.

Fluorescence microscopy requires optical filters that have demanding spectral and physical characteristics. These performance requirements can vary greatly depending on the specific type of microscope and the specific application (Reichman, 2010). These filters are used to select and illuminate only the sample with a certain area of the visible spectrum (MicroPlanet, 2021). The main requirements for filters used in fluorescence detection systems are high shear slope, high transmittance, high positioning accuracy, high depth of cut, and excellent environmental stability (Sigaut and Pietrasanta, 2016). Selecting the proper filters is the key to making this microscopy work. Excitation filters allow only selected wavelengths from the light source to pass through the specimen's illumination path. Barrier filters or emission filters are designed to suppress or block (absorb) excitation wavelengths on their way to the detector. In the **Figure 2.2** is shown the components of a fluorescence microscope.

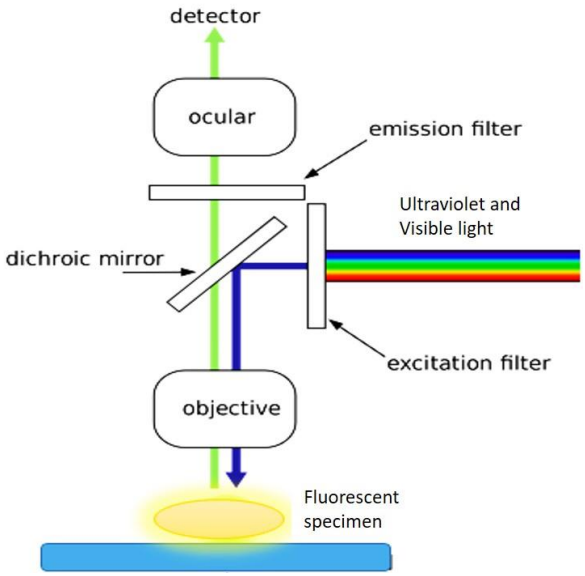

Figure 2.2 Representation of a sample under a fluoresce microscope.

Fluorescence is a good informative technique in photosynthesis study as probes of the inner workings of photosynthetic systems (Blankenship, 2014).

The Olympus® BX63 Motorized Upright microscope has high sensitivity fluorescence detection enables bright, high-contrast imaging with low exposure of cells to the excitation light. This microscope has effective detections of subtle fluorescence emissions even with weak excitation light deliver ideal performance in fluorescence imaging. Moreover, the objectives are made with superior low-autofluorescence glass, antireflection coats, and lens joining materials, improving the S/N ratio (Olympus, n.d.).

The LEICA® DM4000 microscope is ideal for the investigation of biologycal organisms. This equipment has an upright arrangement and comes equipped with light paths for both transmitted and fluorescence microscopy. It counts with a Contrast Manager that manage the methods of fluorescence contrast and the transmitted light. For fluorescence work, the microscope can be equipped with five filter cubes of a wide variety of fluorophores (Biocompare, 2005).

The cubes of filters for Fluorescence Microscopes used are cube of filters for DAPI, cube of filter for GREEN and cube of filter for yellow.

DAPI filter

When DAPI binds to double-stranded DNA, its absorption maximum is at a wavelength of 358 nm (ultraviolet) and its emission maximum is at 461 nm (blue).

Therefore, in fluorescence microscopy, DAPI is excited with ultraviolet light to later be detected through a blue / cyan filter. BP 365 FT 395 LP 397. Xenon, mercury arc lamp, or UV light can be used to excite DAPI during experiments (Surat, 2018). The excitation light of this filter is ultraviolet.

Chlorophyll fluorescence

One of the main photosynthetic pigments that exists into microalgae and cyanobacteria is the Chlorophyll-*a* (Guamán and González, 2016). Chlorophyll fluorescence represents a useful tool in microalgae biotechnology showing a fast evidence of photosynthetic activity of the culture and certain quantification on productivity (Millie et al., 2002).

The advantages of Chlorophyll (Chl) fluorescence to monitor the physiological status of microalgae mass cultures are its non-invasiveness, sensitivity and the wide availability of reliable instruments (Millie et al., 2002).

The fluorescence and photochemical processes that occur in chlorophyll molecules, take place in photosystem II (FSII), a system involved in the photosynthesis process. Chlorophyll molecules are in FSII with other compounds. The chlorophyll reaction center is known as P_{680}. It receives this name because the chlorophyll of this photosystem absorbs light with a wavelength of 680 nm, which is in the red zone of the spectrum (Garcia and Gutierrez, 2015).

A small amount of light energy absorbed by the chlorophyll molecules can be re-emitted as light in the form of fluorescence so that the excess energy does not damage the photosystems. The amount of energy emitted as fluorescence is very small, 1-2% of the total absorbed light (Hernández, 2013). In the **figure 2.3** is shown the fluorescence spectra for Chlorophyll-*a*.

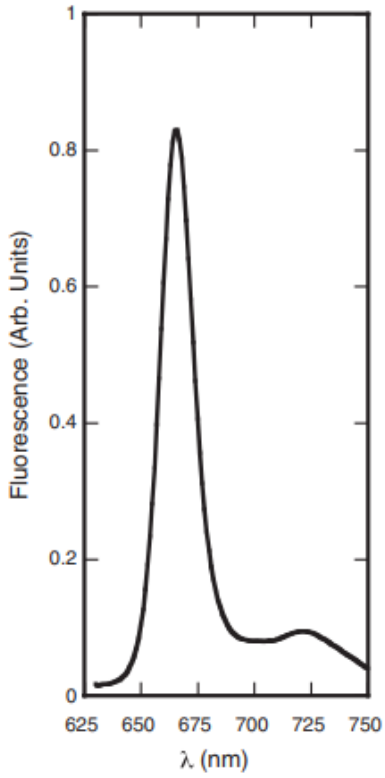

Figure 2.3 Fluorescence spectra of Chlorophyll-*a* (Blankenship, 2014)**.**

The determination of the emission of the fluorescence of chlorophyll, its analysis and the obtaining of the corresponding parameters, constitute a precise way to corroborate the photosynthetic activity of photoautotrophic organisms like microalgae. The auto-fluorescence of pigment granules (chlorophylls) (Zhang et al, 2016) found within microalgae was employed in the present work.

The analysis was performed with a LEICA® DM4000 microscope using a fluorescence lamp with a green filter for (Ex: 450-490 nm, DC: 510 nm, EM: LP 515 nm) and with an Olympus® BX63 Motorized Upright. The objective used for Leica microscope was 10X and for the Olympus the objectives were 4X, 10X, 20X and 40X.

3.1.3 UltraViolet-Visible Spectroscopy

UV-Vis spectroscopy is a cheap, flexible, non-destructive, analytical method that is suitable for analysis of the majority of compounds (Rocha et al, 2018). This technique is very used to different analysis as to derive liquid phase reaction kinetics, and to recognize the absorption mechanism at the molecular scale.

UV-Vis spectrophotometers measure the absorbance or transmittance of light passing through a medium as a function of the wavelength (Rocha et al., 2018). Their

principle is the transfer of electrons from low-energy to high-energy atomic or molecular orbitals when the material is irradiated with light (Weckhuysen, 2004).

UV-Vis spectrophotometers pass a light source through a sample and a detector on the opposite side records transmitted light. The resulting data have the baseline at the bottom with the peaks pointing upward and they report wavelength in nanometers (nm) on the x-axis and absorbance (A) on the y-axis (no units). The transmittance denotes how much light is absorbed at each wavelength and we are most interested in the highest peak-max (Rocha et al., 2018). The device to put the sample inside the equipment are cuvettes, the quartz cuvettes are more used because of their wavelength range and optical qualities.

Spectroscopy properties of chlorophylls

The chlorophylls all have two main absorption bands, one in the blue or near UV region and one in the red or near IR region (Blankenship, 2014). In the **Figure 2.4** is shown the chlorophyll a spectrum and their representative bands.

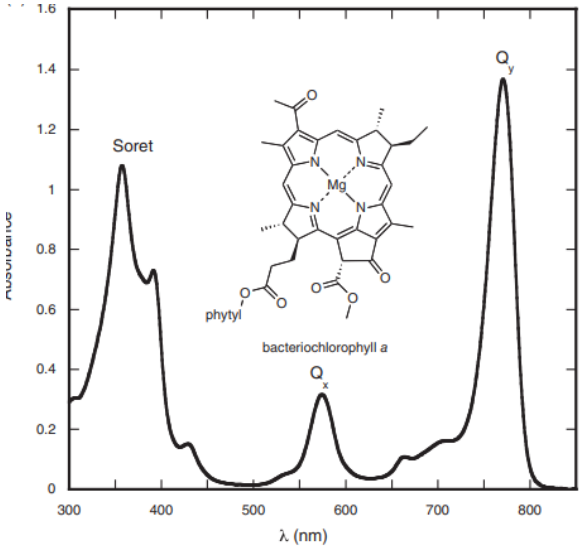

Figure 2.4 Spectrum of absorption of *Chlorophyll-a* (Blankenship, 2014).

The microalgae taken form Yahuarcocha lagoon as it was previously mentioned, were filtrated and put it again into distilled water to be analyzed by UV-Vis Spectroscopy using a quartz cuvette. The equipment to perform this measurements was the spectrophotometer Lambda 1050+UV/Vis/NIR from PerkinElmer®, from 800 nm to 300 nm.

3.1.4 Column Chromatography

Chromatography is an important technique that enables the separation, identification, and purification of the components of a mixture for qualitative and quantitative analysis based on color of the fractions obtained (Coskun, 2016) (Roge et al, 2011). Its principle is that molecules in mixture applied onto the surface or into the solid, and fluid stationary phase is separating from each other while moving with the aid of a mobile phase.

Depending on how the solvent flows down the column it has a name, when solvent is allowed to flow down the column by gravity it is a gravity column chromatography; and when solvent is forced down the column by positive air pressure, it is a flash chromatography (Roge et al., 2011).

Column chromatography have difference characteristic features as size, shape, net charge, stationary phase used, and binding capacity.

The components of this chromatography column are the stationary phase, composed of a solid phase or a layer of a liquid adsorbed on the surface a solid support, the mobile phase which is always composed of liquid or a gaseous component, and the separated compounds.

Traditionally, in the column chromatography a sample to be purified is placed on the top of a column containing some solid support, often silica gel. The rest of the column is after filled with a solvent or mixture of solvents which then runs through the solid support under the force of gravity. The components to be separated go through the column at different rates and then can be collected separately as they drop from the bottom of the column. The rate at which the solvent percolates through the column is slow (Roge et al., 2011).

The most used stationary phase is silica gel SiO_2 40 – 63 µm. The adsorbents are sold in different mesh sizes, as indicated by a number on the bottle label. To illustrate "silica gel 60" the number refers to the mesh of the sieve used to size the silica, the number of holes in the mesh or sieve through which the crude silica particle mixture is passed in the manufacturing process (Roge et al., 2011). Adsorbent particle size affects how the solvent flows through the column. Smaller particles, higher mesh values, are used for flash chromatography; while larger particles, lower mesh values, are used for gravity chromatography.

For n grams of sample, one should use 30 to 100 n grams of silica gel. For easier separations, ratios closer to 30: 1 are effective, for difficult separations more silica gel is needed (Roge et al., 2011).

Regarding solvent systems in a gravity column chromatography is usually carried out with a mixture of two solvents, with a polar and a nonpolar component (Roge et al., 2011). Occasionally, just one solvent can be use: Ether/Petroleum Ether, Ether/Hexane, and Ether/Pentane. The selection of hydrocarbon component depends of its availability.

Cocktail preparation

The cocktail used for the chromatography column was composed by 60 mL of n-hexane (99.9%), 16 m L of petroleum ether (analytical grade), 4 mL of methanol (>99%), 10 mL of acetone (>99%), and 10 mL of ethyl acetate (>99%). These reagents was purchased by Fischer Chemical, with the exception of the petroleum ether which is purchased by ABC Laboratories.

Column assembly

On a stand with the aid of a clamp was set a gravity liquid chromatography column (**Figure 2.5**). First mixed silica with the prepared cocktail was poured into the column. Then, it was added a mixture of 2 mL of acetone and 2.2 g of vacuum filtrated microalgae. The stationary phase (silica) lets to pass the components of interest (pigments) which were evaluated in the spectrophotometer. The fractions were collected and measured with the PerkinElmer® Lambda 1050+UV/Vis/NIR Spectrophotometer.

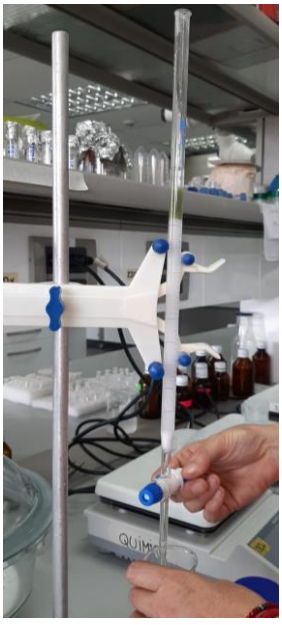

Figure 2.5 Chromatography column performed in laboratory.

3.2 Hybrids silica monolith/microalgae

The solid samples were characterized by Diffuse Reflectance Ultra Violet Spectroscopy and Fluorescence Microscopy.

3.2.1 Diffuse Reflectance Ultraviolet-Visible Spectroscopy

UV-Vis diffuse reflectance spectroscopy is a variant that measures the properties of solids and powders (Rocha et al., 2018). In Diffuse Reflectance Ultraviolet-Visible Spectroscopy (DRS) a solid is placed inside the accessory Praying Mantis allows adding multiple reflections **Figure (2.6)**. The reflected beam intensity, R, is then compared with a reference that gives $R_\infty = \frac{R_{sample}}{R_{standard}}$, which is an approximation of an infinitely thick sample (Rocha et al., 2018).

