UNIVERSIDAD REGIONAL AMAZÓNICA IKIAM

FACULTAD DE CIENCIAS DE LA VIDA

INGENIERÍA EN BIOTECNOLOGÍA

INACTIVATION OF POTENTIALLY TOXIC CYANOBACTERIA BY PHOTOCATALYTIC BISMUTH OXYIODIDE MICROSPHERES

Proyecto de investigación previo a la obtención del Título de:

INGENIERA EN BIOTECNOLOGÍA

AUTORA: VIRGINIA DEL CARMEN RIVADENEIRA ARIAS

TUTOR: PhD. MIGUEL HERRERA ROBLEDO

Napo – Ecuador

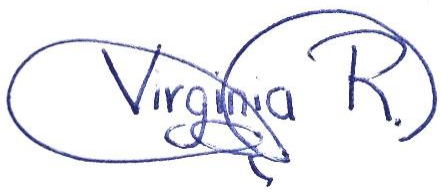
2023

DECLARACIÓN DE DERECHO DE AUTOR, AUTENTICIDAD Y RESPONSABILIDAD

Yo, Virginia del Carmen Rivadeneira Arias con documento de identidad N° 1550018251, declaro que los resultados obtenidos en la investigación que presento en este documento final, previo a la obtención del título de Ingeniería en Biotecnología, son absolutamente inéditos, originales, auténticos y personales.

En virtud de lo cual, el contenido, criterios, opiniones, resultados, análisis, interpretaciones, conclusiones, recomendaciones y todos los demás aspectos vertidos en la presente investigación son de mi autoría y de mi absoluta responsabilidad.

Tena, 11 de mayo de 2023



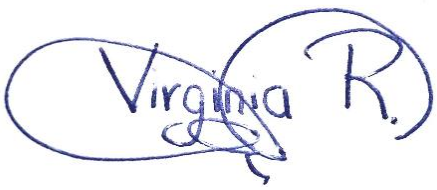
Virginia del Carmen Rivadeneira Arias

AUTORIZACIÓN DE PUBLICACIÓN EN EL REPOSITORIO INSTITUCIONAL

Yo, VIRGINIA DEL CARMEN RIVADENEIRA ARIAS, con documento de identidad Nº 1550018251, en calidad de autora y titular de los derechos morales y patrimoniales del trabajo de titulación: INACTIVATION OF POTENTIALLY TOXIC CYANOBACTERIA BY PHOTOCATALYTIC BISMUTH OXYIODIDE MICROSPHERES de conformidad con el Art.114 del CÓDIGO ORGÁNICO DE LA ECONOMÍA SOCIAL DE LOS CONOCIMIENTOS, CREATIVIDAD E INNOVACIÓN, reconozco a favor de la Universidad Regional Amazónica Ikiam una licencia gratuita, intransferible y no exclusiva para el uso no comercial de la obra, con fines estrictamente académicos.

Así mismo autorizo a la Universidad Regional Amazónica Ikiam para que realice la publicación de este trabajo de titulación en el Repositorio Institucional de conformidad a lo dispuesto en el Art. 144 de la Ley Orgánica de Educación Super.

Tena, 11 de mayo de 2023



Virginia del Carmen Rivadeneira Arias

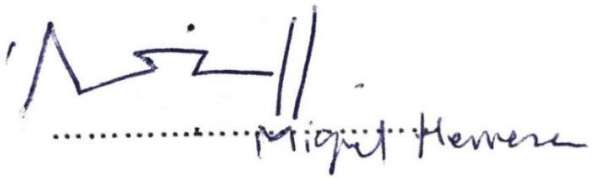
1550018251

CERTIFICADO DE DIRECCIÓN DE TRABAJO DE INTEGRACIÓN CURRICULAR

Certifico que el trabajo de integración curricular titulado: **Inactivation of potentially toxic cyanobacteria by photocatalytic bismuth oxyiodide microspheres**, en la modalidad de: artículo, fue realizado por: Virginia del Carmen Rivadeneira Arias, bajo mi dirección.

El mismo ha sido revisado en su totalidad y analizado por la herramienta de verificación de similitud de contenido; por lo tanto, cumple con los requisitos teóricos, científicos, técnicos, metodológicos y legales establecidos por la Universidad Regional Amazónica Ikiam, para su entrega y defensa.

Tena, 11 de mayo de 2023



Miguel Herrera Robledo

Pasaporte: G07498271

AGRADECIMIENTOS

Agradezco a mi familia, en especial a mi madre Juana Arias, mi abuela Virginia Escobar y mi tía Esperanza Arias, quienes me apoyaron económica, moral y emocionalmente lo que ha permitido culminar esta etapa tan importante de mi vida.

Agradezco infinitamente al doctor Miguel Herrera Robledo, tutor de este trabajo y director del proyecto CEPRA XVI-2022-21 DEGRADACION CONTAMINANTES, quien me involucró en el campo de la Nanotecnología, confió en el potencial desarrollo de este trabajo y me acogió dentro de su grupo de investigación y. Al profesor Miguel Quishpe por el seguimiento durante todo el proceso.

Al proyecto NB-LAB en especial a Michael Suárez quien me guio en cada uno de los procesos de la realización de este trabajo. A todos mis compañeros y compañeras del grupo de investigación por el apoyo constante e intercambio de conocimientos.

