Immobilized Microalgae Bioremediation:
A Sustainable Solution for Contaminated Water

"Water is the principle of all things."
Thales of Miletus.

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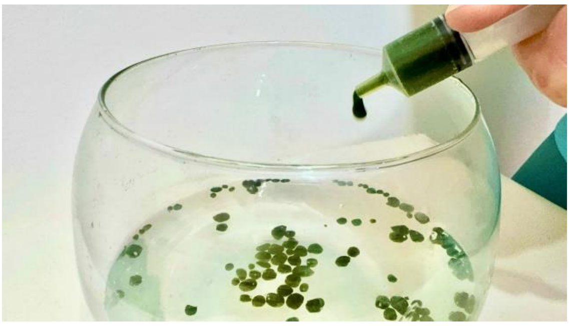
Abstract

This project investigates the application of bioremediation techniques using immobilized Chlorella Vulgaris microalgae in agar-agar spheres to treat surface freshwater contaminated by industrial discharges. Focusing on the challenge of water pollution, this study uses a 2-liter bioreactor to evaluate the effectiveness of the microalga in absorbing organic and inorganic pollutants, including heavy metals.

The experiments were conducted under controlled conditions with water from industrial discharges from the El Clavel stream in Buenos Aires Province. Over a 6-day period, a significant reduction in contaminant concentrations such as ammoniacal nitrogen, total nitrogen, phosphorus, copper, and chromium was documented. The algal biomass not only showed a high capacity to absorb these contaminants but also improved water quality by reducing Biochemical Oxygen Demand (BOD) and promoting the reduction of eutrophication. Furthermore, the bio adsorption of heavy metals was analyzed, highlighting the ability of microalgae to immobilize these elements in their cell walls through chemical interactions. The results indicate that Chlorella Vulgaris, in both active and inactive forms, can be an effective and economical solution for the remediation of water bodies affected by industrial activity.

This project demonstrates the feasibility of implementing low-cost bioremediation systems accessible to low-income communities, providing a sustainable and effective methodology to address the global issue of water pollution.



Biography: Valentina Fontenla is a distinguished 17-year-old student currently in her final year of high school. With a profound interest in science, technology, and environmental conservation, Valentina has participated in numerous projects focused on these areas. Last year, she collaborated with the University of Pennsylvania, where she received a certification of merit for her creative, innovative, accessible, and sustainable project, which led to the creation of a prototype biofilter using natural elements for the capture, collection, and filtration of rainwater and dew, making it suitable for human consumption. Additionally, she worked on a biomimicry project developing prototypes using UV and ionization for water treatment.

Valentina also participated in an environmental toxicology course at UC Davis, California, where she gained hands-on experience working in a university lab on college-level experiments. During this program, she explored the effects of toxic chemical exposure on guppies and honey bees, applied the scientific method to postulate research questions, conducted RNA extractions, used bioinformatics software to design primers, and analyzed gene expression experiments via agarose gel electrophoresis. This experience allowed her to connect with a scientific network of mentors and further solidified her interest in pursuing research in the field of toxicology.

Her passion lies in supporting and aiding communities with limited resources, aiming to provide practical and sustainable solutions to environmental challenges. Valentina believes that our planet belongs to everyone and that it is our collective responsibility to protect and preserve it. She is also deeply interested in technology and its potential to improve lives. As a volunteer, she conducts classes and workshops for senior citizens, helping them become familiar with technology and demonstrating its various benefits in everyday life.

Valentina's dedication to environmental stewardship and technological empowerment reflects her commitment to making a positive impact on both local and global scales.

Keywords: Bioremediation, Chlorella Vulgaris, immobilized microalgae, agar-agar, water contamination, heavy metals, adsorption, eutrophication, vulnerable communities, sustainability.

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INTRODUCTION

In an increasingly urbanized world, water pollution stands as one of the most critical environmental challenges of our time, threatening both the biodiversity of aquatic ecosystems and human health. Population expansion, coupled with urbanization, industrialization, and agro-industry development, has exponentially increased the production and disposal of solid and liquid waste, exacerbating the contamination of our waters. This issue is particularly severe in densely populated areas such as Buenos Aires City, its metropolitan area, and Buenos Aires Province, where urban growth has generated a constant flow of organic and inorganic pollutants into water bodies.

The presence of a wide range of waste, from domestic to industrial, including heavy metals and pathogenic compounds, in our streams and rivers not only compromises water quality but also poses risks to public health and ecosystem integrity. Extreme weather events, such as intense rainfall exacerbated by climate change, further aggravate this situation by facilitating the wash-off of these wastes into stormwater systems and, eventually, our watercourses.

Facing this critical scenario, the need to implement innovative, sustainable, and accessible solutions for the remediation of contaminated waters has become urgent. This project focuses on bioremediation as a key strategy to address freshwater pollution, proposing the use of immobilized microalgae to effectively purify affected water bodies. The goal is not only to mitigate the harmful impacts of pollution on human health and aquatic ecosystems but also to promote more sustainable and conscientious management of our water resources.

OBJECTIVE AND PURPOSE

This project addresses the growing issue of water pollution through an innovative and sustainable solution: the purification of surface freshwater using agar-agar spheres with Chlorella Vulgaris microalgae. Inspired by natural processes, the system aims to improve water quality in areas compromised by organic and inorganic contaminants, especially heavy metals, providing cleaner and safer water for human and agricultural use.

The initiative aims to alleviate the adverse effects of water pollution on health and ecosystems, promoting the recovery of freshwater bodies through an economical and environmentally friendly method. Additionally, the project seeks to encourage the adoption of green technologies, educate, and raise awareness about the importance of protecting our water resources. In collaboration with local communities and organizations, it aims to develop a replicable and adaptable bioremediation solution, aiming for a future where access to clean water is universal.

DEVELOPMENT

This method utilizes living organisms, particularly microalgae such as Chlorella Vulgaris, to clean water contaminated with heavy metals and other harmful compounds. Chlorella Vulgaris stands out for its ability to grow under adverse conditions, absorb heavy metals, and transform nitrates and phosphates into useful biomass. This process not only reduces water contaminants but also captures CO2 and releases oxygen, mitigating climate change.

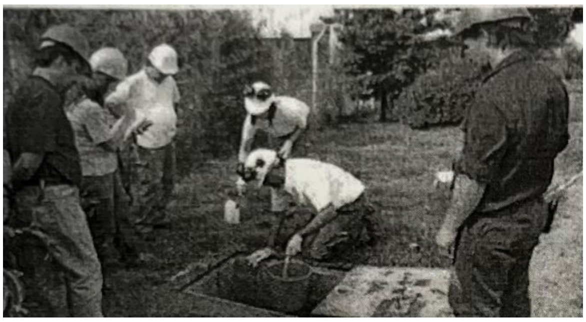
Phycological remediation, a technique that uses microalgae to clean water bodies, has gained new relevance due to the increase in contaminants from industrial and urban activities. Using agar-agar spheres to immobilize these algae, the method optimizes their effectiveness by keeping them stable at the contamination site for prolonged periods, acting as efficient biofilters.

The importance of this technology is especially evident in the streams of Buenos Aires Province, where critical levels of lead, chromium, copper, and phosphate have been detected, exceeding standards for aquatic life protection and affecting public health and local biodiversity. This project not only aims to effectively remove contaminants but also to raise awareness about preserving our aquatic ecosystems through sustainable solutions.