The measurements obtained with the spectrometer are transmittance, and absorbance. The ratio of the intensity of light entering the sample (I_0) to that which is transmitted, not absorbed, is defined as the transmittance and is given by $T = \frac{I_t}{I_0}$.This value is normally represented as a percentage (% T) by multiplying this fraction by 100 (Upstone, 2006). The transmittance value of a sample is not particularly useful, as there is not a straightforward relationship between sample concentration and transmittance.

Absorbance is given by $A = -10log_{10}T$. Absorbance is far more useful, as there is now a direct correlation between concentration and absorbance. If the concentration is doubled, the absorbance will also similarly double. This is defined by the Beer–Lambert law $A = \varepsilon cl$ l where ε is the extinction coefficient for the analyte at a specific wavelength, c is the sample concentration, and l is the path length of the cuvette (Upstone, 2006).

This technique was utilized in order to confirm the effectiveness of microalgae encapsulation within the silica matrix. The hybrid monoliths were pulverized to carry on the analysis. They were measured using a Praying Mantis module in a PerkinElmer® Lambda 1050+UV/Vis/NIR Spectrophotometer (**Figure 2.6**), in a wavelength range of 800 nm to 400 nm. For comparison and to prove that the spectrum changes when no microalgae is present in the monolith, the same measurement was made for the reference sample.

Figure 2.6 Accessory used for DRS.

3.2.2 Fluorescence Optical Microscopy

The Olympus® BX63 Motorized Upright Microscope allows to observe autofluorescence of living microalgae and verify the evolution or not, as well as the preservation of the photosynthetic pigments. All samples were prepared before the analysis by pulverizing around 0.5 g of the hybrid monolith and placed on glass slides. Images were seen with 4X and 20X objective lenses using an excitation blue filter (450 to 490 nm), which allows the green and red emissions. The samples were also analyzed with the Leica microscope using a green filter previously described. The objectives to see the samples were at 4X and 10X.

4. Adsorption of Copper and Nickel ions by the hybrid monolith and microalgae

4.1 Calibration Curves

For the calibration curve were prepare a battery of five copper and nickel solution of 3000, 1500, 1000, 500, and 1 ppm of each metal. Then, the samples were measured in a wavelength of 830 nm to 350 nm. For the nickel identification the main wavelength considered was 394 nm and for copper identification was 810 nm. The Lambda 1050+UV/Vis/NIR Spectrophotometer from PerkinElmer®.

4.2 Kinetics

The adsorption of metals was carried by duplicate, at room temperature, and under agitation during 48 hours at 140 rpm. 2 mL aliquots were collected at 5, 20, 40, 60, 180, 420, 1440 and 2880 minutes. The metal concentration was measured by UV-Vis with a Lambda 1050+UV/Vis/NIR Spectrophotometer from PerkinElmer®, the wavelength range used was the same of calibration curves.

Chapter 3: Results and Discussion

The samples collected from Yahuarcocha's lagoon, denominated in the present work as MAYL01, were characterized by optical and optical fluorescence microscopy, by UV-Vis spectroscopy and by column chromatography are presented in next section. As it was already mentioned before, these techniques can give relevant information about the chlorophyll's and other photosynthetic pigments behavior. They also help to identify (not in a deep way because taxonomy was not performed) these microorganisms.

3.1 Microalgae identification

3.1.1 Optical and Fluorescence Microscopy

3.1.1.1 Optical Microscopy

In the **Figure 3.1** shown the optical microspopy imagen of the sample MAYL01 obtained with the LEICA® DM4000.

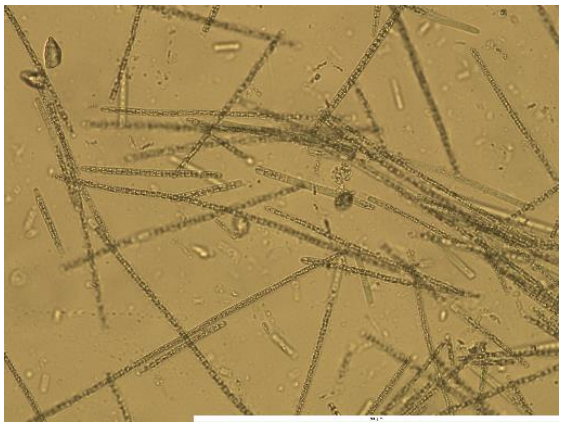

Figure 3.1 Fluorence microscopy image of sample MAYL01 using 10x magnification.

In the **Figure 3.1** are observed elongated microorganisms forming chains-type of different sizes between 20 and 100 µm. They can be recognised as cyanobacteria according to the literature (Dufresne, n.d.; National Museum of Nature and Science, 2011; Kaštovský, n.d.). The most comparable species are shown in the **Figure 3.2.**

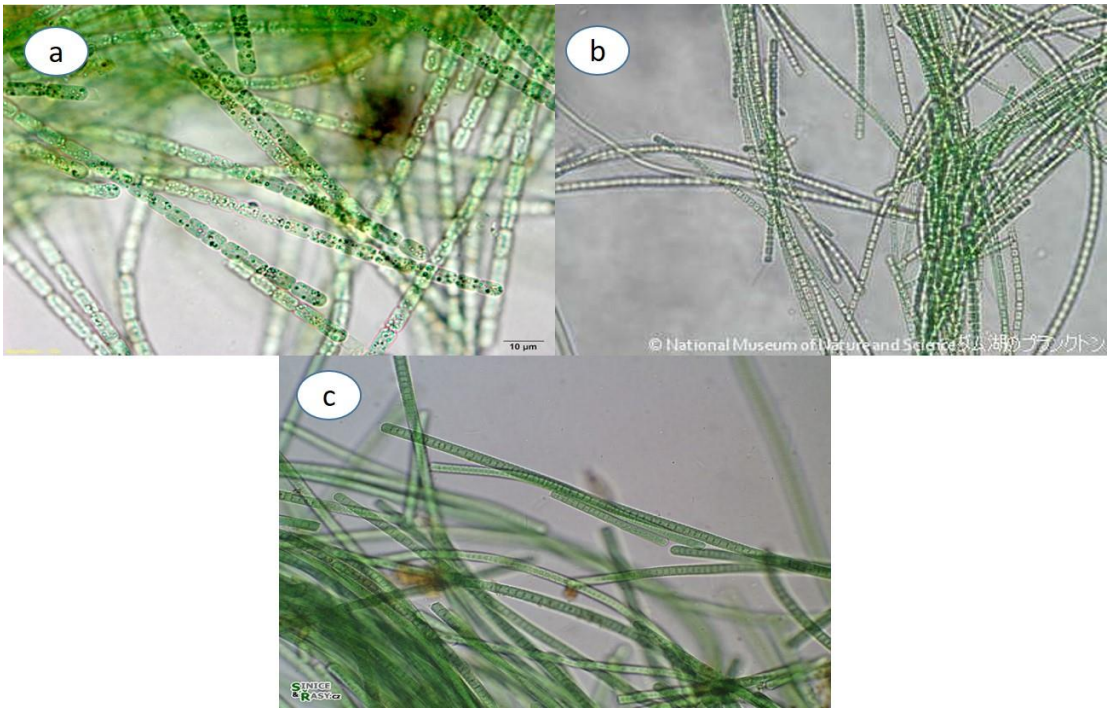

Figure 3.2 Microalgae imagens found in literature: a) *Cylindrospermum* sp. (Dufresne, n.d.). ; b) *Pseudanabaena sp.* (National Museum of Nature and Science , 2011); and c) *Phormidium sp* (Kaštovský, n.d.).

The three images show a lot of similarity with the sample taken from Yahuacocha's lagoon shown in **Figure 3.1**. The *Cylindrospermum* sp., a *Pseudanabaena sp, and Phormiium sp* belong to the *Cyanophyta* specie that is another name of the Cyanobacteria that belongs to the microalgae. *Cyanobacteria* are known also as blue-green microalgae. This microalgae have floatage due to their gas vesicles, and live forming floating blooms near the water's surface. Moreover, these species have *Chlorophyll-a* as a photosynthetic pigment. One can atribuite the collected sample as belonging to microalgae division, and allow to continue the work based on the propierties of these organisms.

3.1.1.2 Fluorescence Microscopy

Due to difficulties with the equipment the following **Figure 3.3** was taken with another device directly from the microscope ocular.

Figure 3.3 Fluorence microscopy image of sample MAYL01 (10x).

The **Figure 3.3** shows great fluoresce of the sample, this is atribuited to the presence of Chlorophylls that upon excitacion (450-490 nm) emits fluorescence in the red region at 660 nm. This fact evidence the presence of microalgae because they have Chlorphylls as photosynthetic pigments (Hernández, 2013).

It is also known that if one excite the electrons of the chlorophyll molecules with the black light (ultraviolet light), with no electron transport they release their energy in the form of red light while returning to their ground state. In **Figure 3.4** it can be appreciated this phenomena.

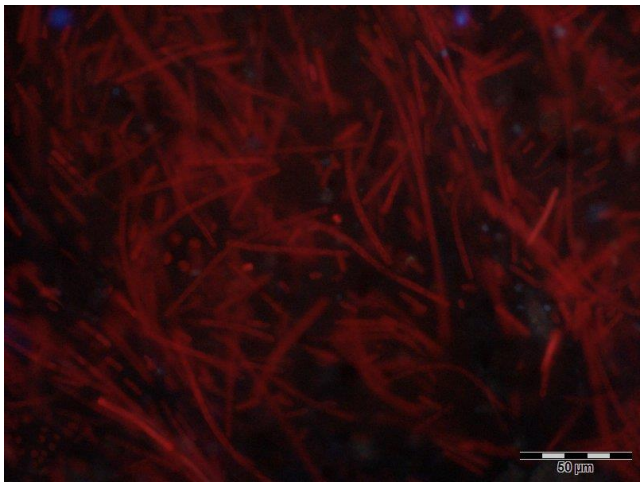

Figure 3.4 Fluoresce microscopy image of sample MAYL01 (4x).

3.1.2 UV-Vis spectroscopy

Microalgae species have *Chlorophyll a* as photosynthetic pigment, the absorption wavelengths of this are specified in the **Table 1.1**. These wavelengths vary according to the solvent used to perform the measurements. The sample MAYL01 presents an absorption band at 680 nm and 440 nm as is shown in the **Figure 3.5**. The peak of Chlorophyll-*a* at 680 nm is the common peak observed when algal suspension is studied. This fact confirms the presence of Chlorophyll-*a*, supporting that microalgae is present in the measured sample. This bathochromic shift usually happens due to the Chlorophyll is not extracted but measured in vivo (Rowan, 1989) as a suspension consisting of microalgal cells grown in water which is considered a polar solvent; this causes the shift to the red region in comparison with values of the extracted chlorophyll from microalgae between 660-665 nm (Blankenship, 2014; Ishii et al., 1995; Manna et al., 2009).

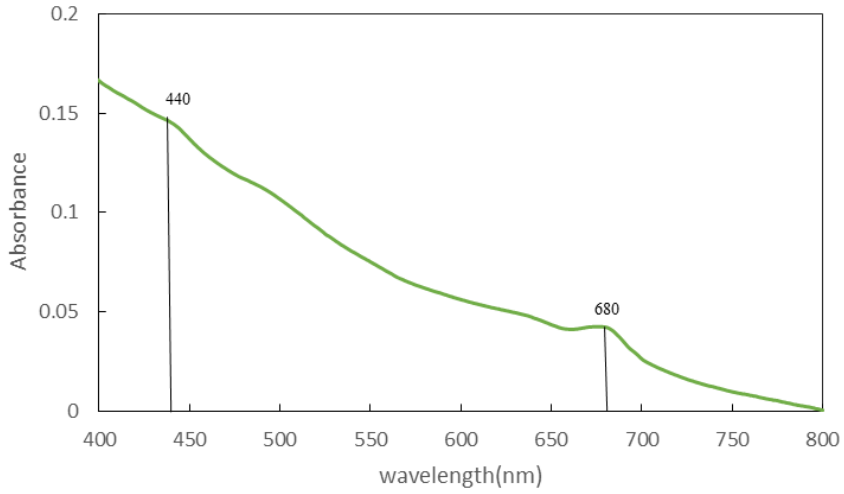

Figure 3.5 UV-Vs absorption spectra of sample recollected in Yahuarcocha lagoon.

As comparison, the spectrum of *Chlorella vulgaris* is reported in **Figure 3.6** and it is evidenced the presence of photosynthetic pigments and similar composition and behavior with the microalgae MAYL01.