De la misma forma agradezco a Evelyn Oña, Carolina Castro, Carolina Ñacato, Giovanna Moran, Anghela Reinoso y Andrea Salgado, técnicas de laboratorios de la Universidad Regional Amazónica Ikiam por su guía fundamental en la parte metodológica para llevar a cabo este trabajo y cumplir a cabalidad los objetivos planteados.

A mi fiel amigo Cristian Sillagana quien con sus consejos y apoyo desde el primer momento que entre a estudiar en la universidad hizo de mi estancia una aventura y de mis objetivos un reto que motivaron la culminación de este trabajo.

Este trabajo se realizó gracias a la Corporación Ecuatoriana para el Desarrollo de la Investigación y la Academia (CEDIA) a través del proyecto CEPRA XVI-2022-21 DEGRADACION CONTAMINANTES y al programa Erasmus + CBHE mediante el proyecto NB LAB (Número de contrato: 619346–EPP–1–2020–1–DE–EPPKA2–CBHE–JP).

ÍNDICE GENERAL

ÍNDICE DE FIGURAS

ÍNDICE DE TABLAS

ÍNDICE DE ANEXOS

RESUMEN

El progresivo deterioro de las aguas superficiales por floraciones de cianobacterias se ha convertido en algo habitual en masas de agua. En este sentido, la fotocatálisis heterogénea surge como herramienta potencial que aprovecha la radiación solar para inactivar microorganismos patógenos presentes en efluentes de Plantas de Tratamiento de Aguas Residuales (PTAR). En ese contexto, el objetivo del presente trabajo fue evaluar la eficacia de las microesferas de BiOI y $Bi_4O_5I_2$ en la inactivación de cianobacterias potencialmente tóxicas bajo luz visible. En primer lugar, se realizó un muestreo en la PTAR de la Universidad Regional Amazónica Ikiam. A continuación, en un medio específico BG-11 para cianobacterias se aisló la especie *Microcystis* sp. según estudios morfológicos. Para confirmar este género se amplificaron los genes 16S, mcyA y ficocianina. Simultáneamente, se masificó la cianobacteria en matraces de 1L que contenían medio de cultivo y bajo las siguientes condiciones: aireación constante, fotoperiodo (16:8), 30W de intensidad de luz; para análisis molecular y fotocatalítico. Finalmente, se realizaron las evaluaciones fotocatalíticas aplicando 50mg de microesferas BiOI en 150ml de cultivo de cianobacterias, el mismo experimento se realizó con $Bi_4O_5I_2$. Los resultados de la identificación tanto morfológica como molecular afirmaron la presencia de cianobacterias potencialmente tóxicas de la especie *Microcystis* sp. por la expresión del gen mcyA, el cual es el encargado de producir cianotoxinas. Por otro lado, los resultados de las pruebas de fotocatálisis demostraron que los fotocatalizadores BiOI y $Bi_4O_5I_2$ son eficientes para la inactivación celular de cianobacterias con porcentajes del 92.97% y 95.49%, respectivamente. En conclusión, el proceso fotocatalítico de las microesferas de BiOI y $Bi_4O_5I_2$ redujo la presencia de cianobacterias potencialmente tóxicas y, por tanto, se propone como un tratamiento complementario del agua residual.

Palabras clave: BiOI; $Bi_4O_5I_2$; cianobacteria; fotocatálisis; *Microcystis* sp.

ABSTRACT

The progressive deterioration of surface waters by cyanobacterial blooms has become a common occurrence in water bodies. In this sense, heterogeneous photocatalysis emerges as a potential tool that takes advantage of solar radiation to inactivate pathogenic microorganisms present in effluents from wastewater treatment plants (WWTP). In this context, the aim of the present work was to evaluate the efficacy of BiOI and $Bi_4O_5I_2$ microspheres in the inactivation of potentially toxic cyanobacteria under visible light. First, sampling was performed at the WWTP of the Ikiam Amazon Regional University. Then, in a specific BG-11 medium for cyanobacteria, the species *Microcystis* sp. was isolated according to morphological studies. To confirm this species, the 16S, mcyA and phycocyanin genes were amplified. Simultaneously, cyanobacteria were massaged in 1L flasks containing culture medium and under the following conditions: constant aeration, photoperiod (16:8), 30W light intensity; for molecular and photocatalytic analysis. Finally, photocatalytic evaluations were performed by applying 50mg of BiOI microspheres in 150ml of cyanobacteria culture, the same experiment was performed with Bi4O5I2. The results of both morphological and molecular identification affirmed the presence of potentially toxic cyanobacteria of the species *Microcystis* sp. by the expression of the mcyA gene, which is responsible for producing cyanotoxins. On the other hand, the results of the photocatalysis tests showed that the BiOI and $Bi_4O_5I_2$ microspheres are efficient for the cell inactivation of cyanobacteria with percentages of 92.97% and 95.49%, respectively. In conclusion, the photocatalytic process of BiOI and Bi4O5I2 microspheres reduced the presence of potentially toxic cyanobacteria and is therefore proposed as a complementary wastewater treatment.

Keywords: BiOI; $Bi_4O_5I_2$; cyanobacteria; photocatalysis; *Microcystis* sp.