SITUATION DIAGNOSIS

El Clavel stream, which flows from Provincial Route 6 to its confluence with Larena stream, passes through residential, industrial, and recreational areas before joining the Luján River.

This water body has been critically affected by pollution for decades due to discharges of domestic and industrial effluents, open-air dumps, and inadequate waste management, contributing to high levels of nitrates, phosphorus, and other contaminants. These factors have resulted in elevated Biochemical Oxygen Demand (BOD) and heavy metal levels that exceed local and international standards, such as the ACUMAR Res. 283/19 Annex A, for uses I.a (suitable for biota protection and recreational use with direct contact) and I.b (suitable for biota protection). The pollution of the stream not only poses a threat to biodiversity and water quality but also represents a risk to public health. Media reports and neighborhood complaints about the presence of solid waste, turbidity, and foul odors in the stream waters reflect the community's concern about this issue.



The situation of the stream reflects the environmental challenges faced by many urban and peri-urban streams in the region. The interaction between accelerated urbanization and inadequate waste and effluent management has exacerbated water pollution, leading to a critical state that requires immediate attention. Despite actions taken by various local administrations and complaints since the early 1990s, the problem persists, aggravated by inconsistency in the application and enforcement of environmental regulations by local industries.

In response to this scenario, this project focuses on the analysis of the El Clavel stream water. Critical parameters such as pH, temperature, dissolved oxygen, BOD, total phosphorus, ammoniacal and total nitrogen, as well as copper and chromium concentrations, are evaluated. Bioremediation is proposed as a viable strategy to restore the health of the El Clavel stream. Using techniques that incorporate microalgae such as Chlorella Vulgaris immobilized in agar-agar spheres, the aim is not only to effectively reduce contaminant levels but also to revitalize the aquatic ecosystem, improve local quality of life, and foster environmental awareness about the importance of preserving our water resources. Before proceeding with the research methodology, a detailed review of each parameter to be studied for effective remediation is provided.

Analysis of Critical Parameters in Contaminated Water

- pH: Measures the acidity or water alkalinity on a scale of 0 to 14, with 7 being neutral. Values less than 7 are acidic and greater, alkaline. This parameter is crucial because it influences the solubility of chemical substances and the survival of aquatic life. An inappropriate pH can harm aquatic ecosystems, affecting everything from microorganisms to fish and plants. Monitoring pH is vital to protect these ecosystems and ensure the water is suitable for use.
- Dissolved Oxygen (DO): The amount of free oxygen in the water, vital for aquatic life such as fish and microorganisms. Adequate levels are essential for respiration and decomposition in

water; low levels can cause anoxic conditions that threaten aquatic life. Fluctuations can indicate pollution or eutrophication. Monitoring is crucial to assess ecosystem health and the effectiveness of wastewater treatments.

- Biochemical Oxygen Demand (BOD): Measures the oxygen used by microorganisms to decompose organic matter in water. It is expressed in milligrams of oxygen per liter (mg O_2/L) and is a key indicator of organic pollution in water bodies. High BOD values suggest a high concentration of organic pollutants, which can lead to hypoxia or anoxia, severely affecting aquatic life. It is commonly measured over a five-day incubation period (BOD_5) to predict future oxygen consumption. Despite being time-consuming and delicate, BOD analysis is crucial to assess water quality, wastewater treatment efficiency, and the impact of discharges on rivers and lakes.
- Total Nitrogen: Encompasses all forms of nitrogen in the water, including organic nitrogen, ammoniacal nitrogen, nitrites, and nitrates. This measure is crucial to evaluate the nutritional load of the water and its propensity to cause eutrophication, a process that can result in excessive algae growth and oxygen depletion, deteriorating aquatic life. Its analysis is essential to detect pollution from agricultural, industrial, and urban sources and to develop effective remediation strategies.
- Ammoniacal Nitrogen: Comes from the decomposition of organic matter and fertilizer use. Indicates organic pollution in water bodies. This compound is especially toxic to aquatic life under high pH and temperature conditions. Measuring it is vital to assess the impact of wastewater and agricultural discharges and is fundamental to adjusting phycological remediation strategies with microalgae. These techniques aim to reduce its concentration, promoting a healthier and balanced aquatic ecosystem.
- Nitrites: An intermediate product in nitrification, formed from the oxidation of ammoniacal nitrogen and the reduction of nitrates. Used as indicators of organic pollution, including fecal contamination, in water bodies. Their presence in surface waters usually signals pollution and can result from the biological decomposition of proteinaceous organic matter. Although small amounts of nitrites are part of normal cycles, elevated levels can be toxic to aquatic life and are indicative of water quality issues.
- Phosphorus: An essential nutrient that plays a crucial role in the ecological balance of water bodies. Though necessary, its excess, typically originating from agricultural runoff containing fertilizers, detergents, and wastewater, can cause eutrophication. This phenomenon triggers uncontrolled algae and aquatic plant growth, reducing available oxygen and threatening fish and other organisms. Therefore, it is crucial to analyze phosphorus to evaluate the water's nutritional health and determine the need for remediation interventions.
- Chromium: A metal with a dual role in the environment and human health, mainly appearing in two forms: Chromium (III), essential for human nutrition, and Chromium (VI), highly toxic and carcinogenic. Its presence in water bodies, especially as Chromium (VI), generally indicates industrial pollution from processes like steel manufacturing, textiles, and paints. Its detection is crucial, as Chromium (VI) can cause cancer, organ damage, and disturbances in aquatic ecosystems, even at low concentrations. Continuous monitoring is essential to assess contamination and the effectiveness of bioremediation measures that aim to convert it into less harmful forms.
- Copper: An essential metal for biological and industrial processes, found in various forms in nature, with uses ranging from alloy manufacturing to agricultural fungicides. Although essential, its presence in water, especially at high concentrations, is concerning. Typically, copper in water bodies comes from industrial discharges and infrastructure corrosion. It is crucial to monitor its levels, as it can be toxic to aquatic life, affecting species growth and the food chain. High concentrations can also indicate industrial pollution, compromising water quality and posing a risk to human health through direct or indirect consumption.

BIOREMEDIATION WITH CHLORELLA VULGARIS

Bioremediation

It is a revolutionary technique that leverages the power of living microorganisms, such as bacteria, fungi, and plants, to decompose, transform, or directly remove chemical contaminants present in soil and water. These natural organisms treat contaminants as a source of energy or food, initiating an environmental detoxification process that results in the reduction or complete cleanup of harmful substances. This ecological process can occur naturally, where microorganisms already present in the environment act on contaminants without human intervention, or it can be stimulated through techniques that increase the efficiency and speed of decontamination.

In freshwater bodies, bioremediation is particularly important due to its potential to restore water quality, ensuring the conservation of biodiversity and the ecological balance of these vital ecosystems. The process involves several steps, from the initial contamination assessment and selection of suitable microorganisms to the implementation and monitoring of the remediation strategy. The key is to find the organism or set of organisms with the specific ability to treat the contaminants present, which may include adapting microbial communities to the contaminated environment or adding nutrients to accelerate the decontamination process.