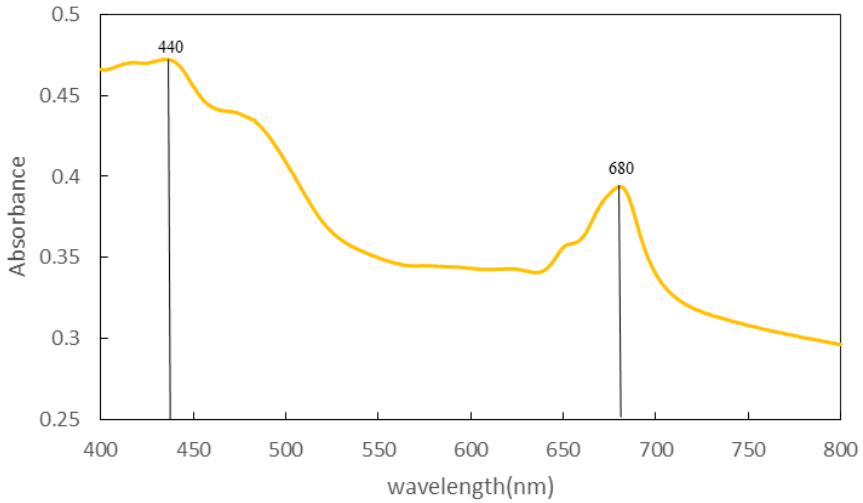

Figure 3.6 UV-Vs absorption spectra of *Chlorella vulgaris.*

3.1.3 Chromatographic column

From the chromatographic column were obtained four fractions, the less polar compounds are obtained sooner than the ones with higher polarity. The first compound obtained is β- carotene, whose three peaks are about 455 nm and its characteristic color is orange. The second fraction obtained was the green pigment which is characteristic of Chlorophyll, the absorption bands of this fraction were 660 nm and 430 nm. This fraction is attributed to a mix of Chlorophyll-*a* and -*d* Chlorophyll *b* is more polar than Chlorophyl-*a*. The third fraction collected have its peak near to 400 nm and have another at 693 nm, these peaks are characteristic of Pheophytins, in this case pheophytin of Chlorophylls. The pheophytin is a derivatives compound from Chlorophyll and it lacks of the central Mg^{2+} ion. The absorption spectra of the described fractions is shown in the **Figure 3.7**.The last fraction achieved was the one belonging to the Xanthophylls, these photosynthetic pigment is the most polar among the photosynthetic pigments. The associated wavelengths were between 450 and 500 nm and another peak at ~670 nm.

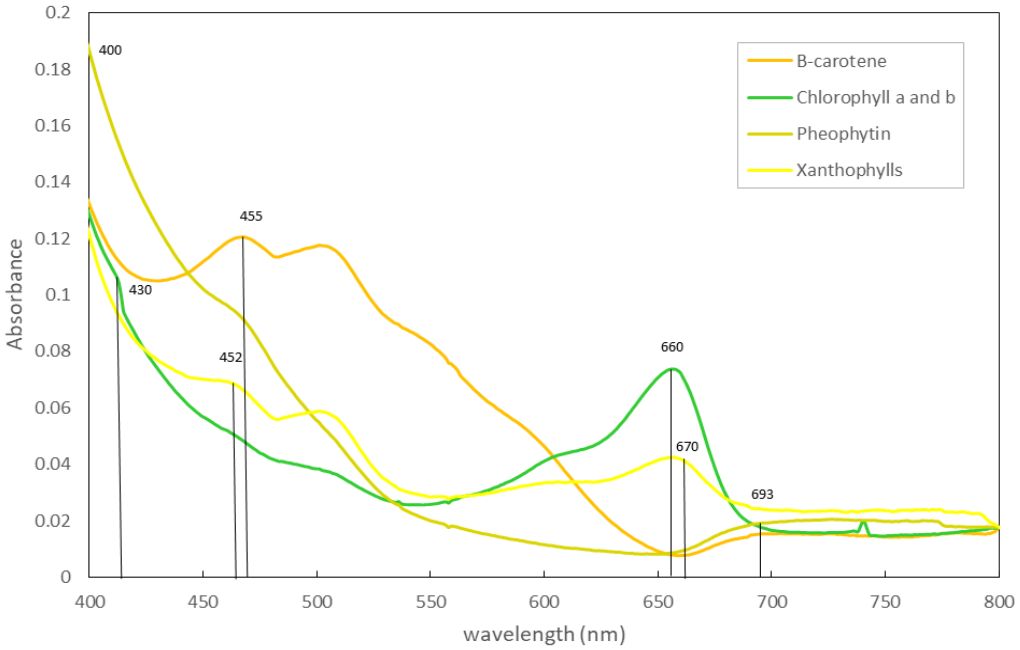

Figure 3.7 UV-Vs absorption spectra of chromatographic column fractions.

In comparison with others works, the spectra shown by Valdez (2019) and Pocock (n.d.) match with the **Figure 3.7** regarding the peaks obtained for Chlorphyll-*a* at 660nm. In the work of Sommer (2014) is shown that the Chlorophyll-*a* in acetone absorbs at about 660 nm, the same solvent was used for the present work. The same fractions are evidenced in another work of Sommer (2013) from spinaches. The obtained spectra of the fractions obtained by chromatography are very similar to the obtained in the present work. It is important to mention that the concentration of photosynthetic pigments decrease along time as show Martínez (2010) where the concentration of Chlorophyll-*a* and -*b* decrease to about the half of the initial concentration in two days, This fact could be manifested in the samples because they were not measured immediately and may be the reason that the peak of Chlorophyll-*b* is not well differentiated. Martinez (2010) obtained the same photosynthetic pigments in the study of microalgae. With these studies is verified the existence of microalgae in the samples studied in the present work.

Thanks to the previous characterization of fresh microalgae MAYL01, a comparison can be performed between the encapsulated ones into the hierarchical silica matrix and monitoring them upon the time.

3.2 Hybrids silica monolith/microalgae

3.2.1 Diffuse Reflectance UV-Vis Spectroscopy

After 3 months of synthesis DRS-UV-Vis technique was performed with the hybrid monoliths in order to determine the resistance of microalgae into the silica monolith. The monoliths measured had 170 mg, 70 mg and 40 mg of microalgae distributed all along the monolith.

In Figure 3.7 are shown the reflectance spectra transformed to Kubelka-Munk units of the samples monolith_ma(170), monolith_ma(70) and monolith_ma(40) .. There is a mixture of the whole pigments found by column chromatography for the Yahuarchocha's samples (MAYL01) that appears in the spectra. The peaks at 660 nm and 430 nm are belonging to Chlorophylls. The peak at 455 nm belong to Carotenenes (β-carotene). The peaks at 400 nm and 693 nm are characteristic of Pheophytins. The peaks at 452 nm and at 670 nm belong to the Xanthophylls. The reference sample (the one without any microalgae) shows no peaks that interfere with the lecture in the selected region (visible region from solar spectrum). That means that the sample reflects the majority of light because of its white color. It is shown, in a qualitative way, that the intensity of "absorption" bands is proportional to the quantity of loaded microalgae (the same quantity of sample was used to perform the measurements). The sample monolith_ma(170), with the highest content of microalgae showed more predominant "absorption" bands than the ones with less amount of microalgae. Followed by the sample monolith_ma(70) and finally by the sample monolith_ma(40).

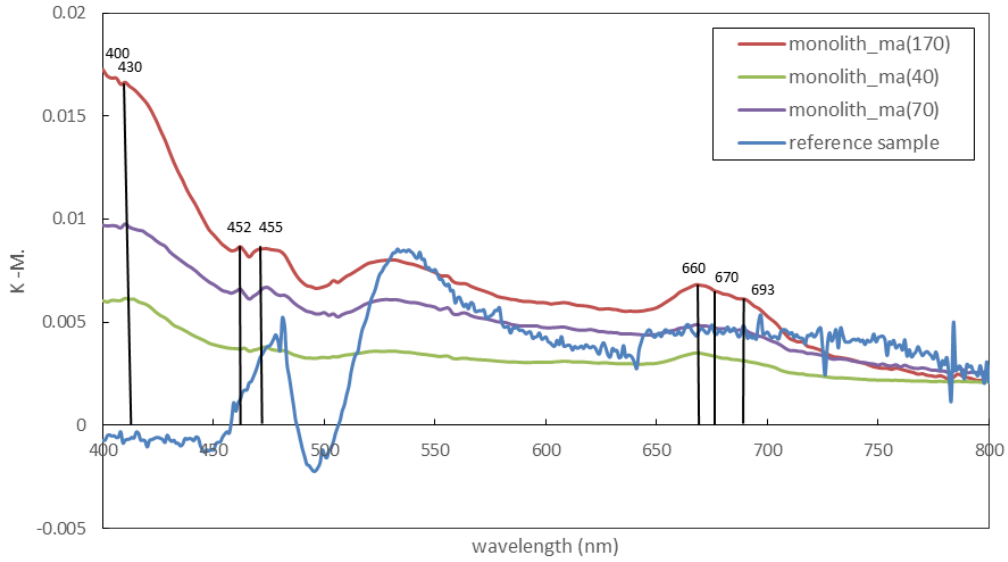

Figure 3.8 DRS UV-Vis spectra of monoliths monolith_ma(170), monolith_ma(70) and monolith_ma(40) and reference sample, after 3 months of synthesis.

These spectra shows the resistance of the microalgae into the silica monolith (at least until the time in which they were taken) and the probably opportunity to use them in metal sorption after months of synthesis. Additionally, it should be noted that the samples were kept without any extra care or feeding. All the samples were kept in a little plastic flask with tiny perforated aluminum foil for covering them. These results are very similar to the ones obtained by (López N., 2020) for *Chlorella vulgaris*. In that work the microorganisms were growth under laboratory controlled conditions and they conclude that they can also growth within the hierarchical silica matrix made by HIPE when low quantity of microalgae is encapsulated. These is also supported by Rooke et al. (2008). They also concluded that Chlorophyll degradation also occurs faster when more microalgae is present.

Hybrid samples with much less quantity of microalgae were synthetized as it was said before, monolith_ma(4), monolith_ma(2.7) and monolith_ma(1) with 4, 2.7 and 1 mg, respectively in order to see if they could growth faster. However, all microalgae was precipitated to the bottom of the monolith during the aging time. The DRS-UV-Vis spectra of these samples after one week of synthesis are presented in **Figure 3.9**. Their behavior is very similar to the reference sample and this is due to microalgae was not well

distributed all along the monolith. Besides, the part with the microalgae (bottom monolith) was used to perform the metal sorption.

The peak at 660 nm attributed to chlorophyll, pigment which is in higher quantity than the others according to literature (Blankenship, 2014; Ruiz et al., 2019; Garcia and Gutierrez, 2015; Hernández, 2013; Rowan, 1989; Ishii et al., 1995; Manna et al., 2009) can slightly be seen in the monolith_ma(4mg) sample. This fact validates again that the high amount of microalgae means major concentration of *Chlorophylls* and it is represented by differentiated peaks at the spectrum.

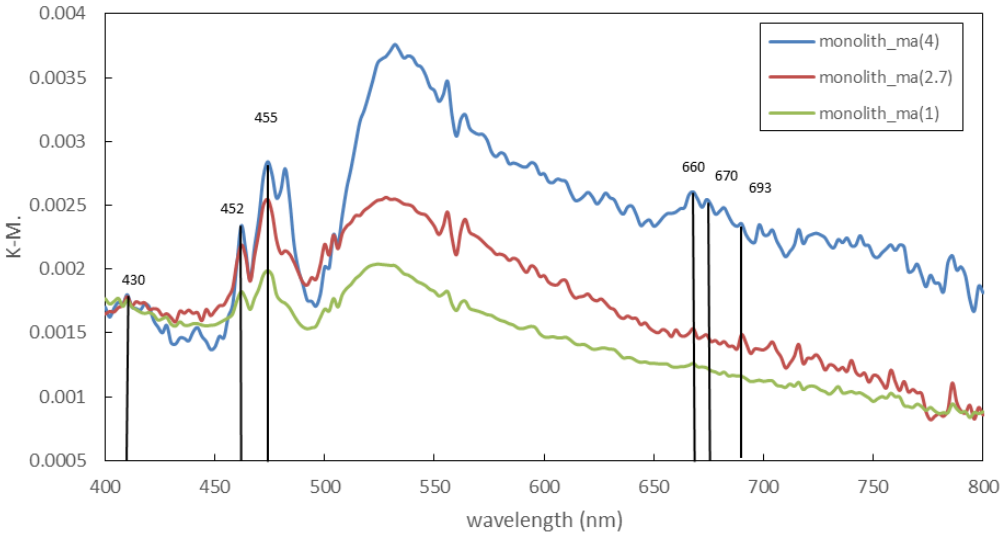

Figure 3.9 DRS UV-Vis of the samples monolith_ma(4), monolith_ma(2.7) and monolith_ma(1) after one week of synthesis.

The spectra of samples with 4 mg, 2.7 mg and 1 mg of microalgae are very similar to the spectra of the reference sample, the difference is due to the peaks of Chlorophylls are less notable than in the samples with great amount of microalgae (170 mg, 70 mg and 40 mg) shown in the **Figure 3.8.** This description confirms that the concentration of *Chlorophylls* is proportional with the amount of microalgae existing.

3.2.2 Fluorescence microscopy

In order to qualitatively evaluate the behavior of the photosynthetic pigments of the microalgae encapsulated into the silica monolith, samples of the hybrid monoliths were observed by fluorescence microscopy. Remembering the background a little it was highlighted that fluorescence is a useful and very important informative technique in photosynthesis research (Blankeship, 2014). Fluorescence measurements are helpful as

inquiry information of the inner workings of photosynthetic systems (Blankeship, 2014; Garcia, 2015, Millie et al., 2002). In the **Figure 3.10** fluorescence images taken at different time from monolith_ma(170), monolith_ma(70) and monolith_(40) are shown.