Inactivation of potentially toxic cyanobacteria by photocatalytic bismuth oxyiodide microspheres

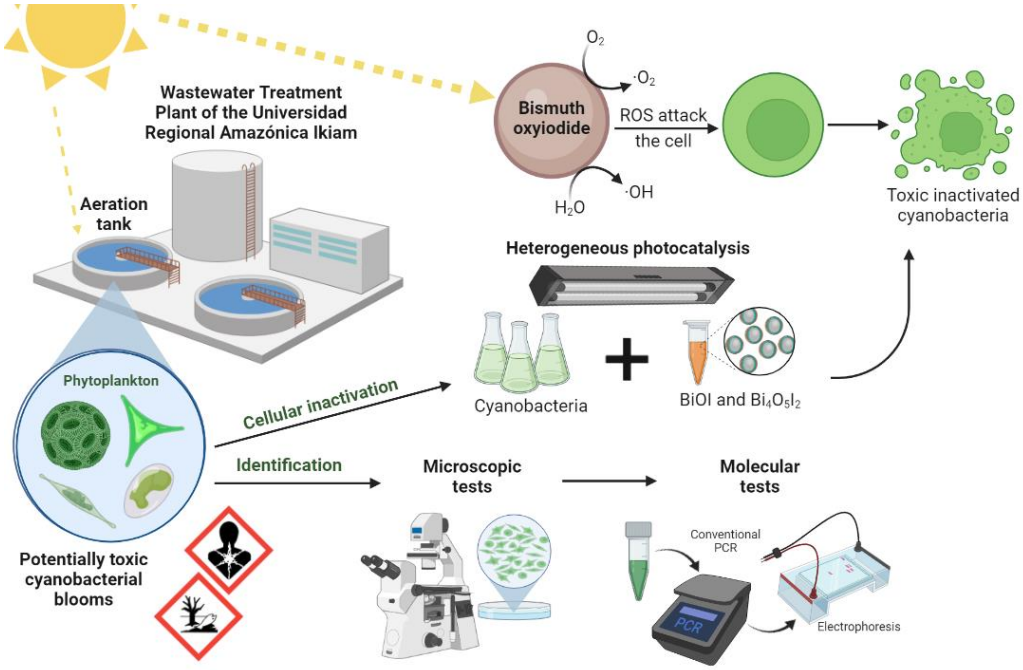
Virginia D.C. Rivadeneira[1], Michael Zuarez-Chamba[2], Miguel Quishpe[3], Miguel Herrera-Robledo[3*]

(1) Facultad de Ciencias de la Vida, Universidad Regional Amazónica Ikiam, Napo-Tena, Ecuador.

(2) Instituto de Nanosistemas, Universidad Nacional de San Martin, Avenida 25 de Mayo 1021, General San Martín, Provincia de Buenos Aires, Argentina

(3) Facultad de Ciencias Socio Ambientales, Universidad Regional Amazónica Ikiam, Napo-Tena, Ecuador.

Graphical abstract



* Corresponding autor

E-mail address: miguel.robledo@ikiam.edu.ec (Miguel Herrera-Robledo)

Abstract

The progressive deterioration of surface waters by cyanobacterial blooms has become a common occurrence in water bodies. In this sense, heterogeneous photocatalysis emerges as a potential tool that takes advantage of solar radiation to inactivate pathogenic microorganisms present in effluents from wastewater treatment plants (WWTP). In this context, the aim of the present work was to evaluate the efficacy of BiOI and $Bi_4O_5I_2$ microspheres in the inactivation of potentially toxic cyanobacteria under visible light. First, sampling was performed at the WWTP of the Ikiam Amazon Regional University. Then, in a specific BG-11 medium for cyanobacteria, the species *Microcystis* sp. was isolated according to morphological studies. To confirm this species, the 16S, mcyA and phycocyanin genes were amplified. Simultaneously, cyanobacteria were massaged in 1L flasks containing culture medium and under the following conditions: constant aeration, photoperiod (16:8), 30W light intensity; for molecular and photocatalytic analysis. Finally, photocatalytic evaluations were performed by applying 50mg of BiOI microspheres in 150ml of cyanobacteria culture, the same experiment was performed with Bi4O5I2. The results of both morphological and molecular identification affirmed the presence of potentially toxic cyanobacteria of the species *Microcystis* sp. by the expression of the mcyA gene, which is responsible for producing cyanotoxins. On the other hand, the results of the photocatalysis tests showed that the BiOI and $Bi_4O_5I_2$ microspheres are efficient for the cell inactivation of cyanobacteria with percentages of 92.97% and 95.49%, respectively. In conclusion, the photocatalytic process of BiOI and Bi4O5I2 microspheres reduced the presence of potentially toxic cyanobacteria and is therefore proposed as a complementary wastewater treatment.

Keywords: BiOI; $Bi_4O_5I_2$; cyanobacteria; photocatalysis; *Microcystis* sp.

1. INTRODUCTION

The progressive deterioration of surface waters around the globe has been observed for many years. Toxic cyanobacterial blooms have become a common occurrence in water bodies around the world [1], as a consequence of eutrophication and climate change, posing a serious threat to the local economy based on tourism or fishing [2]. Cyanobacteria (blue-green algae) are gram-negative photoautotrophic bacteria that are common members of the freshwater phytoplankton community. Their presence in drinking water is therefore of concern because of their ability to produce toxins and odors that can significantly impair water quality [3].