Microalgae: Sentinels and Purifiers of Contaminated Water

Recently, interest in studying microalgae has experienced a notable resurgence, driven by the increase in pollution from nitrates, ammonium, and phosphates, originating from both industrial activities and agriculture due to intensive fertilizer use, as well as from sewage discharges. Microalgae offer a promising solution in this context, given their innate ability to purify these contaminants, using them for their growth and transforming them into valuable biomass. Additionally, this process has the added benefit of capturing carbon dioxide and releasing oxygen into the environment, contributing to climate change mitigation.

Unique Characteristics of Microalgae for Bioremediation

Through their cell walls rich in polysaccharides and proteins, microalgae can interact with various contaminants, absorbing or transforming them. This process is influenced by several factors, including the medium's pH, which alters the availability and form of the contaminants, as well as the charge and binding capacity of the algal cells.

The cell walls of microalgae contain functional groups such as carboxyl, hydroxyl, sulfate, phosphate, and amine, which are crucial for the biosorption process. These groups can bind to heavy metals and other contaminants, facilitating their removal from the water. The biosorption capacity varies with pH, affecting the ionization of functional groups and the solubility of metals. At low pH, competition for binding sites can decrease biosorption, while at high pH, certain metals may precipitate, reducing their bioavailability.

In addition to Chlorella, genera such as Spirulina and Scenedesmus are recognized for their ability to efficiently remove nutrients and heavy metals from contaminated waters. Each genus has unique adaptations that make it suitable for treating different types of contaminants under varied environmental conditions.

Biosorption: A Physicochemical Cleaning Process

Biosorption is a biological process through which microorganisms, including various species of algae, have the ability to clean water contaminated with heavy metals. This process occurs naturally and leverages the intrinsic properties of these organisms to effectively remove contaminants from the environment.

Metal ions present in the water move towards the cells of microorganisms through active and passive transport mechanisms. Passive transport allows metals to flow from areas of high concentration to areas of lower concentration, approaching the cell surface without requiring energy. In contrast, active transport uses cellular energy to capture metal ions from lower concentrations and move them to higher concentrations within the cells. Once the metal ions reach the cell surface, they adhere to it due to specific sites that show a chemical affinity for these metals. This adhesion phenomenon can take various forms, such as simple adsorption, where ions stick directly to the cell surface, or more complex processes like ion exchange, complexation, chelation, where rings are formed that trap the metals, and microprecipitation, resulting in the formation of small precipitates on the cell surface.

Although initial adsorption is rapid and reversible, it is only the beginning. A slower and often irreversible process follows, where metals form stronger bonds with the biomass. This can include the formation of robust covalent bonds, precipitation on the cell surface, redox reactions that alter the metal's valence, and crystallization. Additionally, some metal ions can even diffuse into the cell's interior, where they bind to proteins and other intracellular components, serving as a more permanent form of storage or detoxification. The algae's capacity for biosorption varies depending on the type of algae, the nature of the metal, and the composition of the aqueous solution, highlighting the specificity and adaptability of this process. Therefore, biosorption not only stands out for its effectiveness in contaminant removal but also for the possibility of adapting this process to different conditions and types of contamination.

Biosorption does not solely depend on cellular metabolism and can occur even with dead or inactive biomass. This allows the use of microalgae and other biosorbents in a wide range of conditions, offering an environmentally friendly and economically viable remediation technique. Contaminants are trapped by the biomass through chemical and physical interactions, which can result in their recovery and reuse.

Microalgae offer several advantages in bioremediation, including low operational costs and the possibility of recovering contaminants as valuable metals. They present an innovative and sustainable solution for the bioremediation of contaminated waters, making them valuable tools in environmental management.

Chlorella Vulgaris: Distinctive Characteristics

Chlorella Vulgaris is a unicellular green microalga that stands out in the field of bioremediation for its robustness and effectiveness in purifying a wide range of contaminants. It is valued for its adaptability, allowing it to thrive in diverse environments and facilitating its cultivation and application in various contamination contexts. Additionally, it has a rapid growth rate, which facilitates biomass production in a short time, essential for large-scale remediation projects.

It is effective in biosorption. This ability is due to the presence of functional groups such as carboxyl, phosphate, and sulfate on the cell surface, which interact effectively with heavy metals and nutrients. The alga is notable for the removal of nitrogen and phosphorus, whose excessive presence can cause eutrophication in water bodies. It uses these nutrients for its growth, converting them into biomass and reducing their concentration in the water, which also makes it a valuable source of nutrient-rich biomass, usable even for bioenergy production. Moreover, it has an outstanding capacity to sequester heavy metals such as lead, cadmium, mercury, copper, arsenic, and chromium. This biosorption process is influenced by factors such as pH, the presence of other ions, and the composition of its cell wall.

Recent research has shown that it can also be genetically modified to enhance its biosorption capacity, expanding its utility in the bioremediation of an even wider range of contaminants. The combination of flexibility, effectiveness, and sustainability makes Chlorella Vulgaris a model of choice for this remediation study.

Immobilized Microalgae: Method and Choice

Agar-Agar Spheres

Studies have shown that immobilizing microalgae cultures significantly improves bioremediation efficiency. In this project, Chlorella Vulgaris was encapsulated in agar-agar spheres, selecting this polysaccharide extracted from red algae for its gel-forming ability, accessibility, and economic advantages compared to other agents such as alginate, cellulose, silica gel, even polyacrylamide and various cross-linking agents. Agar-agar not only facilitates the formation of spheres that allow for the uniform and efficient distribution of algal cells but also creates an optimal environment for the microalga, protecting it against toxins and variations in pH and temperature.

Additionally, the encapsulation technique in agar-agar ensures more efficient handling of the microalgae, with spheres approximately 5 mm in diameter promoting a homogeneous distribution in the bioremediation system. This characteristic improves the system's operational stability and manipulation, making agar-agar particularly attractive for large-scale bioremediation projects.

The use of agar-agar offers a low-cost and sustainable solution. The choice of this material reflects a commitment to responsible innovation and economic sustainability, opening new possibilities for the efficient and accessible purification of contaminated waters.

Reason for Immobilization in Agar-Agar

The choice to immobilize microalgae in agar-agar spheres is strategic and responds to multiple operational and environmental advantages. This technique offers an effective barrier that protects the microalgae from toxic contaminants in the water while maintaining optimal pH and temperature conditions. This protection is essential to optimize biomass production and ensure the microalgae's survival, allowing for greater efficiency in bioremediation processes.

Immobilization confines the microalgae to a specific and reduced space, significantly facilitating their handling and providing stability to the system. This confinement is crucial to control the dispersion of cells in the aquatic environment, thus preventing eutrophication and promoting a more regulated and safe water treatment.

Moreover, the immobilization technique favors intense intercellular communication through chemical signals dispersed in the agar-agar matrix. This communication is crucial to enhance the cellular interactions essential for the bioremediation process. Immobilization not only improves biomass production but also simplifies the recovery and reuse of microalgae at the end of the treatment.

Bioreactor Design with Agar-Agar Spheres and Chlorella Vulgaris

Bioreactor

Watch the process and operation video at the link: https://youtu.be/CN5u9jpXozM

This project implements an experimental bioreactor specifically designed for the bioremediation of contaminated waters. It uses agar-agar spheres to immobilize Chlorella Vulgaris. This system allows for precise control of cultivation conditions and optimizes water purification.