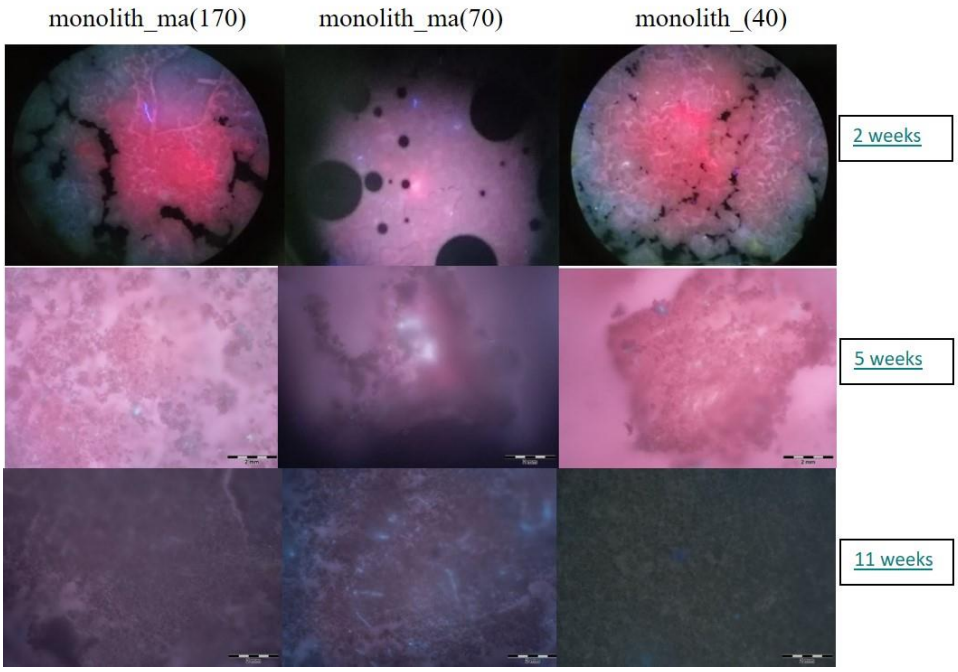

Figure 3.10 Fluorescence microcopy of monoliths with 170 mg (left), 70 mg (center), and 40 mg (right) with an objective of 20X and DAPI Filter.

Figure 3.10 highlights the preservation of the encapsulated microalgae through the time because of the natural red fluorescence appreciated once excited with a DAPI filter (UV region). The fluorescence emission stills remains strong after 5 weeks of their synthesis for the three samples. However after 11 weeks of synthesis they started to lose their photoactivity being more noticeable for the monolith_ma(40). This can be attributed to the intensity of light which can cause that fluorescence do not remain longer during the measurements which can be seen in the light dependency of various photosynthetic parameters (Blankeship, 2014; Grigoryeva, 2020). In addition, not only light quantity, but also light quality (wavelength) is an important factor (Papageorgiou, 2011). Also can be attributed to microalgae death.

Before it was pointed out that when a large amount of microalgae is loaded to the monolith they can degrade faster if there is no more place to growth. It seems that is neccesary a certain amount of microorganisms to allow this to happen, i.e. it is necessary not too much microalgae but also not too low quantity. Therefore that is why when just 40 mg were used no longer florescence can be appreciated. However to study the

photosynthetic cells either in microorganisms or higher plants, some additional specific techniques are needed to carry out measurements and to perform data processing (Grigoryeva, 2018; Grigoryeva, 2020). One of the principal problems of *in vivo* measurements is to protect the object under consideration from light and heat damage. UV light used in this experiment and the intervals between the illuminations could be an important factor to influence photodamage and saturation of photosynthesis (Grigoryeva, 2020).

To compare the energy of used light in fluorescence response of the samples and the highest and lowest microalgae loaded, fluorescence microscopy was performed after 11 weeks but with a green filter (excitation: 450-490 nm). The images are found in **Figure 3.11**.

monolith_ma(170) monolith_ma(40)

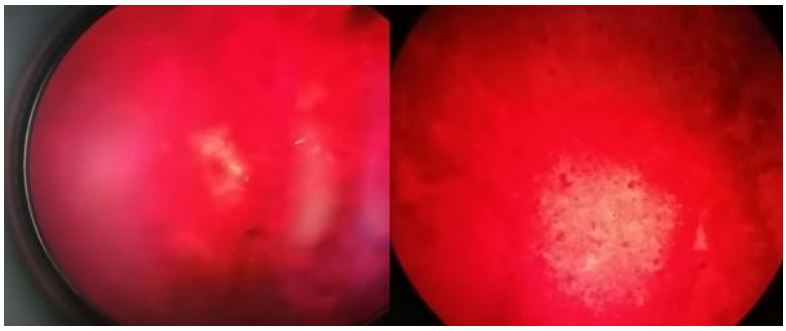

Figure 3.11 Fluorescence microcopy of monolith_ma(170) and monolith_ma(40) after eleven weeks of synthesis (20X).

The previous images confirm that fluorescence response is very dependent on the energy of the used light. A further explanation is given by Blankeship (2014) where he says that through many studies, researchers concluded that “Photosystem II exhibits a phenomenon known as variable fluorescence, in which the intensity of fluorescence reports on the properties of the reaction center complex and associated electron transport chain in a remarkably detailed way”. In this case for both monoliths fluorescence intensity is clearly observed in equality. The quantity of loaded microorganism for this region of excitation has no influence.

The same samples were observed after 15 weeks of synthesis under a green (excitation: 450-490 nm) filter and a yellow filter (excitation: 515-561 nm) in a Leica microscope, **Figure 3.12**.

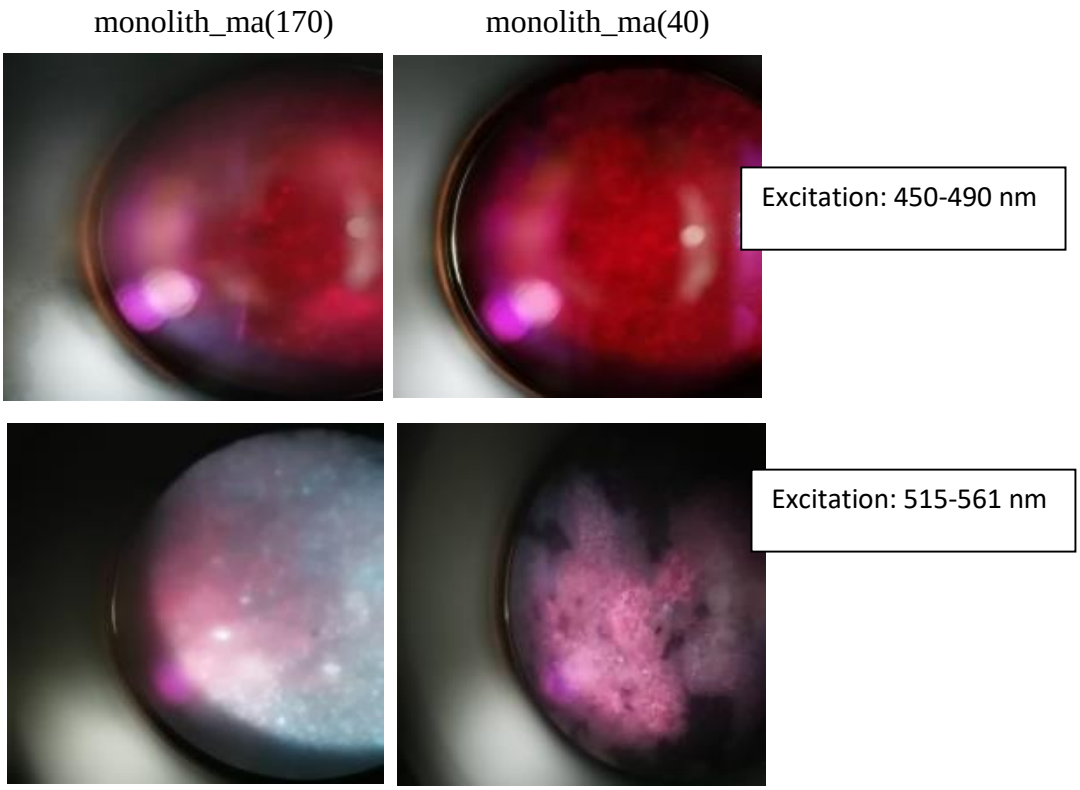

Figure 3.12 Fluorescence microcopy of monoliths with 170 mg (left), and 40 mg (right) after fifteen weeks of synthesis (10X).

Once more it is demonstrated that 1) the diversity in fluorescence responses on different excitation wavelength in cyanobacterial cells (Grigoryeva, 2020) and 2) microorganisms still alive after 15 weeks of encapsulation, therefore the encapsulation procedure is very suitable. This fact is a promising opportunity to use the monoliths in the objectives of the present work that is the heavy metal removal.

3.3 Sorption of Copper and Nickel ions by the hybrid monoliths and microalgae

In order to determinate the amount of metal M^{2+} (Cu or Ni) adsorbed by the synthetized hybrid monoliths and by the microalgae collected from Yahuarcocha's lagoon a kinetic and a thermodynamic experiment was carried out. The sorption data obtained from the linear regression of calibration curve was used to determinate the metal concentration (See Appendix B).

For Copper the resulting parameters from the linear regression of the calibration curve are (See Appendix C):

Slope	0.00005
y-intercept	0.0072
r^2	0.9918

For Nickel solution the parameters of linear regression of the calibration curve are (See Appendix C):

Slope	0.00002
y-intercept	0.0036
r^2	0.9909

The concentration figures shown in this section was performed using the average values of the metal concentration obtained from the duplicated experiment mentioned in the Section 4.2.

3.3.1 Sorption of Copper and Nickel ions by microalgae

3.3.1.1 Sorption of Copper ions by microalgae

The volume of the used metal solution was kept at 50 mL. The concentration was 3000 ppm of Copper (II) nitrate trihydrate solution. In the **Table 3.1** is detailed the amount of algae used for the kinetical and thermodynamic experiment performed.

Table 3.1 Amount of microalgae used for Copper sorption and percentage of removal.

Amount of microalgae (mg)	Amount of Cu sorbed from initial solution (%)
4	28.7
2.7	23.5
1	23.6

In the **Figure 3.13** it is shown the change in Copper concentration through the time, from 0 to 60 minutes at constant agitation and at room temperature. On the top right of the figure, the whole kinetic (from 0 to 48 h) is showed.

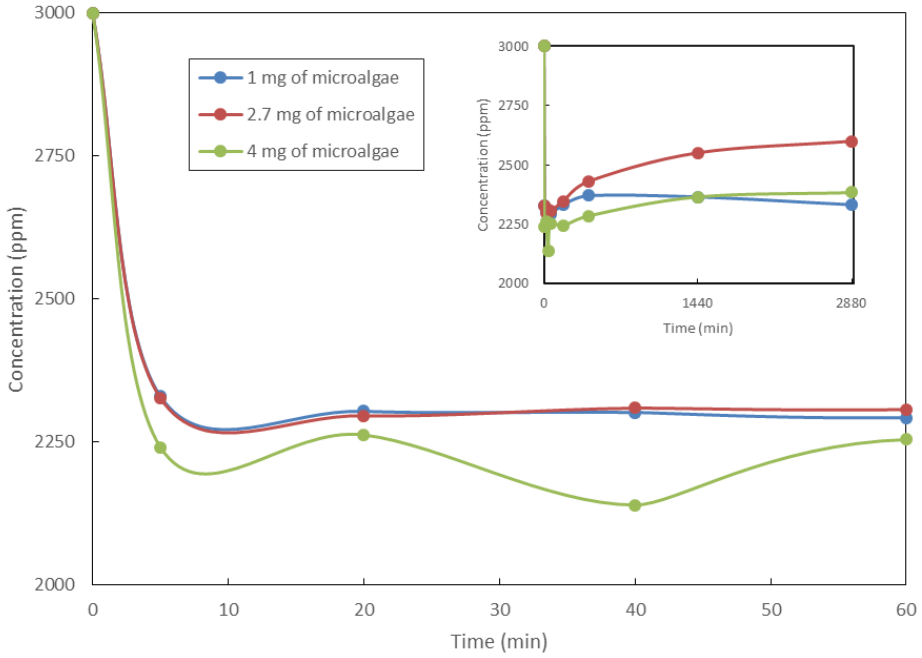

Figure 3.13 Copper concentration through 1 and 48 hours using microalgae.

The kinetics of MAYL(4*)* show that the major amount of microalgae can sorb the greatest quantity of Copper, reducing its concentration on a 28.7%. The sample MAYL(2.7) and the MAYL(1) have a very similar behavior. The major removal in the three samples is obtained before the 10 minutes. After 10 minutes the concentration in the three cases increases, this can be due to the osmotic shock caused by the metal to the algae membrane cell (Meunier, 2010) therefore the metal ions were released again to the solution. For the samples where 4 mg and 2.7 mg of algae were used, the copper concentration increased after 24 hours and then equilibrium was reached.

3.3.1.2 Sorption of Nickel ions by microalgae

For the Nickel sorption, in **Table 3.2** is the information about the quantity of microalgae used into the 50 mL of 3000 ppm Nickel chloride hexahydrate solution and the amount of Nickel sorbed.

Table 3.2 Amount of microalgae used for Nickel sorption and the percentage of metal removal.

Amount of microalgae (mg)	Amount of Ni absorbed from initial solution (%)
4	27.6
2.7	25.4
1	25.0

In the **Figure 3.14** it is shown the variation of Nickel concentration of the samples through 1 and 48 hours at constant agitation.