To identify and monitor cyanobacteria and their toxins in WWTPs, water utilities use a variety of techniques such as microscopy, flow cytometry, ELISA, chemoanalytical methods and, more recently, molecular techniques [4]. Conventional PCR amplifies genes present in potentially toxic cyanobacteria, such as 16S rDNA, mcyA and phycocyanin [5]. Compared to morphological identification that recognizes the genus, molecular identification by sequencing can discover new species [6]. Among the cyanobacterial genera that release cyanotoxins are the following *Microcystis*, *Anabaena*, *Planktothrix* and *Nostoc* [7]. Cyanobacteria of the genus *Microcystis* are the main producers of cyanotoxins such as microcystin (MC). This type of cyanotoxins are cyclic heptapeptides possessing five invariant peptides (Adda-Glu-Mdha-Ala-MeAsp) and two XY variant peptides (LR, RR, YR, LY, LW, LF). Among these variants, microcystin-LR (MC-LR) is the most toxic and its main producer species is *Microcystis aeruginosa* [8].

Physical (e.g., ultrasound, adsorption, UV irradiation and membrane separation), chemical (e.g., chemical algaecides, coagulation and flocculation) and microbial (e.g., bacteria, fungi and protozoa) technologies have been developed and applied to eliminate these types of cyanobacteria [2]. Although these methods have shown promise in cyanobacteria removal, they have limitations, such as high operating costs and low capacity to remove cyanobacterial secondary metabolites. In this regard, advanced oxidation processes (AOPs) have been considered as a potential alternative to the aforementioned technologies, as they have several advantages, such as high efficiency, lower costs and positive contribution to

the environment [9]. Among AOPs, heterogeneous photocatalysis stands out for its ability to harness solar radiation, which is a renewable energy source [2].

Heterogeneous photocatalysis is a process based on the use of semiconductor materials and sunlight as an energy source. The interaction of light with the semiconductor material favors the generation of an electron-hole pair, which can participate in a variety of redox reactions leading to the formation of reactive oxygen species (ROS) such as hydroxyl radical, perhydroxyl radical, hydrogen peroxide and superoxide. ROS, due to their high oxidative capacity, can degrade and mineralize organic compounds (e.g., dyes, cyanotoxins) to carbon dioxide and water [10]. In recent years, much work has been done with titanium oxide (TiO_2)-modified materials. However, since this material works with ultraviolet light, the development and application of materials that work with visible light is necessary [11], as the three-dimensional structures of bismuth oxyiodide (BiOI).

BiOI three-dimensional microspheres, due to their morphology, have a high specific surface area, the large specific surface area could provide more active sites and collect light efficiently during the photocatalytic process and increase ROS, which are the key factors for the enhancement of photocatalytic activity [12]. Although these types of microspheres have been studied for the degradation of organic compounds, their efficiency has not been evaluated in the inactivation of potentially toxic cyanobacteria. Therefore, the objective of this work was to evaluate the efficiency of BiOI and $Bi_4O_5I_2$ microspheres in the inactivation of potentially toxic cyanobacteria under visible light guaranteeing a sustainable and economic technology that ensures the recreational use of water.

2. MATERIALS AND METHODS

2.1. Sampling Site

The sample of visible cyanobacterial blooms at the Wastewater Treatment Plant (WWTP) of the Ikiam Amazon Regional University was collected on December 13, 2021, from the following sampling site: aeration tank (S 00°57.106', W 77°51.769'). The collection site flows

into the Tena River, which is the water intake cradle of the Muyuna sector, where in 2021 inhabitants of the sector reported the presence of a large algal bloom (Fig. 1), which resulted in a breakdown of the wastewater treatment system.

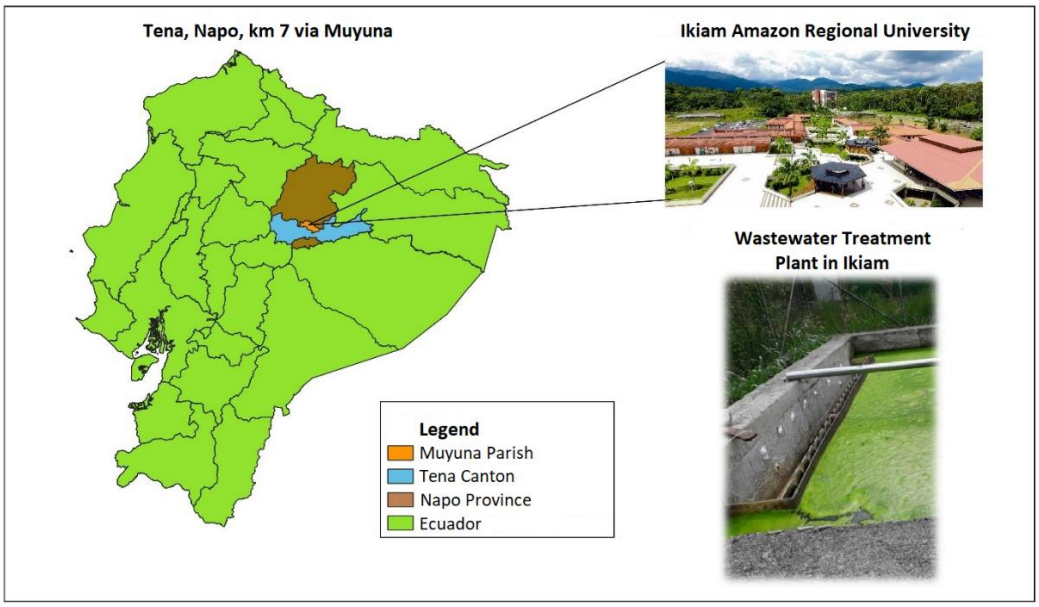


Figure 1. Wastewater Treatment Plant of the Ikiam Amazon Regional University.
Made by: Rivadeneira-Arias, 2023

The water sample was collected approximately 5 to 10 inches (1 inch = 2.54 cm) below the water surface in 1-liter, wide-mouth, amber-colored, autoclaved, screw-capped, wide-mouth, glass bottles [13]. The samples were not transported in a cold chain since the distance to the laboratory was close, and were processed the same day.