In the study, water samples were taken from the stream affected by industrial discharges. While one liter of this water was sent to the laboratory for immediate analysis, two additional liters were introduced into the bioreactor, where the agar-agar pearls with C. Vulgaris were already placed. During the following six days, optimal water quality conditions were monitored to ensure bioremediation. At the end of the experiment, the treated water was analyzed again in the laboratory to evaluate the remediation process's effectiveness. These analysis results were compared with the initial sample to determine the reduction of contaminants.

Additionally, the algal biomass obtained at the beginning and end of the experiment was compared, providing a concrete measure of C. Vulgaris's bioremediation activity and effectiveness.

Container

A 2-liter transparent glass aquarium was selected for its optical clarity that optimizes light penetration, essential for the algae's photosynthetic process. Equipped with aeration and agitation systems, it simulates optimal growth conditions and facilitates effective water treatment. The transparency of the aquarium also allows for constant monitoring of the treatment progress, observing both algal biomass growth and changes in water quality, allowing for real-time adjustments to optimize the bioremediation process.

Aeration

The bioreactor includes a bubble aeration system designed to replicate the conditions of stirred tank fermenters used in laboratories, thus optimizing the circulation and oxygenation necessary for microalgae cultivation. An adjustable air pump with a capacity of 4 to 20 liters per minute was installed on the aquarium's side. Equipped with a diffuser, this pump ensures a constant and uniform air flow, eliminating dead zones and facilitating homogeneous oxygen distribution. This allows for adequate CO_2 and air dispersion, essential for photosynthesis and gas exchange, keeping the algae in suspension to maximize their exposure to light and nutrients without causing mechanical stress.

pH

A digital hydroponic meter was used to control the pH, maintaining a constant value of 7.5. This level is crucial as pH directly affects the biosorbent capacity of the biomaterial. At acidic pH, the functional groups on the cell walls become protonated, reducing biosorption efficiency, while at alkaline conditions, metals can precipitate, decreasing the metal's availability in solution. The removal of metal ions is more effective at a higher pH. Based on the robustness of Chlorella Vulgaris and previous results, a pH of 7.5 was chosen. Sodium bicarbonate was used to increase the pH and ascorbic acid to decrease it, both economical and safe methods for aquatic systems and microalgae cultures.

Temperature

A thermometer was installed to maintain the temperature controlled at 24 ± 2°C, an optimal range for C. Vulgaris growth, which typically varies between 20 and 30°C. Although an aquarium heater with an integrated thermostat was available to increase the temperature, it was not necessary as the temperature remained constant during the experiment.

Illumination

To promote photosynthesis within the bioreactor, low-intensity LED lighting with a photoperiod of 16 hours of light and 8 hours of darkness was used. This light regime is crucial to optimize photosynthesis and, therefore, maximize the system's contaminant removal capacity.

DO Measurement

Dissolved oxygen was measured using a professional analyzer that allows in-situ measurements. This parameter is crucial, not only as an indicator of the potential for aquatic life but also because it reflects the biological dynamics of the system, including photosynthesis and respiration processes. Measurement was particularly essential in this study, given that the initially contaminated water had low oxygen levels. The air pump incorporated into the system was adjusted to optimize gas exchange, with the goal of improving dissolved oxygen levels.

Evaporation

To minimize water evaporation, a transparent cover that allowed light to pass through, essential for photosynthesis, was used. The cover included an opening to facilitate gas exchange and prevent system overheating, thus ensuring optimal conditions within the bioreactor.

Placement of Agar-Agar Pearls in the Bioreactor

Determination of the Number of Pearls in the Bioreactor

To calculate the number of 5 mm diameter agar-agar pearls needed in a 2-liter bioreactor, the volume each pearl would occupy within the bioreactor was first estimated. It was considered important to leave additional space between the pearls to ensure optimal distribution and adequate access to light and nutrients. Therefore, a 20% extra space per pearl was calculated, allowing for the creation of two layers/levels of pearls at the bottom of the bioreactor – the area of the sphere's meridian was calculated. It is important to clarify that the calculated placement of the pearls is solely for initial quantity and volume calculations. Once the contaminated water was added to the bioreactor, the pearls began to disperse and float or suspend due to the aeration process.

Calculation of the Number of Pearls

<u>First Level (Bioreactor Base):</u>

The bioreactor used has base dimensions of 19 cm x 19 cm and a height of 14 cm (without filling completely).

- Base Area = Length x Width = 19 cm x 19 cm = 361 cm^2.
- Converting to mm^2, we obtain 36,100 mm^2.

To completely cover the bottom with 5 mm diameter pearls, maintaining a 1 mm separation between each pearl, approximately 1,276 pearls were required (see volume and surface calculations detailed below), equivalent to about 35 pearls per side.

<u>Second Level:</u>

Since the pearls in the second layer are placed intercalated with respect to the first, 34 pearls per side were used, totaling 1,156 pearls for this level.

In total, **2,432 pearls of 5 mm were placed in the bioreactor.**

<u>Addition of Water:</u>

After positioning the pearls, 2 liters of water were added for bioremediation.

<u>Note on Counting Methodology:</u>

Given the large number of produced spheres, counting them individually would be impractical. Therefore, an alternative method was implemented where the number of pearls per syringe was determined, and the total number of syringes needed to reach the desired number of pearls for the study was estimated. This method provided an efficient and accurate way to plan the production of spheres.

Calculation of Volume and Surface Area Occupied by an Agar-Agar Pearl

Pearl Diameter: 5 mm

<u>Meridian Area of a Pearl</u>

- Pearl Radius: r = 2.5 mm
- Area Formula: $\pi r^2 = 3.1416 \times (2.5\text{mm})^2 =$ **Area ≈ 19.635 mm^2**

<u>Meridian Area Considering Pearl Separation</u>

- Additional Separation: 1 mm
- Total Radius = (r + separation) = (2.5 mm + 0.5 mm) = 3 mm
- Area Formula with Separation = $\pi (r + 0.5\text{ mm})^2 = 3.1416 \times (3\text{ mm})^2 =$ **Area ≈ 28.27 mm^2**
- Bioreactor Surface Area / Pearl Area = 36,100 mm^2 / 28.27 mm^2 = **1,276 pearls**

Volume Calculation of a Pearl

- Volume Formula: $V = 4/3\ \pi\ r^3$
- Calculation: $V = 4/3\ \pi\ (2.5\ mm) = V \approx 4/3 \times 3.1416 \times 2.5^3\ mm^3$

$$V \approx 4/3 \times 3.1416 \times (15.625)\ mm^3 \approx \mathbf{26.18\ mm^3}$$

Notes

Meridian Area: Refers to the area of a cross-section through the center of the sphere.

Pearl Separation: Considers additional space around each pearl for calculating the area affected by an individual pearl in the bioreactor.

Pearl Volume: This value is crucial to determine how much space each pearl will occupy within the bioreactor and will help calculate the total number of pearls that can be placed.

Preparation of Agar-Agar Spheres

For the production of biospheres, a 2% agar-agar concentration was used. This concentration ensures adequate gelation, fundamental for the structural integrity of the spheres. In practical terms, this translates to 2 grams of agar-agar per 100 ml of solution.