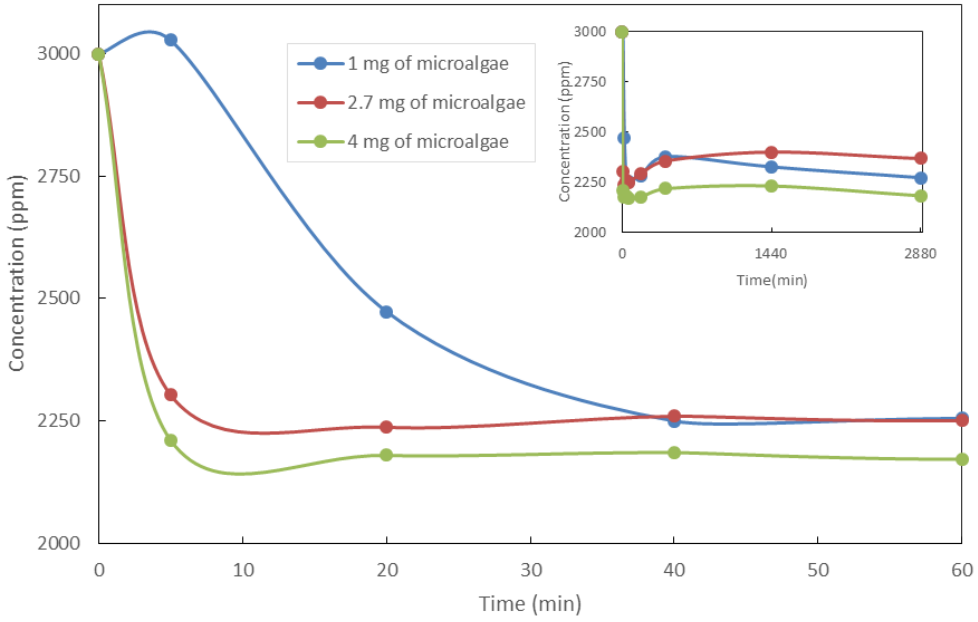

Figure 3.14 Nickel concentration through 1 and 48 hours using microalgae.

As the same as before, the major amount of microalgae sorbs the greatest quantity of Nickel from the initial solution, reducing its concentration about 27.6%. The highest removal represented as the less Nickel concentration is obtained before the 10 minutes of the experiment. After 40 minutes, the Ni concentration in the three cases increase, similar to copper ions. After 24 hours it is observed that the microalgae do not adsorbed more metal, keeping a less variable concentration until 48 hours.

3.3.2 Sorption of Copper and Nickel ions by hybrid monoliths

10 DAYS OLD HYBRID MONOLITHS WITH HIGHER QUANTITY OF MICROALGAE

The amount of 112 mg of hybrid monolith was used in the kinetic and thermodynamic experiment, with 50 mL of 3000 ppm Copper (II) nitrate trihydrate solution and 3000 ppm of Nickel chloride hexahydrate solution. In **Table 3.3** is the information of the amount of algae into the monoliths used on the experiment and the percentage of metal removed. The monoliths reported in the **Table 3.3** were 3 months old.

Table 3.3 Percentage of copper and nickel removed by hybrid monolith according the microalgae mass during one hour of the performed experiment.

Metal for removal	Monolith	Amount of microalgae(mg)	Amount of metal sorbed from initial solution (%)
Copper	reference sample	0	0.7
	monolith_ma(5)	5	32.4
	monolith_ma(2.83)	2.83	32.0
	monolith_ma(1.65)	1.65	29.7
Nickel	reference sample	0	4.2
	monolith_ma(5)	5	31.4
	monolith_ma(2.83)	2.83	30.8
	monolith_ma(1.65)	1.65	27.2

In the **Figure 3.15** is shown the decrease of Copper ions in the evaluated solutions using the hybrid monoliths with different amount of microalgae.

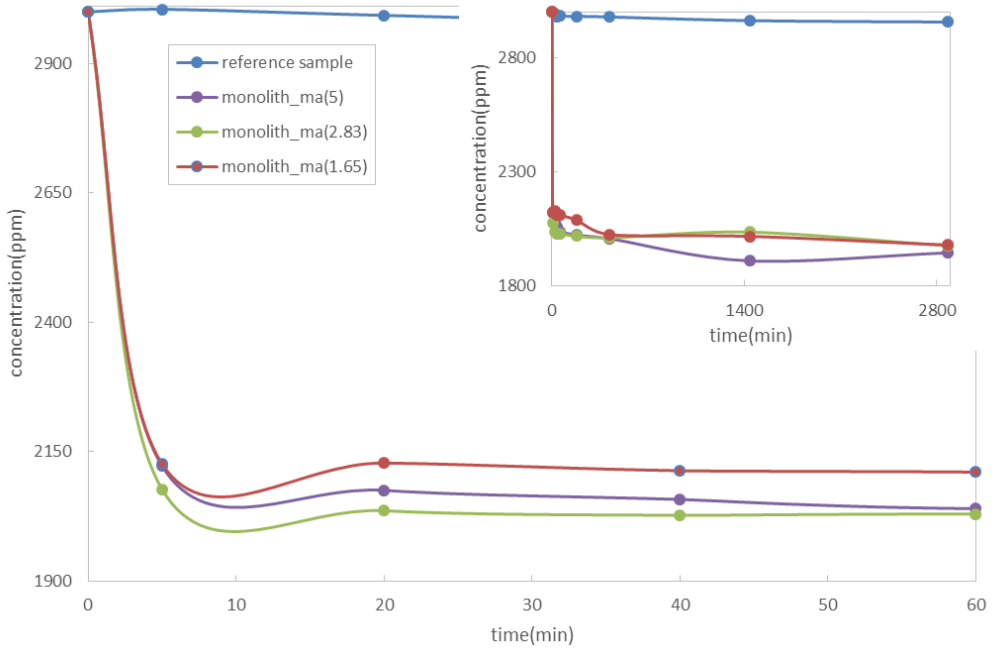

Figure 3.15 Concentration of Copper through 1 and 48 hours of experiment using monolith_ma(5), monolith_ma(2.83) and monolith_ma(1.65) and reference sample.

In the **Figure 3.16** is shown the Nickel concentration through time using hybrid samples: monolith_ma(5), monolith_ma(2.83) and monolith_ma(1.65), and the reference sample (without microalgae).

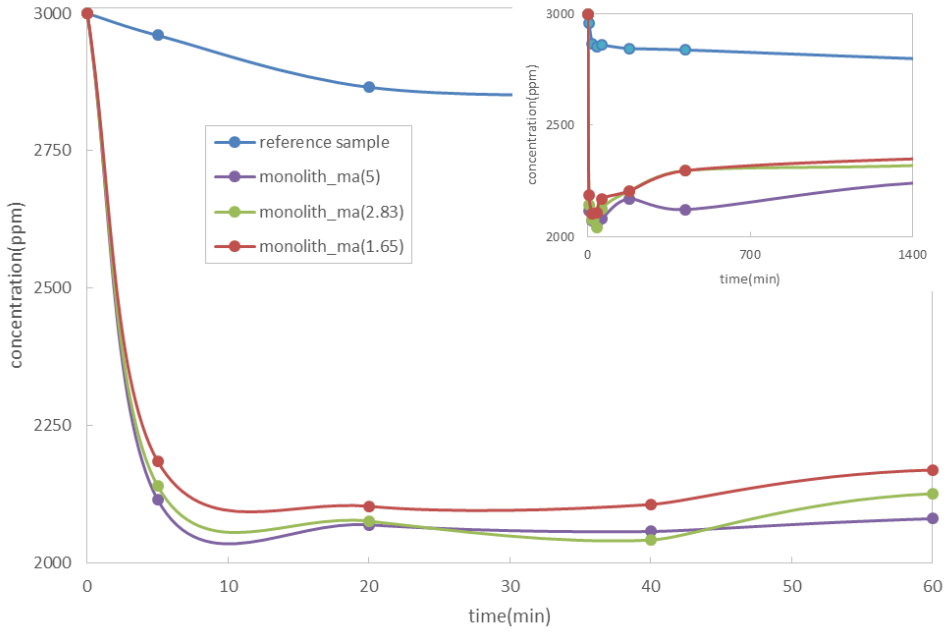

Figure 3.16 Concentration of Nickel through 1 and 48 hours of experiment using monolith_ma(5), monolith_ma(2.83) and monolith_ma(1.65) and reference sample.

It is demonstrated that the Copper has been removed (32.4%) slightly more than Nickel (31.4%). This fact is because of the affinity of microalgae to the Cu (II) ion that is highest than with Nickel (de-Bashan and Bashan, 2010) (Reyes et al., 2009).

10 DAYS OLD HYBRID MONOLITHS WITH LOWER QUANTITY OF MICROALGAE

The kinetic and thermodynamic experiment was also performed with monoliths after a week of having been synthesized, also 112 mg of hybrid monolith was used. In the **Table 3.4** is detailed the amount of microalgae into the used monoliths for removal of Nickel and Copper.

Table 3.4 Percentage of copper and nickel removed by hybrid monoliths according the algae mass during one hour of the performed experiment.

Metal for removal	Monolith	Amount of algae (mg)	Amount of metal sorbed from initial solution (%)
Copper	reference sample	0	0.7
	monolith_ma(4)	4	22.0
	monolith_ma(2.7)	2.7	22.4
	monolith_ma(1)	1	19.7
Nickel	reference sample	0	5.0
	monolith_ma(4)	4	29.8
	monolith_ma(2.7)	2.7	29.2
	monolith_ma(1)	1	25.5

In the **Figure 3.17** is shown the decrease of Copper ions in the evaluated solutions using the hybrid monoliths with different amount of microalgae.

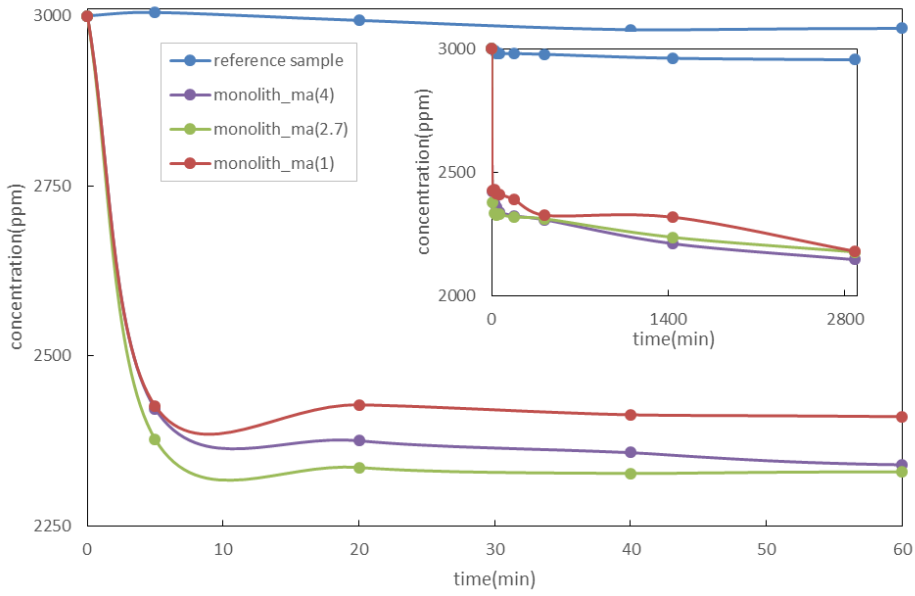

Figure 3.17 Concentration of Copper through 1 and 48 hours of experiment using monolith_ma(4), monolith_ma(2.7) and monolith_ma(1) and reference sample.

The removal using the reference sample, which does not have microalgae, is very low with 0.7 % from the initial concentration. In the cases where hybrid monoliths are used, the main decrease of metal concentration was given before the 10 minutes. The increase on concentration is lower than in the monoliths synthetized 3 months ago. The sample with the major amount of microalgae, monolith_ma(4), is which adsorbed the highest amount of metal ion. In contrast, the monolith with 1 mg of microalgae absorbed less amount of Copper

In **Figure 3.18** is shown the Nickel concentration through time using hybrid samples: monolith_ma(4), monolith_ma(2.7) and monolith_ma(1) and the reference sample.

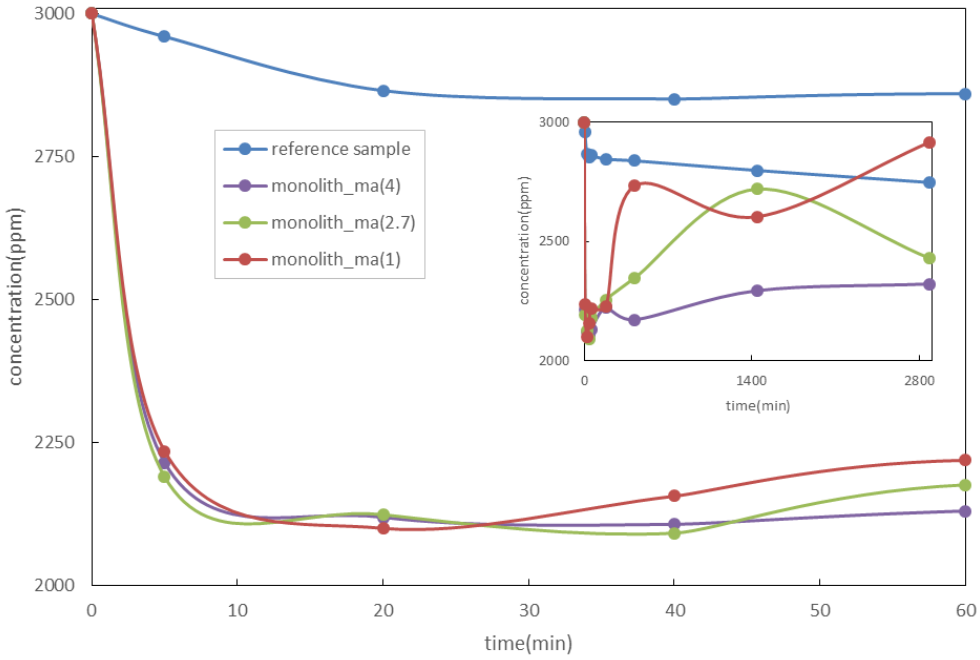

Figure 3.18 Concentration of Nickel through 1 and 48 hours of experiment using monolith_ma(4), monolith_ma(2.7) and monolith_ma(1), and reference sample.