2.2. Isolation and Culture Conditions

Once the sampling was performed at the WWTP. For isolation, a selective medium BG-11 (blue-green medium 11) was prepared in solid medium with 1.5% agar. The water sample was then inoculated in Petri dishes using the striated depletion technique, and incubated for a period of 21 days under the following conditions: room temperature, LED lamp illumination at a low intensity of 30W, photoperiod 16h light/8h dark (16:8). At the end of the established time, replicates were carried out consecutively until the strains were pure, thus obtaining axenic cultures [14], [15].

2.3. Microscopic Identification

For the identification of the isolated strains, an optical microscope was used for 40x and 100x visualization. For the phenotypic characterization of the phytoplanktonic species, the following taxonomic keys were used [16]–[20].

On the other hand, to differentiate cyanobacteria from microalgae, a gram staining was performed, which consisted of staining the sample with different dyes that allowed staining its cell wall and distinguishing it from microalgae [21].

Subsequently, after microscopic analysis of the isolated (axenic) strains, they were inoculated in 250 mL flasks for 15 days under the following conditions: 100mL of BG-11 with a pure axenic agar slice, constant aeration, photoperiod (16:8), light intensity of 30W. Inoculated consecutively in a ratio (1:1). 20 days after inoculation, the 200ml of biomass was transferred to a 500ml flask, adding 200ml of fresh BG-11 medium. Then 15 days later, the 400 ml of biomass was transferred to a 1L flask with 400 ml of fresh BG-11 obtaining a final volume of 800 ml of biomass with a chlorophyll a concentration of 100µg/L [14].

2.4. Molecular Identification

The extraction of bacterial genomic DNA was performed using the commercial kit "Wizard® Genomic DNA Purification Kit" according to the protocol proposed by several authors [22]–[24]. The concentration and purity of the extracted DNA was determined using NanoDrop™ (Thermo Scientific).

DNA extractions were amplified by conventional PCR using the GoTaq® Flexi DNA Polymerase kit (PROMEGA), reactions were carried out in a final volume of 25µL: 0.5 X buffer green, 1.5 mM $MgCl_2$, 0.1mM each dNTPs, 0.5µM of each primer, 0.625 U GoTaq polymerase and 2 µL of DNA (>100 ng/µL) [25].

The 27F and 809R primers were used for general cyanobacterial amplification. MSF and MSR primers examined the potential toxigenicity of cyanobacteria [26], as well as PCβF and PCαR [27] The primer sequences and thermal profiles are listed in Table 1.

Table 1. Primers used in this study and thermal profiles

Gene region and primer	Sequence 5' to 3'	Thermal profile				
		ID	**D**	**A**	**E**	**FE**
16S rDNA:		1 cycle	35 cycles			1 cycle
27F	AGAGTTTGATCCTGGCTCAG	94ºC 2min	94ºC 10s	64ºC 20s	72ºC 1min	72ºC 5min
809R	GCTTCGGCACGGCTCGGGTCGATA					
mcyA TNM:		1 cycle	30 cycles			1 cycle
MSF	ATCCAGCAGTTGAGCAAGC	95ºC 2min	95ºC 1.5min	58ºC 30s	72ºC 50s	72ºC 7min
MSR	TGCAGATAACTCCGCAGTTG					
Phycocyanin:		1 cycle	40 cycles			-
PCβF	GGCTGCTTGTTTACGCGACA	94ºC 5min	94ºC 20s	50ºC 30s	72ºC 1min	-
PCαR	CCAGTACCACCAGCAACTAA					

ID: initial denaturation; D: denaturation; A: annealing; E: extension; FE: final extension
Made by: Rivadeneira-Arias, 2023

PCR products were separated following normal procedures by 1 % agarose gel electrophoresis in Tris-borate-EDTA (TBE) buffer for the 16S and mcyA genes. While for the phycocyanin protein gene it was performed at 2.5% agarose in TBE buffer. The gels were photographed after being clarified and contrasted [25]–[27]. The following equation was used to calculate the percentage of potential toxigenicity [28]:

$$(Eq.1) \qquad Percent\ potential\ toxigenicity = \frac{toxic\ sample}{total\ samples} \times 100\%$$

2.5. Synthesis of Bioi and $Bi_4O_5I_2$ Microspheres

The BiOI and $Bi_4O_5I_2$ microspheres were synthesized by the solvothermal method proposed by Zuarez (2022). In the process for the synthesis of BiOI, a solution A was prepared by dissolving 3 mmol (1.455 g) of $BiNO_3 * 5H_2O$ in 30 ml of ethylene glycol with sonication for 30 min, and then stirred constantly (500 rpm) for 30 min. Simultaneously, a solution B was prepared by dissolving 3 mmol (0.498 g) of KI in 30 ml of ethylene glycol under continuous stirring (500 rpm). Then, solution B was added dropwise (1 ml/min) to solution A under constant stirring. The mixed solution was left for 30 min under constant stirring and then transferred to a 100ml teflon-lined autoclave. The autoclave was placed in a reactor and then placed in an oven and heated at 126 °C for 18 h. After being cooled to room temperature, the resulting precipitate was collected by vacuum filtration and washed with deionization water and ethanol. Finally, the product was dried at 60 °C for 24 h. $Bi_4O_5I_2$ microspheres were obtained by calcining BiOI microspheres previously obtained by the solvothermal method at a temperature of 160 °C and 3h of reaction [29].