Each pearl has a volume of 26.18 mm³. Considering that the production method is not industrial and is more prone to variations, a 15% deviation was estimated. This adjusts the average volume per pearl to 30 mm³. For the study, an initial total of 2,432 pearls was calculated. Additionally, a possible waste of 10% was anticipated, which increases the necessary number of <u>pearls to 2,675.</u>

Calculation of the Total Volume of Agar-Agar Solution

<u>Volume Requirement:</u> Multiplying 30 mm³ by 2,675 pearls, a total volume of 80,250 mm³ of prepared solution is needed.

<u>Solution Preparation:</u> To cover this requirement, 100 ml (100,000 mm³) of water with algae and 2 grams of agar-agar were used.

<u>Conclusion</u>: Meticulous preparation and considerations for deviations and waste are essential to ensure sufficient availability of biospheres for the study, thus guaranteeing consistency and reliability of the experiment.

Steps for Agar-Agar Sphere Preparation

- ✓ Agar-agar was dissolved in 50 ml of distilled water and the mixture was heated to boiling to ensure complete dissolution.
- ✓ The agar-agar solution was cooled by removing it from heat to a temperature that does not harm C. Vulgaris but keeps the agar-agar liquid. The temperature was 30°C.
- ✓ The Chlorella Vulgaris solution was incorporated into the agar-agar solution. It was quickly mixed to distribute the algae evenly in the solution and avoid exposing them too long at that temperature.
- ✓ Sunflower oil was cooled to a temperature of 4°C. It was then placed in an ice bath.
- ✓ The agar-agar solution was dropped into the cold oil using a 50 ml syringe. The density difference caused the solution drops to submerge and begin to solidify as they descended, forming spheres.
- ✓ The spheres were allowed to solidify completely in the oil. This took a few minutes. Once solidified, the spheres were recovered from the oil with a fine mesh strainer.
- ✓ Excess oil was removed from the spheres with distilled water.

Cell Concentration/ml

Previous studies and literature estimate the use of microalgae for the bioremediation of contaminated waters in a range that varies significantly, from an approximate and typical solution concentration of 10,000 cells/ml to 6,000,000 cells/ml. For this study, the total donated amount of Chlorella Vulgaris was used: 50 ml solution (40,000 cells/ml). Therefore, 2,000,000 cells were used. For the production of agar-agar spheres, 50 ml of distilled water was used, which was heated for the agar-agar dilution, combining both liquids resulted in 100 ml with a final concentration of 20,000 cells per ml. Since 2,432 pearls of 26.18 mm^3 were produced, only 63.66 ml were used, meaning 1,273,200 cells. The rest was kept refrigerated for future use.

Materials and Costs

Bioreactor Materials and Costs:

Transparent glass container: $5,000 + Lid: $1,700

Air pump for 20l with hose and diffuser: $5,480

Low-consumption LED lights: $6,956

Dissolved oxygen meter: Digital Oxygen Meter D09100. Borrowed for this study. (Kit O2 Profi test Oxygen with 50 tests): Approx. $15,000

pH meter: Digital pH meter for hydroponics: $9,831

Nitrite meter: Tropical test NO2 with 50 tests. Donated. Approx. $16,950

Thermometer: Rs Glass thermometer with suction cup: $2,020

Substances to adjust pH - sodium bicarbonate 50 gr: $500, ascorbic acid 50 gr: $1,925.

Total Bioreactor Cost: $65,362

Microbead Materials and Costs:

Microalgae Chlorella Vulgaris: Donated for this research (Available from universities, laboratories, public research organizations, and even the Japanese Garden. Market value unknown).

Agar-Agar: $2,062 for 10 gr

Distilled water: $1,100 for 2.5 liters

Sunflower oil 900 CC: $1,600

Inactive microalgae: Chlorella powder 50 gr: $24,000

Total Agar-Agar Beads with Active C. Vulgaris: $4,762

Total Agar-Agar Beads with Inactive C. Vulgaris: $28,762

Various Materials Used:

50 ml syringes: $1,700 each. Total 2 syringes: $3,400

Industrial gloves for handling contaminated water: $5,600 for two pairs

Medium funnel: $1,800

Protective goggles for handling contaminated water: $1,928

Face masks x 5: $1,000

Handheld plastic strainer: $1,515

Total Various Materials: $15,243

Total General with Active C. Vulgaris: $85,367

Total General with Inactive C. Vulgaris: $109,367

Results

This study evaluated the effectiveness of the microalga Chlorella Vulgaris, immobilized in agar-agar spheres, in the bioremediation of contaminated water from the El Clavel stream with industrial discharges. A specific bioreactor was designed for this purpose, and the results after six days of observation include:

- **Growth of Chlorella Vulgaris:** A significant 40% increase in Chlorella Vulgaris biomass was observed. This value represents the percentage difference between the biomass at the beginning (156 g) and the end of the study period (218 g). This increase evidence the effectiveness of immobilization in agar-agar to facilitate algal growth under controlled conditions.
- **Dissolved Oxygen (DO):** The initial level of 1.6 mg/l DO, which can cause hypoxia affecting biota, increased to 2.1 mg/l by the third day, recording a value of 5.4 mg/l at the end of the study, indicating active and efficient photosynthesis, with the algae consuming CO_2 and producing oxygen. ACUMAR establishes optimal values > 5 mg/l (See graph).
- **Water pH:** The initial pH of 8.5, attributed to industrial effluents from metalworking and paper industries using calcium oxide and sodium sulfide, which, when discharged as liquid effluents, tend to significantly increase the water's alkalinity, was adjusted and maintained at 7.5, decreasing the water's alkalinity.
- **Contaminant Removal:** During the study, a significant reduction in the concentration of various contaminants was observed. Biochemical Oxygen Demand (BOD) experienced a notable reduction of 75%, likely due to a constant increase in system aeration, a key factor for achieving this purification efficiency. Regarding nitrogen, ammoniacal nitrogen removal reached 93%, while total nitrogen showed an approximate reduction of 82%. Additionally, total phosphorus decreased by 62%. For heavy metals, significant biosorption was achieved, with a 90% reduction for copper and 96% for chromium. These results highlight the remarkable ability of the microalgae used in the study to effectively absorb both nutrients and contaminants, demonstrating their potential in the bioremediation of contaminated waters. (See graph)
- **Eutrophication:** The consumption of nitrogen and phosphorus by the algae contributed to the prevention of eutrophication, improving water quality and supporting a healthier aquatic ecosystem. The bioreactor utilized the algae's natural ability to absorb these nutrients, which in excess can cause eutrophication, depleting oxygen and destabilizing the aquatic ecosystem, contributing to more sustainable contaminated water treatment.
- **Nitrite Levels**: Nitrite levels in the study showed no significant changes. The technique used to measure these levels was colorimetry, which did not allow detection of slight variations due to the method's resolution. Initially, nitrite was recorded at 0.05 mg/l, according to the homemade test's color scale. In subsequent measurements, the color did not vary, remaining at the same level, with adjacent categories marked at 0 mg/l and 0.15 mg/l. Therefore, it was not possible to confirm whether there were slight changes in nitrite concentration through this method or the reliability of the test.
- **Heavy Metals:** The concentration of metals such as chromium and copper showed a significant decrease, with a reduction of up to 96%, demonstrating the method's effectiveness in heavy metal removal, contributing to a more sustainable and economical treatment methodology. Copper had an initial value of 0.10 mg/l, ending at <0.01 mg/l. Chromium started at a value of 0.25 mg/l, registering a final value of 0.01 mg/l at the end of the study. (See graph)

Trial 2: With Dry-Inactive Chlorella Vulgaris

The efficacy of inactive Chlorella Vulgaris, immobilized in agar-agar spheres, to remove heavy metals in contaminated water was evaluated. Microbeads with inactive and powdered microalgae were prepared and exposed to contaminated water in the same bioreactor. After 48 hours of treatment, the results were evaluated.