The Nickel concentration using the monolith reference sample, which do not have algae mass, was reduced in 5.0%. This reduction can be attributed to the porosity of the monolith. The hybrid monolith with major quantity of microalgae (monolith_ma(4)) sorbs the biggest amount of metal, with a 29.8% of removal from the initial concentration. The major removal of Nickel is obtained before 10 minutes for the three samples. The release of this metal into the solution is given by the same phenomenon described before for the Copper.

3 MONTHS OLD HYBRID MONOLITHS WITH HIGUER QUANTITY OF MICROALGAE

The kinetic and thermodynamic experiment was also performed with monoliths synthesized 3 months ago. The amount used of hybrid monolith for the experiment was 112 mg. In the **Table 3.5** is detailed the amount of microalgae into the used monoliths for removal of Nickel and Copper.

Table 3.5 Percentage of copper and nickel removed by hybrid monolith, 3 months old, according the algae mass during one hour of the performed experiment.

Metal for removal	Monolith	Amount of algae(mg)	Amount of metal sorbed from initial solution (%)
Copper	reference sample	0	11.1
	monolith_ma(5)	5	90.6
	monolith_ma(2.83)	2.83	84.7
	monolith_ma(1.65)	1.65	77.7
Nickel	reference sample	0	12.3
	monolith_ma(5)	5	50.4
	monolith_ma(2.83)	2.83	41.9
	monolith_ma(1.65)	1.65	33.4

The **Figure 3.19** shows the concentration of copper through 48 hours of kinetic and thermodynamic experiment performed 3 months after the microalgae encapsulation.

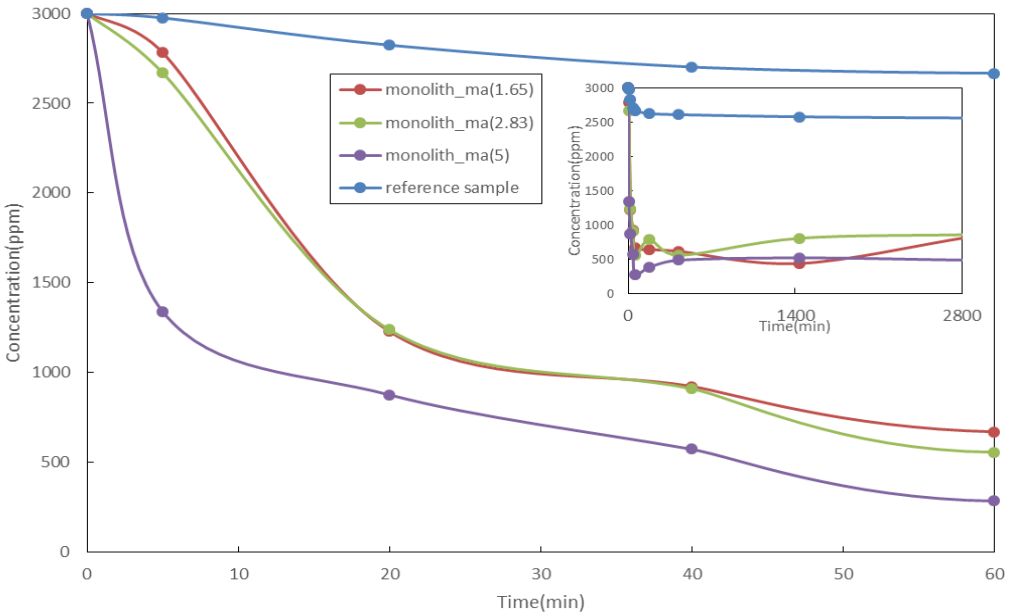

Figure 3.19 Concentration of Copper through 1 and 48 hours of experiment using monolith_ma(5), monolith_ma(2.83) and monolith_ma(1.65), and reference sample.

In the previous figure is observed that the major decrease in Copper concentration is where the sample monolith_ma(5) was used because of its highest amount of microalgae. This sample removes 90.6 % of the initial metal ion present in the solution. The main decrease of the concentration is gotten before 60 minutes for the three samples being monolith_ma(5) the fastest. The monolith with the lowest amount of microalgae removes less percent of metal ions (77.7%). The reference sample (without microalgae) removed a minor amount (11.1%). After a couple of hours the concentration increase for the three hybrid cases, it is because of the monolith fragmentation because of the agitation and consequently, a biggest exposure of the microalgae to the metal solution. Therefore, osmotic shock happened and the metal ions were released to the solution.

The same conditions were performed for the kinetic and thermodynamic experiment with Nickel. The **Figure 3.20** shows the variation on Nickel concentration in the solution through time using hybrid monoliths synthetized 3 months ago.

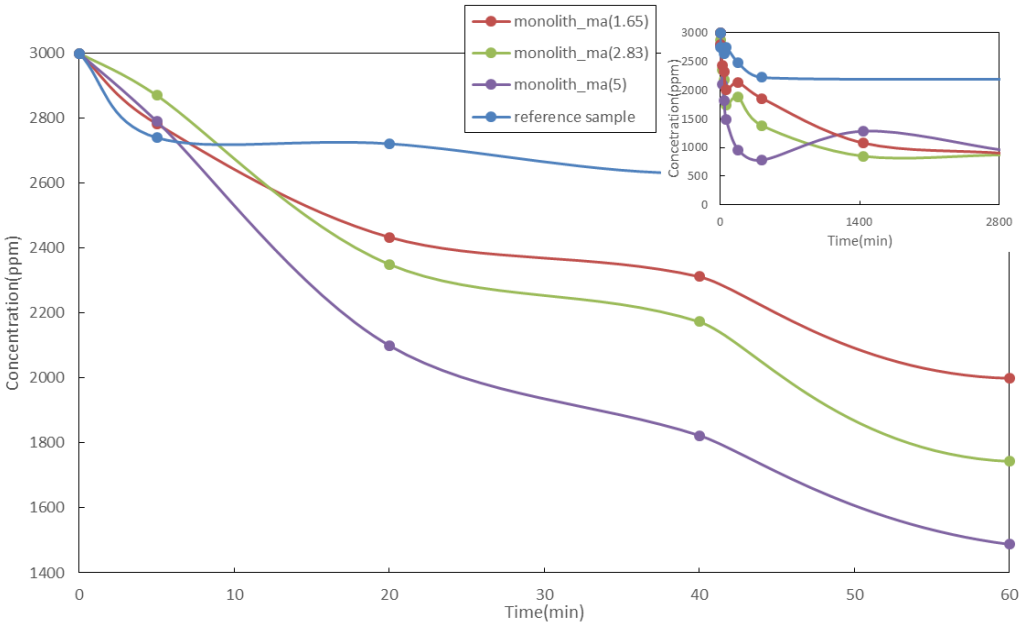

Figure 3.20 Concentration of Nickel through 1 and 48 hours of experiment using monolith_ma(5), monolith_ma(2.83) and monolith_ma(1.65), and reference sample.

The removal of Nickel ion seen in the **Figure 3.20** evidence the same behavior as in the Copper removal, where the amount of metal removal is proportional to the amount of microalgae encapsulated into the monolith. The sample with the highest amount of microalgae, monolith_ma(5), remove 50.4% of Nickel from the initial concentration; the sample with the intermediate amount of microalgae, monolith_ma(2.83), remove 41.9% of Nickel; and the sample with lowest microalgae presence remove less metal ions with 33.4%. The three samples shows an increment of the Nickel concentration after a couple of hours, because of the fragmentation of the monolith by the agitation. The reference sample sorbed a minimum quantity corresponding to 12.3% from the initial concentration of the solution.

Comparing the removal of Copper and Nickel obtained one week and three months after synthesis, it is shown that the removal is highest when the samples are 3 months older. This can be attributed to the microalgae stabilization (lag phase) yet reached and possible growth within the matrix as demonstrated in a previous work where the same encapsulation procedure was used (López, N., 2020; Vaca et al, 2020). Monoliths present a fast metal sorption before 10 minutes but in this case, the first 60 min are critical for the maximum adsorption of the samples with a slower process. After 48 h is seen that part of the sorbed metals are release again to the solution. It has to be

highlighted that the monolith is a little bit destroyed after 48 h and is due to the agitation way.

Additionally, the removal of the metal with the oldest monoliths corroborates the viability to use the monolith after several weeks, and confirm the fluorescence microscopy analysis that evidence the activity of photosynthetic pigments and the consequently maintenance of the microalgae into the silica matrix.

Some articles and reviews show good results in Nickel and Copper removal using immobilized microalgae. In the work performed by Reyes et al (2009) the use of a higher amount of algae, 50 mg, results in the fast removal of the metal ion before the 5 minutes of experiment. In the same work is presented the assumption that the increment of microalgae mass do not increase significantly the sorption capacity. This fact evidence that an increase of active sites do not affect the equilibrium nor the metal removal. This fact would occur due to the formation of adsorbent aggregates (oligomers) that decrease the active surface of the adsorbent given the closeness between the particles of the marine algae in solution. Moreover, it is reported by de-Bashan and Bashan (2010) a Copper removal of 97% and the 91% of Nickel removal during 30 minutes. The same authors reports that the metal removal is proporcional to the micralgae density used. Mehta and Gaur (2001) reported 53% of nickel removal and 60% of Copper removal using microlage during 45 min, they argumented that free cells show lower Nickel and Copper removal. Al-Rub et al (2004) also reported that the inmobilization of microalgae enhace the Nickel removal than free microalgae and that the maximum nickel removal with immobilized microalgae was reached at 120 minutes. These works confirm that in the present work were achieved similar removal percentages, similar times of maximum removal and the high affinity of the microalgae specie to sorb Copper over Nickel ions by using a very low cost and suitable process of microalgae encapsulation as it was already shown by Lopez,2020; Vaca, 2020).

Chapter 4: Conclusions

- The immobilization of Yahuarcocha's microalgae into a hierarchical porous silica matrix is a friendly method of synthesis and it serves to protect the microalgae from the environmental conditions and to make use of their qualities.
- With the use of UV-Vis Spectroscopy, Column Chromatography and Optical and Fluorescence Microscopy the samples collected from Yahuarcocha's lagoon were classified into the *blue-green microalgae specie*. Established the classification of the microalgae used, the development of the present work was based on the qualities of this specie.
- The Fluorescence Microscopy and Diffuse Reflectance UV-Vis spectroscopy allow to obtain information about the photosynthetic pigments of the encapsulated microalgae into the hybrid monoliths. With both techniques the photosynthetic activity of the microalgae was confirmed.
- Using the Fluorescence Microscopy was evidenced the fluorescence activity of the microalgae into the silica monolith verifying the maintenance of the microalgae immobilized through the time (15 weeks after synthesis).
- The samples with encapsulated microalgae (even the ones with the same quantity of encapsulated microalgae as the fresh used microalgae) showed an enhancement in the metal removal than the free organisms.
- The removal of Nickel and Copper ions using the hybrid silica-microalgae monoliths showed high percentage of metal sorption
- It was demonstrated a higher affinity of microalgae for the Copper than for the Nickel ions.
- The percentage of Copper removal was 90.6% and 50.4% for the Nickel ions using monoliths of 3 months old.

Recommendations

It is recommended for futures project based on this work to request for cooperation from the GAD of Ibarra city, because of the relevance of the Yahuarcocha wellness and the efficient use of the polluting species that habit in this emblematic lagoon.

Furthermore, it is recommended the evaluation of the hybrid material obtained for the sorption of metals in mixed solutions of two or more metal ions. Other metal ions should be used for evaluate the sorption capacity of the monoliths.

A good and interesting evaluation should be performed with wastewater, measuring how much metal ions could sorbed the hybrid monoliths. In this experiment would be also evaluated the affinity of determinate metal ions in a complex solution.

References

(1) Abdel-Raouf, N., Al-Homaidan, A., and Ibraheem, I. (2012). Microalgae and wastewater treatment. *Saudi J Biol Sci*(19), 257-275.

(2) Al-Rub, F., El-Naas, M., Benyahia, F., and Ashour, I. (2004). Biosorption of nickel on blank alginate beads, free and immobilized algal cells. *Process Biochemistry*, *39*(11), 1767–1773. doi:10.1016/j.procbio.2003.08.002

(3) Balasubramani, R., Gupta, S., Cho, W., Kim, J., Lee, S., Jeong, K., . . . Choi, H. (2016). Microalgae potential and multiple roles-current progress and future prospects-an overview. *Sustainability*, *8*(12), 1215.

(4) Biocompare. (2005). *The Leica DM4000 B Digital Microscope.* Retrieved from www.biocompare.com/Product-Reviews/41165-The-Leica-DM4000-B-Digital-Microscope/

(5) Blankenship, R. (2014). *Molecular Mechanism of Photosynthesis.* Missouri, USA: Wiley Blackwell.

(6) Cerón, V., Madera, C., and Peña, M. (2015). Uso de lagunas algales de alta tasa para tratamiento de aguas residuales. *Ingeniería y Desarrollo*, *33*(1), 98-62. Retrieved from dx.doi.org/10.14482/inde.33.1.5318

(7) Chisti, Y. (2007). Biodiesel from microalgae. *Biotechnol Adv*, *25*(3), 294-306.

(8) Cobbett, C. S. (2000). Phytochelatins and their roles in heavy metal detoxification. *Plant physiology*, *123*(3), 825-832.