2.6. Inactivation of Potentially Toxic Cyanobacteria

The experiments were evaluated in a 40cm x 60cm box, inside was a 250ml Erlenmeyer flask as a batch photoreactor with a 400W lamp. The BG-11 medium was changed to 0.9% saline solution by centrifugation to discard the residual medium. The reason was because we only wanted to know the inactivation behavior against cyanobacteria as a biological contaminant and that there was no interference with other compounds that could alter the effect to be identified. In the flask, 50mg of BiOI were deposited in a volume of 150mL of potentially toxic cyanobacteria biomass (100µg/L of chlorophyll a) with constant agitation for 11 h. It was subjected to 1 hour of darkness to allow the necessary time for the microspheres to mix with the biomass, and the remaining time in the presence of visible light at a distance of 20cm from the lamp with the experiment. Under the same conditions, another batch was carried out with the $Bi_4O_5I_2$ microspheres, and as a negative control without microspheres. The amount of chlorophyll a that decreased during the 11h was quantified as an indicator of cell inactivation [30]. Aliquots of 10 ml of the sample were taken and then filtered with the vacuum filtration system on glass fiber membranes (0.47 µm), this membrane was placed in 15ml falcon tube with 6ml of acetone and refrigerated overnight at 4°C (5-18 hours) for

chlorophyll a extraction. For subsequent reading in UV-VIS spectrophotometer, the extract was placed in a 1 cm optical path cuvette and the data of the whole spectrum was taken on a USB. Finally, the following formula is used to calculate chlorophyll a [31]:

$$(\mathrm{Eq.}\,2) \qquad (\mathrm{mg/L}) = \frac{[11.85(OD_{664} - OD_{750}) - 1.54(OD_{647} - OD_{750}) - 0.08(OD_{630} - OD_{750})]V_1}{2V_2 L}$$

Where:

OD is the optical density, V1 represents the volume of the extract (mL), V2 is the volume of the water sample (mL), and L is the optical path of the cuvette (cm).

Finally, to determine the inactivation percentage of each microspheres used, the following equation was used [32]:

$$(Eq.3) \qquad Percentage\ of\ inactivation\ of\ cyanobacteria = \frac{Ci - Cf}{Ci} \times 100\%$$

Where Ci represents the initial chlorophyll a concentration (µg/L), Cf the final chlorophyll a concentration (µg/L).

3. RESULTS AND DISCUSSION

3.1. Morphological Characterization of Cyanobacterial Isolates

Figure 2A shows the cyanobacterial colonies after one week, while Figure 2B after one month. It can be observed macroscopically that the colonies acquire a green pigmentation after one month, so it is in its exponential growth.