Research reveals that heavy metal adsorption begins 24 hours after exposure. This adsorption process is based on the physicochemical properties of the algal cell walls, which are endowed with functional groups such as carboxyl, amines, and phosphates. These groups exhibit a negative charge that attracts and sequesters positively charged metal ions in the water, resulting in their immobilization on the cell surface. This action mechanism does not require active metabolic activity, allowing even dried Chlorella Vulgaris cells to maintain a remarkable biosorption capacity.

Quantitatively, the study documented a 16% reduction in Chromium concentration and an 8% reduction in Copper. To ensure adsorption process efficacy and avoid phenomena like metal precipitation, the system's pH level was kept constant at 7.5 throughout the treatment period. This pH management facilitated an environment conducive to optimal interactions between the algal cell wall functional groups and the metal ions, maximizing biosorption capacity without altering the essential physicochemical conditions of the process. Although these results are promising, they highlight the need for further research to deepen the understanding of inactive Chlorella Vulgaris's capacity to remove heavy metals. The study demonstrates the potential of inactive algae for the bioremediation of heavy metal-contaminated waters. The interactions between metal ions and the algal cell wall functional groups indicate an effective and sustainable approach to contaminant treatment, providing a foundation for future applications in remediation technologies. (See graph)

Conclusions

This study effectively demonstrated that the microalga Chlorella Vulgaris, immobilized in agar-agar spheres, can bioremediate contaminated waters efficiently. Using a specially designed bioreactor, a notable decrease in levels of critical contaminants such as phosphorus, nitrogen, copper, and chromium was observed in just 6 days. These results are partly due to the agar-agar immobilization strategy, which not only protects the algae from adverse environmental conditions but also facilitates effective interaction with contaminants through adsorption and bioaccumulation processes. The algae's ability to absorb nutrients, such as nitrogen and phosphorus, significantly contributed to the potential reduction of the system's eutrophication. Additionally, the growth of algal biomass not only helps eliminate these nutrients from the water but also provides a usable biomass source for other industrial or environmental processes, leveraging their capacity to metabolize high concentrations of CO_2.

The agar-agar spheres used in this study offer multiple advantages: they maintain cell integrity, facilitate biomass recovery, and prevent accidental release of cells into the environment, minimizing the risk of additional eutrophication. This immobilization system also promotes robust cellular communication, crucial for the bioremediation process's effectiveness. In other words, the agar-agar pearls allow the algae to exchange chemical signals that coordinate their metabolism to more efficiently absorb contaminants. This work not only reaffirms the possibility of using Chlorella Vulgaris to remove heavy metals and nutrients in aquatic systems but also highlights the potential of this technique to be applied in field conditions, offering a sustainable and efficient solution for managing contaminated waters. Future trials will focus on validating the efficacy of this bioprocess on a larger scale and in different environmental contexts.

This research establishes an important precedent for the use of biotechnologies based on microalgae like Chlorella Vulgaris, demonstrating their effectiveness under controlled conditions and indicating a promising path toward broader applications in sustainable water resource management.

The Vital Role of a Web Platform: www.bioesferas.com

This project was enriched by the creation and maintenance of a dedicated web platform designed to record and document each step of the research in real-time. This initiative not only allowed for total transparency in progress but also provided a space for awareness and education about the global water issue.

The website, designed and developed by the project author, has served as a meeting point for the community interested in topics related to water quality and environmental sustainability. In addition to offering updates on ongoing work, it provided a platform for exchanging knowledge, ideas, and solutions among experts, academics, and activists dedicated to addressing water challenges.

In this space, previous biomimicry projects for water filtration of contaminated water were also highlighted, emphasizing the importance of nature-inspired innovation to find creative and sustainable solutions to contemporary problems.

For more information on the detailed development of this project, including more graphics, results, tables, and a thorough analysis of practical and theoretical aspects, please visit the website: www.bioesferas.com

Awareness Proposals

1. **Educational Workshops:** Educate the community about the importance of clean water and how contaminants affect health and the environment. Practical demonstrations of how to build and maintain home bioreactors using accessible and economical materials. Sessions on how microalgae work in bioremediation and how they can be cultivated and used locally.
2. **"Learning by Doing" Programs:** Encourage community participation by allowing residents to be directly involved in the construction and operation of home bioreactors. Explore the potential of wooden boxes and C. Vulgaris algae to purify community-involved streams in an innovative and sustainable manner. This future project aims to offer an accessible and effective solution, improving water quality and benefiting both communities and the environment.
3. **School Collaborations:** Work with local schools to integrate educational programs that teach children about sustainability and water treatment. School projects involving the creation and monitoring of small bioreactors. Science competitions focused on innovative methods to purify water.
4. **Awareness Campaigns:** Use local media and social networks to communicate community events and disseminate information about the importance of water treatment and how contamination affects daily life. Success stories and testimonies from community members who have seen improvements in their quality of life thanks to bioreactors.
5. **Partnerships with Local and Government Organizations:** Seek support from non-governmental organizations (NGOs), scientific entities, and local authorities that can provide funds, resources, or infrastructure to expand the bioremediation program's reach and effectiveness.

Introduction to the Biofilter Project

Implementing a biofiltration system using wooden boxes filled with Chlorella Vulgaris microalgae represents an innovative and sustainable strategy for the bioremediation of low-flow streams. This method is not only effective in water purification, removing contaminants and heavy metals, but also economical and easy to implement, making it ideal for communities with limited resources.

Biofilter System Design Details

Key Components

- **Wooden Boxes:** Water-resistant and durable wood, such as cedar or environmentally treated pine wood, to construct boxes 50 cm long, 30 cm wide, and 20 cm high. These dimensions can be adjusted according to the stream's specific needs and available space. **Microalgae Chlorella Vulgaris.**

Construction Process

1. **Box Fabrication:** Construct boxes according to dimensions and assemble each box using stainless steel screws. The boxes will have perforations on their sides to allow water flow through them.

a. **<u>Box Lining:</u>** To keep the algae inside the boxes and allow water flow, line the boxes with fine mesh or shade cloth. This will enable water passage while retaining the algae inside the box.
b. **<u>Internal Division:</u>** Install internal divisions in the boxes to create compartments that help distribute the algae evenly. This prevents them from accumulating in one area, ensuring more uniform exposure to the flowing water.