(9) Correa, I. D. (2017). Deep Learning for Microalgae Classification. *16th IEEE International Conference on Machine Learning and Applications*. doi:10.1109/icmla.2017.0-183

(10) Coskun, O. (2016). Separation Tecniques: CHROMATOGRAPHY. *Northern Clinics of Istanbul.* doi:10.14744/nci.2016.32757

(11) Dave, B. C., Dunn, B., Valentine, J. S., and Zink, J. I. (1994). Sol-gel encapsulation methods for biosensors. *Analytical Chemistry*, *66*(22), 1120A–1127A. doi:10.1021/ac00094a001

(12) de-Bashan, L. E., and Bashan, Y. (2010). Immobilized microalgae for removing pollutants: Review of practical aspects. *Bioresource Technology*, *101*(6), 1611-1627. doi:10.1016/j.biortech.2009.09.043

(13) Dufresne, J. (n.d.). *Cylindrospermum sp.* USA. Retrieved from cfb.unh.edu/phycokey/Choices/Cyanobacteria/cyano_filaments/cyano_unbranched_fil/untapered_filaments/heterocysts/no_visible_sheath/CYLINDROSPERMUM/Cylindrospermum_Image_page.htm

(14) Garcia, J., and Gutierrez, R. (2015). Red Fluorescence of Chlorophyll. *Sociedad Española de Óptica, 48*(2), 93-97.

(15) García, P. (2016). *Microalgas y biocombustibles.* Retrieved from Escuela de Organización Industrial: https://www.eoi.es/blogs/merme/microalgas-y-biocombustibles/

(16) González, E. J., and Roldán, G. (2019). Eutrophication and phytoplankton: some generalities from lakes and reservoirs of the Americas. In *Microalgae-From Physiology to Application.* IntechOpen.

(17) Grigoryeva, N. (2020). Self-fluorescence of photosynthetic system—A powerful tool for investigation of microalgal biological diversity. In: Vítová M, editor. Microalgae—From Physiology to Application. *IntechOpen,* 3-26. doi:10.5772/intechopen.887

(18) Grigoryeva, N. (2020). Studying Cyanobacteria by Means of Fluorescence Methods: A review. *IntechOpen*. doi:10.5772/intechopen.93543

(19) Grigoryeva, N., and Chistyakova, L. (2020). Confocal Laser Scanning Microscopy for Spectroscopic Studies of Living Photosynthetic Cells. *Color Detection*. doi:10.5772/intechopen.84825

(20) Grigoryeva, N., Chistyakova, L., and Liss, A. (2018). Spectroscopic techniques for estimation of physiological state of blue-green algae after weak external action. *Oceanology, 58*(6), 896-904. doi:10.1134/S0001437018060061

(21) Guamán, M., and González, N. (2016). Catálogo de microalgas y cianobacterias de agua dulce del Ecuador. *Corporación para la Investigación Energética. Laboratorio de Biotecnología Energética, Quito, Ecuador*.

(22) Guamán, M., and González, N. (2016). *Catálogo de Microalgas y Cianobactrias del Ecuador.* Retrieved from energia.org.ec/cie/wp-content/uploads/2017/09/Catlogo-de-Microalgas-y-Cianobacterias-del-Ecuador.pdf

(23) Gupta, S., Ansari, F., Shriwastav, A., Sahoo, N., Rawat, I., and Bux, F. (2016). Dual role of Chlorella sorokiniana and Scenedesmus obliquus for comprehensive wastewater treatment and biomass production for bio-fuels. *J Clean Prod*, *115*, 255–264.

(24) Hernández, J. (2013). *Fluorescencia de clorofilas.* Retrieved from antioxidantsgroup.wordpress.com/2013/10/10/fluorescencia-de-clorofilas/#:~:text=La%20fluorescencia%20es%20un%20fen%C3%B3meno,esto%20tiene%20en%20el%20proceso

(25) Ishii, A., Itoh, T., Kageyama, H., Mizoguchi, T., Kodera, Y., Matsushima, A., . . . Inada, Y. (1995). Photostabilization of Chlorophyll a Adsorbed onto Smectite. *Dye. Pigment*, *28*(1), 77–82. doi:10.1016/0143-7208(95)00005-Z

(26) Jacome, G., Valarezo, C., and Yoo, C. (2018). Assessment of water quality monitoring for the optimal sensor placement in lake Yahuarcocha using pattern recognition techniques and geographical information systems. *Environmental Monitoring and Assessment*, *190*(4). doi:10.1007/s10661-018-6639-x

(27) Jankowska, E., Sahu, A., and Oleskowicz-Popiel, P. (2017, Agust). Biogas from miroalgae: Review on microalgae´s cultivation, harvesting and pretreatment for anaerobic. *Renewable and Sustainable Energy Reviews*, *75*, 692-709.

(28) Kaštovský, J. (n.d.). *Phormidium sp.* Retrieved from galerie.sinicearasy.cz/galerie/cyanobacteria/jeddnoduche-vlaknite-simple-trichal/phormidium?image_id=9251

(29) Larsdotter, K. (2006). Wastewater treatment with microalgae - A literature review. *Vatten*(62), 31-38.

(30) Livage, J., Coradin, T., and Roux, C. (2001). Encapsulation of biomolecules in silica gels. *Journal of Physics: Condensed Matter*, *13*(33), R673-R691. doi:10.1088/0953-8984/13/33/202

(31) López, N., Sommer, A., and Navarro, R. (2020). Algae Encapsulation into Silica Monoliths Synthesized by High Internal Phase Emulsions (HIPE). Urcuquí, Ecuador: Universidad de Investigación de Tecnología Experiental Yachay Tech.

(32) Mainstone, C., and Parr, W. (2002). Phosphorus in rivers-ecology and management. *The Science of the Total Environment, 282-283*(1-3), 25-47. doi:10.1016/S0048-9697(01)00937-8

(33) Manna, J. S., Basu, S., Mitra, M. K., Mukherjee, S., and Das, G. C. (2009). Study in the Biostability of Chlorophyll a Entrapped in Silica Gel Nanomatrix. *J. Mater. Sci.Mater Electron, 20*(11), 1068-1072. doi:10.1007/s10854-008-9827-6.

(34) Maridueña, A., Chalén, N., Coello, D., Cajas, E., Solís-Coello, P., and Aguilar, F. (2011). Mortandad de peces en la Laguna de Yahuarcocha, Cantón Ibarra, Provincia de Imbabura. Febrero 2003. *Boletín especial*, *2*(1), 1-128.

(35) Martínez, A. (2010). *Evaluación del Crecimeinto Celular y de los Pigmentos Obtenidos ede la Microalga Haematoccus plucialis (Chlorophyta: Volvocales) Cultivada en Diferentes Medios.* Instituto Politécnico Nacional, México. Retrieved from tesis.ipn.mx/bitstream/handle/123456789/8226/EVALCREC.pdf?sequence=1and isAllowed=y

(36) Masojídek, J., Vonshak, A., and Torzillo, G. (2010). Chlorophyll Fluorescence Applications in Microalgal Mass Cultures Chlorophyll a Fluorescence in Aquatic Sciences: Methods and Applications. 277–292. doi:10.1007/978-90-481-9268-7_13

(37) Mehta, S. K., and Gaur, J. P. (2001). Removal of Ni and Cu from single and binary metalsolutions by free and immobilized Chlorella vulgaris. *European Journal of Protistology, 37*(3), 261–271. doi:10.1078/0932-4739-00813

(38) Meunier, C. F., Dandoy, P., and Su, B.-L. (2010). Encapsulation of cells within silica matrixes: Towards a new advance in the conception of living hybrid materials. *Journal of Colloid and Interface Science, 342*(2), 211-224. doi:10.1016/j.jcis.2009.10.050

(39) Meunier, C. F., Rooke, J. C., Léonard, A., Van Cutsem, P., and Su, B.-L. (2010). Design of photochemical materials for carbohydrate production via the

immobilisation of whole plant cells into a porous silica matrix. *Mater. Chem, 20*(5), 929–936. doi:10.1039/B919763J

(40) *MicroPlanet*. (2021, febrero 26). Retrieved from ¿Qué es un microscopio de fluorescencia?: https://www.bioindicacion.com/blog/que-es-microscopio-fluorescencia/

(41) Millie, D. F., Schofield, O. M., Kirkpatrick, G. J., Johnsen, G., and Evens, T. J. (2002). Using absorbance and fluorescence spectra to discriminate microalgae. *37*(3), 313-322. doi:10.1017/s0967026202003700

(42) Ministerio de Agricultura, A. y. (2016). Protocolo de Análisis y Cálculo de Métricas de Fitoplancton en Lagos y Embalses. Retrieved from https://www.miteco.gob.es/es/agua/temas/estado-y-calidad-de-las-aguas/mfitv2-2013_20_01_2016_tcm30-175294.pdf

(43) Moreno-Garrido, I. (2008). Microalgae immobilization: current techniques and uses. *Bioresource technology, 99*(10), 3949-3964.

(44) Nakanishi, K. (2011). Synthesis Concepts and Preparation of Silica Monoliths. *Monolithic Silicas in Separation Science, 9-33*. doi:10.1002/9783527633241.ch2

(45) Nassif, N., Bouvet, O., Noelle Rager, M., Roux, C., Coradin, T., and Livage, J. (2002). Living bacteria in silica gels. *Nature Materials, 1*(1), 42–44. doi:10.1038/nmat709

(46) National Museum of Nature and Science . (2011). *Pseudanabaena sp.* Japan. Retrieved from www.kahaku.go.jp/research/db/botany/dam/105-1-0.html

(47) Olympus. (n.d.). *System Microscope BX 63/BX53* . Retrieved from www.olympus114.com/ctlg/(2)BX63_53%20Catalog.pdf

(48) Osundeko, O., Ansolia, P., Gupta, S. K., Bag, P., and and Bajhaiya, A. K. (2019). Promises and Challenges of Growing Microalgae in Wastewater. *Water Conservation Recycling and Reuse: Issues and Challenges*, 29-53. doi:10.1007/978-981-13-3179-4_2

(49) Papageorgiou, G. (2011). Fluorescence Emission from the Photosynthetic Apparatus. Advances in Photosynthesis and Respiration. 415-443. doi:10.1007/978-94-007-1579-0_18

(50) Pérez-Esteve, É., Ruiz-Rico, M., de la Torre, C., Villaescusa, L. A., Sancenón, F., Marcos, M. D., . . . Barat, J. (n.d.). Encapsulation of folic acid in different silica porous supports: A comparative study. *Food Chemistry, 196*, 66-75. doi:10.1016/j.foodc

(51) Perullini, M., Jobbágy, M., Bermúdez Moretti, M., Correa García, S., and Bilmes, S. A. (2008). Optimizing Silica Encapsulation of Living Cells: In Situ Evaluation of Cellular Stress. *Chemistry of Materials*, *20*(9), 3015–3021. doi:10.1021/cm703075b

(52) Pocock, T., Król, M., and Huner, N. (n.d.). The Determination and Quantification of Photosynthetic Pigments by Reverse Phase High-Performance Liquid Chromatography, Thin-Layer Chromatography, and Spectrophotometry. *Photosynthesis Research Protocols*, 137-148. doi:10.1385/1-59259-799-8:137

(53) Prasanna, R., Sood, A., Suresh, A., Nayak, S., and Kaushik, B. (2007). Potentials and applications of algal pigments in biology and industry. *Acta Botanica Hungarica, 49*(1-2), 131–156. doi:10.1556/abot.49.2007.1-2.14

(54) Priyadarshani, I., and Rath, B. (2012). Commercial and industrial applications of micro algae – A review. *Journal of Algal Biomass Utilization, 4*(3), 89-100.

(55) Promdaen, S. W. (2014). Automated Microalgae Image Classification. *Procedia Computer Science*(29), 1981–1992. doi:10.1016/j.procs.2014.05.182

(56) Qi, W., Li, H., and Wu, L. (2007). A Novel, Luminescent, Silica-Sol–Gel Hybrid Based on Surfactant- Encapsulated Polyoxometalates. *Advanced Materials, 19*(15), 1983–1987. doi:10.1002/adma.200602430

(57) Reichman, J. (2010). *Handbook of optical filters for fluorescence microscopy.* Chroma Technology Corporation.

(58) Reimann, R. Z. (2020). Classification of dead and living microalgae Chlorella vulgaris by bioimage informatics and machine learning. *Algal Research, 48*(101908).

(59) Reyes, Ú. F., Navarro, A. E., and Llanos, B. P. (2009). Algas Marinas del Litoral Peruano como Biosorbentes Potenciales de Ion Cu (II) en Trataiento de Efluetes Industriales. *Revista de la Sociedad Química del Perú, 75*(3), 353-361.

(60) Rizwan, M. M. (2018). Exploring the potential of microalgae for new biotechnology applications and beyond: A review. *Renewable and Sustainable Energy Reviews, 92*, 394–404. doi:10.1016/j.rser.2018.04.034

(61) Rocha, F. S., Gomes, A. J., Lunardi, C. N., Kaliaguine, S., and Patience, G. S. (2018). Experimental Methods in Chemical Engineering: Ultraviolet Visible Spectroscopy|UV-Vis. *The Canadian Journal of Chemical Engineering.* doi:10.1002/cjce.23344

(62) Roge, A. B., Firke, S. N., Kawade, R. M., Sarje, S. K., and Vadvalkar, S. M. (2011). Brief Review on: Flash Chromatography. *International Journal of Pharmaceutical Sciences and Research, 2*(8), 1930-1937. Retrieved from https://d1wqtxts1xzle7.cloudfront.net/32542166/Flash_Chromatography_review.pdf?1386895012=andresponse-content-disposition=inline%3B+filename%3DBrief_Review_on_Flash_chromatography.pdfandExpires=1616919667andSignature=SA-mSilsqFqhy69kD1GCQPxtwN2OjeIuxiO64FxAG94Y

(63) Rooke, J. C., Meunier, C., Léonard, A., and Su, B.-L. (2008). Energy from Photobioreactors: Bioencapsulation of Photosynthetically Active Molecules, Organelles, and Whole Cells within Biologically Inert Matrices. *Pure Appl. Chem, 80*(11), 2345–2376. doi:10.1351/pac200880112345

(64) Roucher, A., Bentaleb, A., Laurichesse, E., Dourges, M.-A., Emo, M., Schmitt, V., . . . Backov, R. (n.d.). First Macro-Mesocellular Silica SBA-15-Si(HIPE) Monoliths: Conditions for Obtaining Self-Standing Materials. *Chemistry of Materials, 30*(3), 864–873. doi:10.1021/acs.chemmater.7b04483

(65) Rowan, K. S. (1989). *Photosynthetic pigments of algae.* Cambridge University Press.