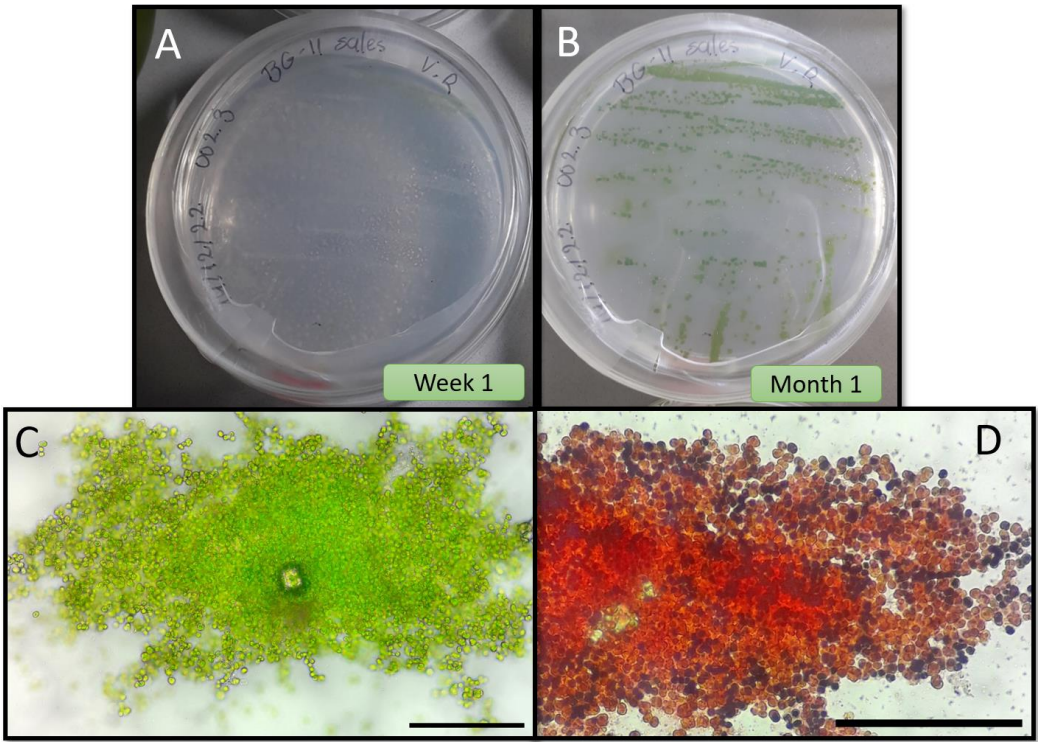


Figure 2. Colonies of *Microcystis* sp. on a 1.5% w/v agar plate with BG-11 medium A) after 1 week of incubation, B) after 1 month of incubation. C) Micrographs of *Microcystis* sp. under light microscope without gram staining, D) with gram staining; scale bar = 80 µm.
Made by: Rivadeneira-Arias, 2023

According to the catalogs used for morphological identification, Figure 2C shows a group of spherical to subspherical colonies, in the form of sacs or bags, forming macroscopic colonies. It is seen to be composed of subcolonies or clustered with mucilage present. The mucilage is thin and colorless. Its cellular content is rarely homogeneous and light green to pale gray, such as the species *Microcystis* sp. which usually presents with numerous gas vesicles [17], [18]. Since their morphology is similar to other microalgae species such as *Chlorella vulgaris*, a staining was performed which was positive for gram-negative bacteria due to the reaction to stain red (Fig. 2D) and was expected for cyanobacterial colonies of the species *Microcystis* sp. [33].

3.2. Molecular Characterization

The Wizard® DNA extraction kit used in this work gave a good yield, as the amount of DNA and the level of DNA purification were good at the A260/A230 ratio and poor at the A260/A280 ratio.

Table 2. DNA concentration of the three flasks used in molecular assays

# Flask	Concentration (ng/µl)	A260/A280	A260/A230
001	44,6	1,89	1,65
002	308,4	1,80	1,18
003	221,1	1,85	1,26

Made by: Rivadeneira-Arias, 2023

Three flasks shown in Appendix 1 were used in the molecular assays, whose DNA quantification is detailed in Table 2, with flask 002 having the highest DNA concentration. The A260/A280 and A260/A230 ratios were used to evaluate the purity of cyanobacterial DNA. According to research by DNA Bank (2020), the optimal purity in the A260/280 ratio ranges from 1.8-2.1, while in the A260/A230 ratio it is 2-2.2. In this case the three samples present optimal purity in the A260/280 ratio; while in the A260/A230 ratio, DNA contaminated with salts, phenol and carbohydrates (<1.8) is observed in flask 001, and highly contaminated (<1.5) in flasks 002 and 003 [34] .

3.3. Amplification of the 16S rDNA, mcyA and Phycocyanin Gene

Figure 3 shows the PCR amplification of the genomic DNA of the isolated *Microcystis* sp. strain using specific primers. PCR products using the 16S rDNA primer were approximately 782 bp, while with the mcyA specific primer it was approximately 1300 bp. In the study by Saker et al. (2005) the same size PCR products were amplified for both the 16S gene and the mcyA gene in molecular tests of a pure strain of *M. aeruginosa*, so it is considered that the same strain is being studied [26]. Two of the three flasks were positive for the 16S rDNA molecular test, which is used to identify the presence of cyanobacteria, meaning that flasks 001 and 003 contain the species *M. aeruginosa*, while flask 002 possibly other cyanobacterial species with the same genus that were carried over in the isolation, meaning that this flask is not isolated and does not contain the species *M. aeruginosa* [35]. The DNA was fragmented because an extra band is observed in sample 001 and 003 of the 16S gene.

The fragmented DNA hindered the amplification of high molecular weight PCR products and affected the reproducibility of the techniques [36].

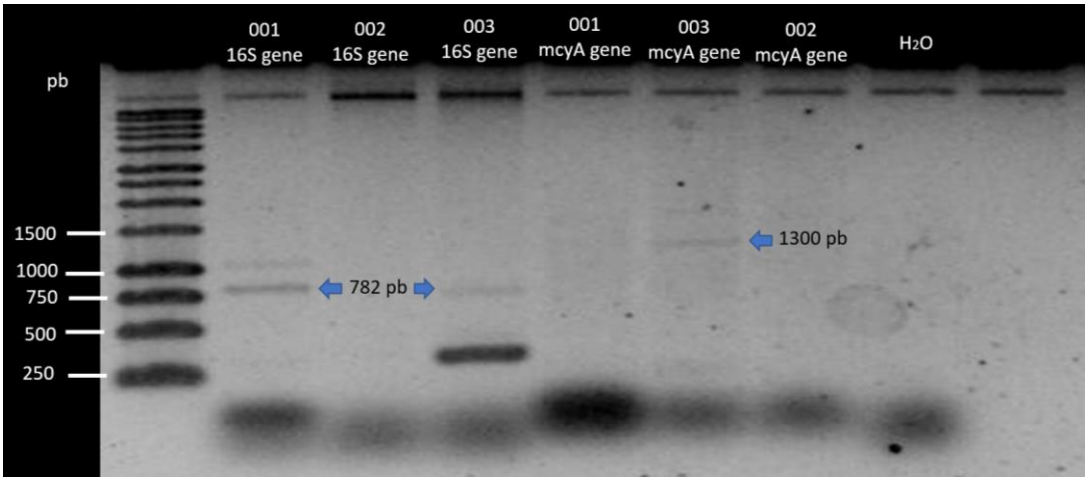


Figure 3. Photographic record of the first electrophoresis run of the different PCR products, using the primers 27F/809R of the 16S gene, to obtain 782bp fragments, and the MSF/MSR primers of the microcystin synthetase gene (mcyA) to obtain 1300bp fragments in the potentially toxic cyanobacteria samples. Water as a negative control.
Made by: Rivadeneira-Arias, 2023