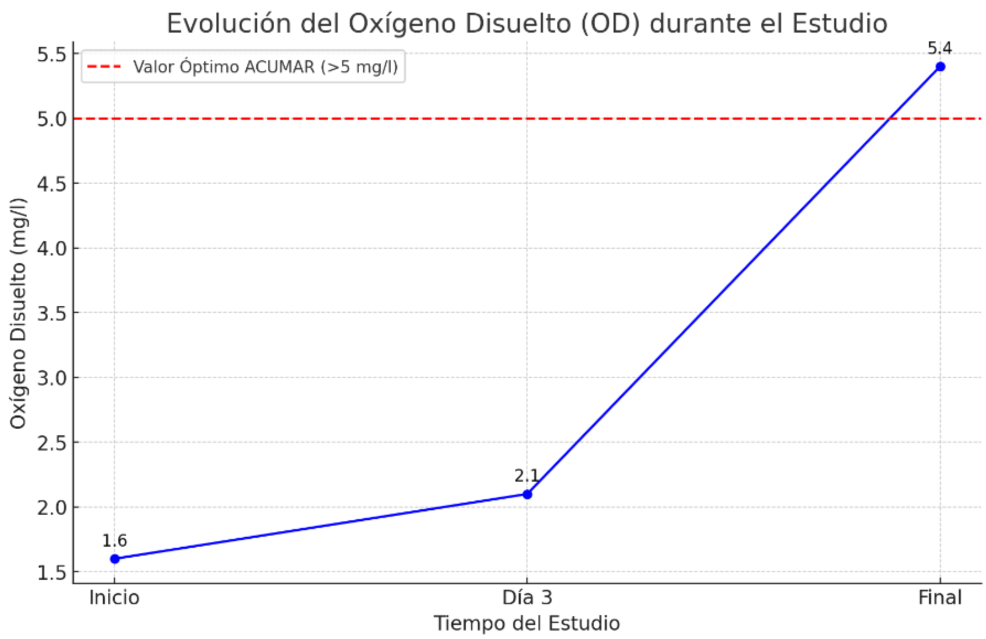
2. **<u>Algae Preparation</u>**: Start cultivating Chlorella Vulgaris in a controlled environment several weeks before installing it in the boxes.
    a. Use transparent tanks or containers under direct sunlight or artificial light to stimulate growth.
3. **<u>Installation in the Stream:</u>** Determine strategic points for placing the boxes, ensuring uniform distribution along the stream. The boxes should be placed so that the water flow passes through them, allowing water contact with the algae.
    a. **<u>Anchoring:</u>** Secure the boxes firmly to the shore or stream bed to prevent them from being carried away by the current.
4. **<u>Maintenance and Monitoring</u>**: Periodically check the boxes to ensure the algae are healthy and that the structures are not clogged with sediments or debris.
    a. **<u>Algae Replacement:</u>** Depending on the algae's efficacy and life cycle, determine the frequency of replacing them to maintain effective bioremediation operation.
5. **<u>Documentation and Improvement:</u>** Document changes in water quality before and after box installation to assess the system's effectiveness.
    a. **<u>Adjustments Based on Results:</u>** Adjust algae density, box configuration, or positioning based on results obtained to optimize the bioremediation process.

For more detailed information and to see the model, visit the website: www.bioesferas.com

GRAPHS

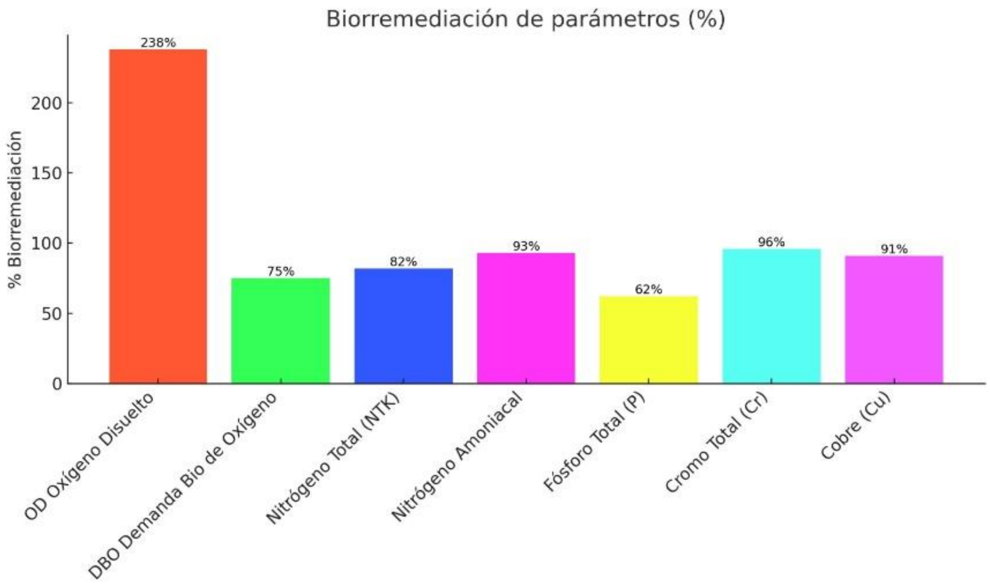
(See all complete graphs at www.bioesferas.com)

Evolution of Dissolved Oxygen During the Experiment



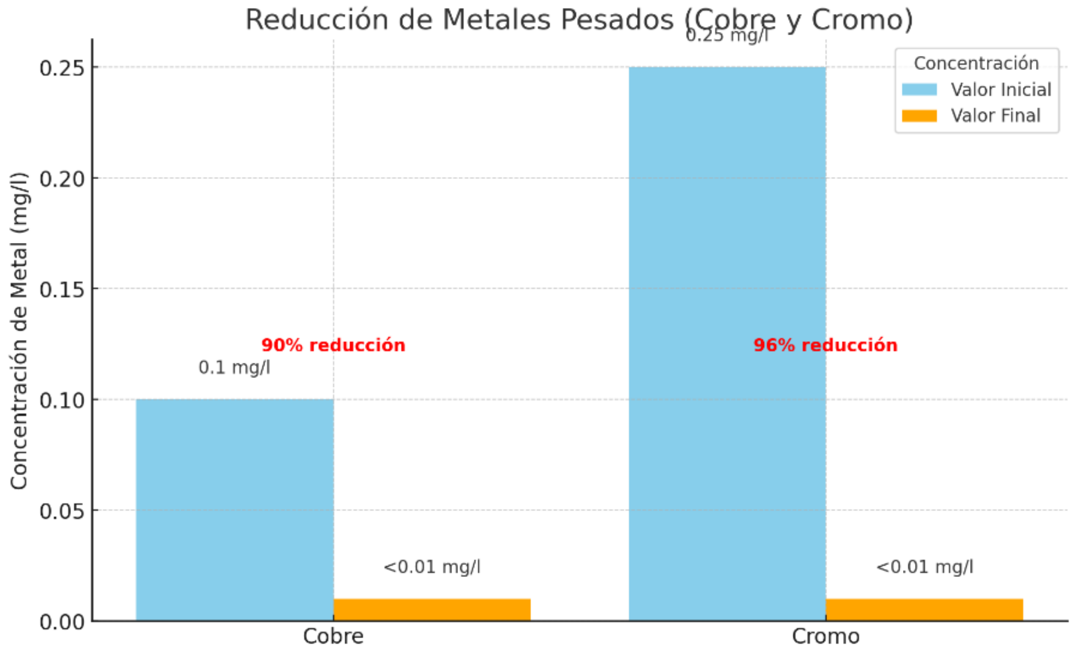
Graph showing improvement in dissolved oxygen levels from the beginning to the end of the study, indicating effective photosynthesis by the microalgae.