(66) Ruiz, F., Ruiz, J., Hernández, B., García, R., and Valadez, A. (2019). Extracción y cuantificación de clorofila en hojas comestibles del estado de Tabasco. *Investigación y Desarrollo en Ciencia y Tecnología de Alimentos, 4*, 891-896. Retrieved from http://www.fcb.uanl.mx/IDCyTA/files/volume4/4/10/126.pdf

(67) Saelens, P. (2015). Ecological functioning of a shallow lake in Ecuador, Lagua Yahuarcocha. Master thesis. KU Leuven, Belgium, 96 pp.

(68) Sigaut, L., and Pietrasanta, L. (2016). *Guía 9: Microscopía óptica y de fluorescencia.* Retrieved from http://materias.df.uba.ar/f2bygaa2016c1/files/2012/07/Gu%C3%ADa_9_FII_ByG_Fluorescencia_2016.pdf

(69) Smith, V., and Schindler, D. (2009). Eutrophication science: where do we go from here? *Trends in Ecology and Evolution, 24*(4), 201–207. doi:10.1016/j.tree.2008.11.009

(70) Sommer, A. (2013). *Retención de Clorofila en Hidrotalcitas.* Benem´rita Universidad Autónoma de Puebla.

(71) Sommer, A., Lerner, D., G, F., Bosch, P., Tichit, D., and Palomares, E. (2014). Preparation of layered double hydroxide/chlorophyll a hybrid nano-antennae: a key step. *Dalton Transactions, 43*, 10521–10528 . doi:10.1039/c4dt00113c

(72) Sommer-Marquez, A., Mansas, C., Talha, N., Rey, C., and Causse, J. (2016). Reinforced silica monoliths functionalised with metal hexacyanoferrates for cesium decontamination: a combination of a one-pot procedure and skeleton calcination. *RSC Advances, 6*(77). doi:10.1039/c6ra16980e

(73) Surat, P. (2018). *DNA etiqueta usando DAPI: Metodología*. Retrieved from News Medical Life Sciences: www.news-medical.net/life-sciences/DNA-Labelling-Using-DAPI-Methodology-(Spanish).aspx

(74) University of California Museum of Paleontology. (n.d.). *Photosynthetic Pigments*. Retrieved from Glossary: https://ucmp.berkeley.edu/glossary/gloss3/pigments.html

(75) Upstone, S. L. (2006). Ultraviolet/visible light absorption spectrophotometry in clinical chemistry. In *Encyclopedia of Analytical Chemistry: Applications, Theory and Instrumentation.* © 2013 John Wiley and Sons, Ltd. doi:10.1002/9780470027318.a0547.pub2

(76) Vaca, A., Causse, J., and Sommer-Márquez, A. (2020). Immobilization of chloroplasts from grass within a silica matrix synthetized by HIPE method. *MRS Advances*, 1–7. doi:10.1557/adv.2020.46

(77) Valdez, E. (2019). *Extracción, Absorción in vivo y Monitoreo de Cultivos de pico y nano Plancton con Ficobiliproteínas acondicionados a diferentes espectros de luz.* Centro de Investigación Científica y de Educación Superior de Ensenada, Baja California. Retrieved from cicese.repositorioinstitucional.mx/jspui/bitstream/1007/3055/1/tesis_Valdez%20Silva%20Esperanza%20Guadalupe_05_dic_2019.pdf

(78) Weckhuysen, B. (2004). Ultraviolet-visible spectroscopy.

(79) Whitton, B. A., and Potts, M. (2012). Introduction to the Cyanobacteria. *Ecology of Cyanobacteria II*, 1-12. doi:10.1007/978-94-007-3855-3_1

(80) Wilde, E. W., and Benemann, J. R. (1993). Bioremoval of heavy metals by the use of microalgae. *Biotechnology Advances, 11*(44), 781-812. doi:10.1016/0734-9750(93)90003-6

(81) Wong, M., and Pak, D. (1992). Removal of Cu and Ni by free and immobilized microalgae. *Biomed. and Environ Sci, 5*, 99-108.

(82) Xu, H., Yan, F., Monson, E. E., and Kopelman, R. (2003). Room-temperature preparation and characterization of poly (ethylene glycol)-coated silica nanoparticles for biomedical applications. *Journal of Biomedical Materials Research, 66A*(4), 870-879. doi:0.1002/jbm.a.10057

(83) Yang, S. H., Lee, K. B., Kong, B., Kim, J. H., Kim, H. S., and Choi, I. S. (2009). Biomimetic Encapsulation of Individual Cells with Silica. *Angewandte Chemie International Edition, 48*(48), 9160–916. doi:10.1002/anie.200903010

(84) Yang, X., Wu, X., Hao, H., and He, Z. (2008). Mechanisms and assessment of water eutrophication. *Journal of Zhejiang University SCIENCE B, 9*(3), 197-209. doi:10.1631/jzus.b0710626

(85) Zhang, B.-B., Wang, L., Charles, V., Rooke, J., and Su, B.-L. (2016). Robust and Biocompatible Hybrid Matrix with Controllable Permeability for Microalgae Encapsulation. *ACS Applied Materials and Interfaces, 8*(14), 8939–8946. doi:10.1021/acsami.6b00191

Apendix A

Conditions

For removing metals, the most used microalgae are green algae like *Chlorella, Scenedesmus, Cladophora; cyanobacteria* like *Spirulina, Oscillatoria, Anabaena* or consortiums of both (Gupta et al., 2016; Cerón et al., 2015; de-Bashan and Bashan, 2010; Wilde and Benemann, 1993)

Active uptake systems can reduce the residual metal concentrations to very low, sub-ppb, levels (Wilde and Benemann, 1993) Chemical and physical parameters make a great influence on metal binding, especially on the groups with Co, Cu, Hg, Ag, and U, whose mobility is associated with their water solubility (Wilde and Benemann, 1993).

The most important parameter in bioremoval is pH, but this condition depends of the type of algae used and this parameter can be adjusted to be more favorable with certain metals. Wilde and Benemann (1993) showed that the typical optimal pH value for green algae is higher than 5 for removal of aluminum, cadmium, chromo, copper and mercury.

Additionally, the temperature influences the metal bioremoval, the biosorption is improved when the temperature increase; to illustrate the reduction of Cr(VI) to Cr(III) was increased as temperature increases from 25°C to 55°C (Wilde and Benemann, 1993).

Often a chemical of physical treatment like heat or acids treatment in the microalgae biomass make greater their biosorption capacity, probably the reason is the uncovering of masked binding sites (Wilde and Benemann, 1993).

Most of the heavy metal captured by biosorption has a great economic value, for this reason it is recommended to desorb the metal from the used hybrid material.

Elution

For the recovery, disposal or reuse of the binding-algae metal some eluents are used. To reduce the ion bond there are used a metal chelator, high pH solution, salt solution, or washing with EDTA (ethylene diamine tetra-acetic acid) (Wilde and Benemann, 1993).

Economic considerations

The costs of the most available microalgae species, *Chlorella* and *Spirulina*, are above 10 hundred per ton, it means a high value for the metal removal. At the present work, it is used the "waste" microalgae which represents a great economic benefit but a high variability on its quality.

Microalgae have the potential to remove metal ions to very low concentrations, to grow on light energy, and to collect large amounts of specific toxic elements (Wilde and Benemann, 1993). Immobilized *Chlorella* showed significantly lower efficiency for removing Cu and Ni in all experimented concentrations than free cells of the same species (Wong and Pak, 1992).

Copper removal

This metal is essential in the living microorganism and has a wide use in the industry, also represents a dangerous environmental hazard at huge concentrations. According to de-Bashan and Bashan (2010), some studies had demonstrated that a great amount of immobilized algae results on more Copper removal with 91% of removal. The recovery of this valuable element is an important challenge after sorption, it has been performed by *HCl* as eluent, increment of pH of eluent means less desorption rate and more time for this objective. It is important to highlight that the material for bioremoval can be regenerate to be applied in multiples sorption cycles.

Nickel removal

Nickel has a huge applicability in the industry and also represent an important element in living organisms, some nickel compounds are toxic for human health. The removal with immobilized algae gets 91% from the initial metal concentration, the increment of pH means an improvement for the nickel sorption. This metal is recovered too because of its high value (de-Bashan and Bashan, 2010).

Appendix B. Determination of metals concentration

For the determination of copper and nickel concentration in kinetics, absorption data obtained from the linear regression of calibration curve was used according the formula:

$$Cm = \frac{A - a}{b} * F_d$$

Where: A is the absorbance of the sample

a is the y-intercept of the calibration curve

b is the slope of the calibration curve

F_d is the dilution factor

Appendix C. Calibration curves for Copper and Nickel.

The data obtained for the calibration curves of the metal concentrations are shown in the **Table A.1** for copper, the **Table A.2** are the statistics parameters of its regression and the and in the **Table A.3** is shown the variance analysis for the data for this metal.

Table A.1 Copper concentration data obtained for calibration curve.

Cu concentration (ppm)	Absorbance
500	0.0096
500	0.0103
500	0.0101
1000	0.04347
1000	0.04899
1000	0.04255
1500	0.0705
1500	0.0715
1500	0.0715
3000	0.1463
3000	0.1483
3000	0.1483

In the **Figure A.1** is shown the calibration curve for copper concentration.

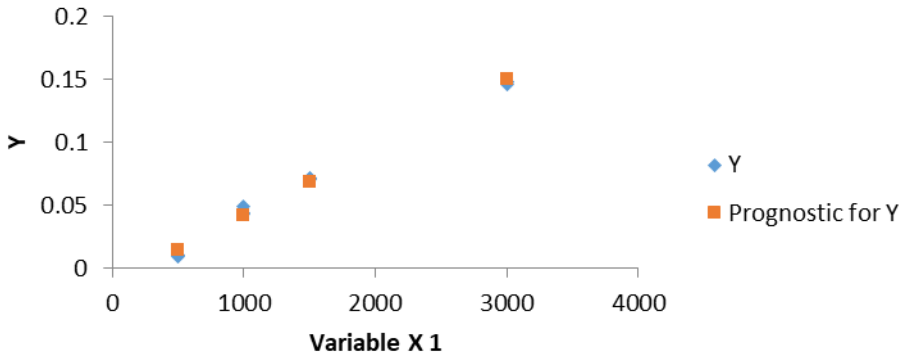

Figure A.1 Fitted regression curve for Copper concentration.

Table A.2 Statistics parameters of the regression for copper.

	Coefficients	Typical Error
Interception	-0.0072527024	0.002162878
Variable X 1	5.39852E-05	1.22351E-06
Coefficient of determination R^2	0.9918897	0.0039646

Table A.3 Variance analysis for copper data obtained.

	Degrees of freedom	Sum of squares
Regression	1	0.030601262
Residual	10	0.000157182
Total	11	0.030758444

The absorbance obtained for Nickel concentrations is shown in **Table A.4**, in the **Table A.5** are shown the statistic parameters of the regression and in the **Table A.6** is the variance analysis of nickel data.

Table A.4 Nickel concentration data obtained for calibration curve.

Ni concentration (ppm)	Absorbance
500	0.0038
500	0.0037
500	0.0037
1000	0.009
1000	0.0089
1000	0.0095
1500	0.03
1500	0.03
1500	0.0303
3000	0.0629

3000	0.0638
3000	0.0638

The calibration curve for nickel concentration is shown in the **Figure A.2**.

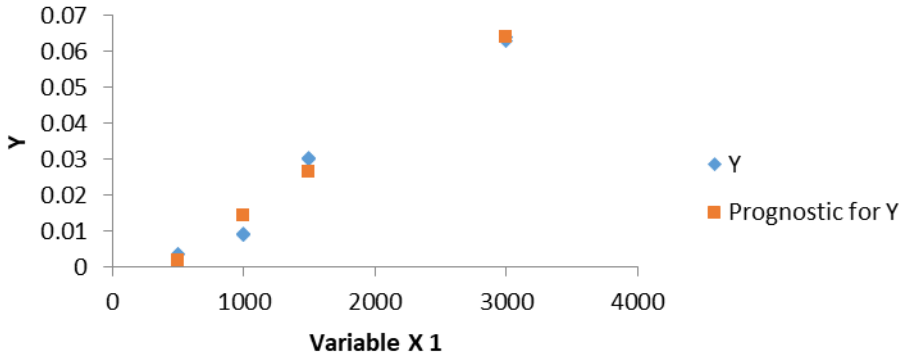

Figure A.2 Fitted regression curve for Nickel concentration.

Table A.5 Statistics parameters of the regression for Nickel.

	Coefficients	Typical Error
Interception	0.00361065	0.001936779
Variable X 1	2.48429E-05	1.09561E-06
Coefficient of determination R^2	0.9909	0.00355017

Table A.6 Variance analysis for Nickel data obtained.

	Degrees of freedom	Sum of squares
Regression	1	0.006480259
Residual	10	0.000126037
Total	11	0.006606297