Contaminant Reduction



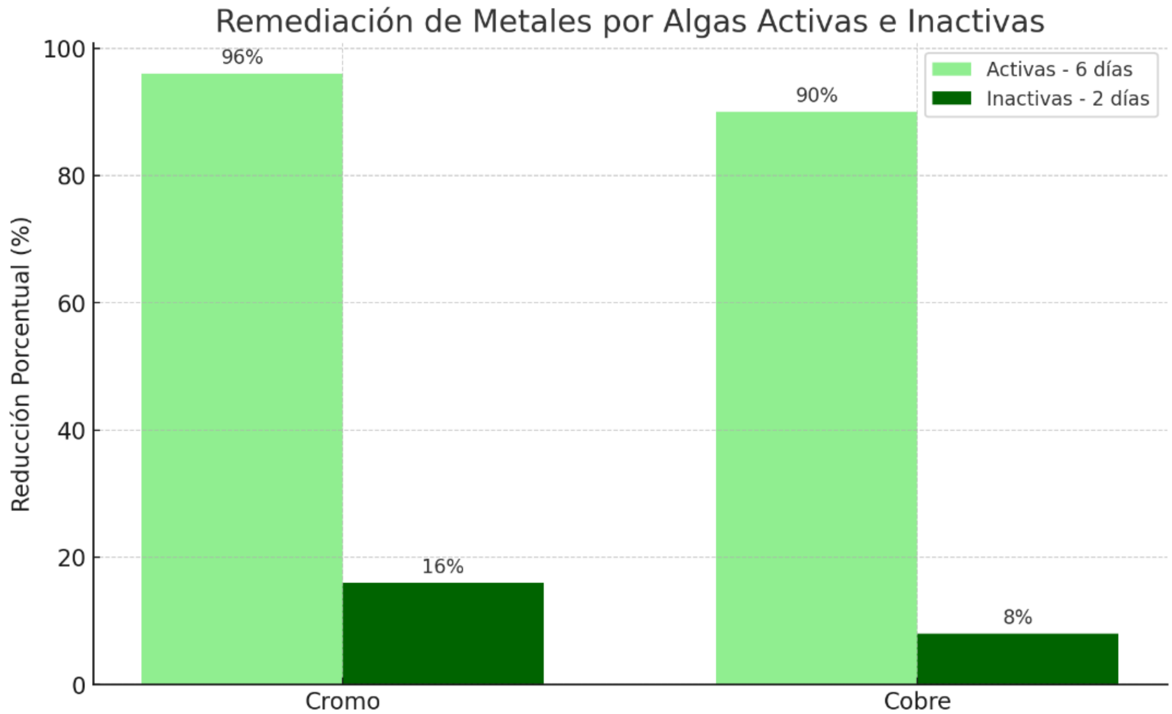
Graph highlighting the removal of various contaminants, contributing to improved water quality.

Heavy Metal Removal



Graph showing up to a 96% reduction in heavy metal concentrations, demonstrating the effectiveness of bioremediation with immobilized microalgae.

Metal Remediation: Active and Inactive Algae



Graph comparing metal removal efficacy between active and inactive algae.

TABLES

ACUMAR. Resolution 283/2019 (Annex A)

Parámetro	Unidad	1.a	1.b	Resultado Laboratorio M1	Resultado Laboratorio M2
OD Oxíg. Disuelto	Mg/l	>5	>5	1.6	5.4
DBO Demanda Bioq. Oxígeno	Mg/l	<5	<5	19.70	4.90
Nitrógeno Total (NTK)	Mg/l	S/D	S/D	14.98	2.73
Nitrógeno Amoniacal	Mg/l	<0.6	<0.6	11.70	0.79
Fósforo Total (P)	Mg/l	<0.01	<0.01	1.63	0.62
Cromo Total (Cr)	Mg/l	<0.02	<0.02	0.25	0.01
Cobre (Cu)	Mg/l	<0.09	<0.09	0.10	<0.01
Temperatura	°C	<35	<35	18.5	24
pH	UpH	6.5-9	6.5-9	8.5	7.5

ACUMAR (Authority of the basin Matanza Riachuelo) is an autonomous, self-governing, inter-jurisdictional entity formed by the Nation, the Province of Buenos Aires, and the City of Buenos Aires. Originally, the geographic boundaries of its control jurisdiction included the entire extent of Buenos Aires City and those municipalities of the Province of Buenos Aires that had part of their territory within the Basin. Subsequently, the geographic scope of the organization was reduced to territories physically related to the basin (Resolution No. 1113/2013). In its Resolution No. 283/2019 (Annex A), ACUMAR established its limits for permissible parameters in waters.

M1: Water sample from the El Clavel stream of the Luján River. Provincial Route No. 6, Buenos Aires Province.

M2: Post-bioremediation water sample from the El Clavel stream of the Luján River. Provincial Route No. 6, Buenos Aires Province.

Laboratory Results

BELQUIM LABORATORIO
Análisis y Mediciones Industriales

O.P.D.S Laboratorio Habilitado Nº 110
RELADA Laboratorio Habilitado Nº 36
SEDE Bs As: Carlos Pellegrini 1034/36 Vicente López
Sede Neuquen: Chaele Choel, Casa 3 Lote Z2 Ciudad Neuquen

Protocolo Nº **112684** Lugar **Efluente crudo MUESTRA 1**

RESULTADOS DEL ANALISIS

PARÁMETRO	MÉTODO DE ANÁLISIS	UNIDAD	LQM	RESULTADOS
DBO	SM-5210-B	mg/l	10,00	19,70
Nitrógeno Total (NTK)	SM 4500 Norg - B	mg/l	1,00	14,98
Nitrogeno amoniacal	SM 4500 NH3- F	mg/l	0,10	11,70
Cromo total (Cr)	SM 3111 B	mg/l	0,01	0,25
Cobre (Cu)	SM 3111 B	mg/l	0,01	0,10
Fosforo total (P)	SM 4500 PO43- B/C	mg/l	0,10	1,63

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Protocolo Nº **112685** Lugar **Efluente Tratado MUESTRA 2**

RESULTADOS DEL ANALISIS

PARÁMETRO	MÉTODO DE ANÁLISIS	UNIDAD	LQM	RESULTADOS
DBO	SM-5210-B	mg/l	10,00	4,90
Nitrógeno Total (NTK)	SM 4500 Norg - B	mg/l	1,00	2,73
Nitrogeno amoniacal	SM 4500 NH3- F	mg/l	0,10	0,79
Cromo total (Cr)	SM 3111 B	mg/l	0,01	0,01
Cobre (Cu)	SM 3111 B	mg/l	0,01	<0,01
Fosforo total (P)	SM 4500 PO43- B/C	mg/l	0,10	0,62

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See the full PDF of the laboratory analysis at www.bioesferas.com

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<u>Legal and confidentiality notice:</u>

- The sending of the sample and the analysis report of laboratory results with active microalgae were carried out by me, author of the project, in a private capacity, recording the requested data under the name of my legal guardian due to my minority.
- On the other hand, the studies with inactive microalgae had the financial support of a local law firm, who covered the costs associated with sending results to the laboratory. For reasons of confidentiality agreed with said study, the specific details of these results with inactive microalgae cannot be disclosed, for this reason only their percentages are shown.