The individual strains comprising the cyanobacterial bloom populations at the Ikiam WWTP can be evaluated for their potential toxigenicity using molecular techniques targeting the enzyme microcystin synthetase mcyA. One of the three strains tested (flask #003) produced PCR results when the primer pair used to find the microcystin synthetase mcyA gene was applied. In other research, such as that of Saker et al. (2005), which showed a 50% incidence of toxigenicity of colonies isolated and tested directly from lakes, in this study the measure of potential toxigenicity was 33% and is consistent with reports of previous studies (Eq.1). The finding that only one of the strains has microcystin synthetase genes supports the idea that natural blooms of *M. aeruginosa* are composed of chemically and genetically diverse strains [28], [37].

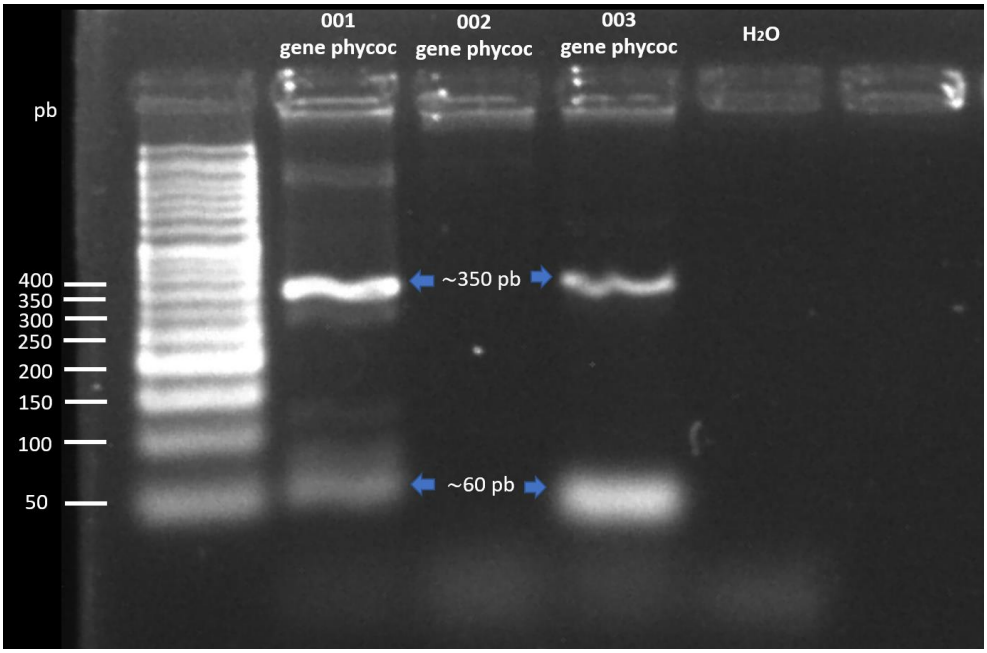


Figure 4. Photographic record of the second electrophoresis run of the different PCR products, using the primers PCβF/PCαR of the PcA gene of the protein pigment phycocyanin, to obtain fragments of 60bp for the PC island and 350bp of the total fragment in the potentially toxic cyanobacterial samples. Water as a negative control.
Made by: Rivadeneira-Arias, 2023

Phycocyanin protein is an indicator of the presence of cyanobacteria by its blue-green color. From the *Microcystis* sp. strain, the PCR PC-IGS PCR product length (with the primers and reaction conditions described in the methodology) is 350 bp, with an IGS of 60 bp (Fig. 4). The molecular technique was positive for sample 001 and 003, while in 002 no presence of this pigment was recorded, so it is presumed that the latter contains another type of pigmentation that does not belong to the cyanobacteria of the species *Microcystis* sp. [38].

3.4. Chlorophyll a Depletion in the Photocatalytic Process

Since the biomass of flask 003 tested positive for potentially toxic cyanobacteria, the experiments were performed only with this sample. The age of sample 003 was 13 days of incubation, the initial chlorophyll a concentration was always the same 100µg/L. Three replicates were performed with this flask for each microsphere and the average of the results was considered. Figure 5A shows the decrease in the absorbance curves of chlorophyll a in the presence of the microspheres $Bi_4O_5I_2$, with an initial absorbance of 0.0483 and a final

absorbance of 0.0037 at the wavelength of 664nm, during 10 hours of light with no dark period.

While Figure 5B presents a comparison of two microspheres BiOI and $Bi_4O_5I_2$ in the inactivation of potentially toxic cyanobacteria considering another equal experiment without microspheres as negative control. In which it can be evidenced that the biomass of cyanobacteria tends to increase since they are photosynthetic microorganisms that feed on visible light. However, exponential growth cannot be observed because, being immersed in a saline solution, they do not have the necessary nutrients to continue reproducing, but they do have the necessary nutrients to avoid the osmotic pressure of the cells, i.e. to survive [4], [18], [38].

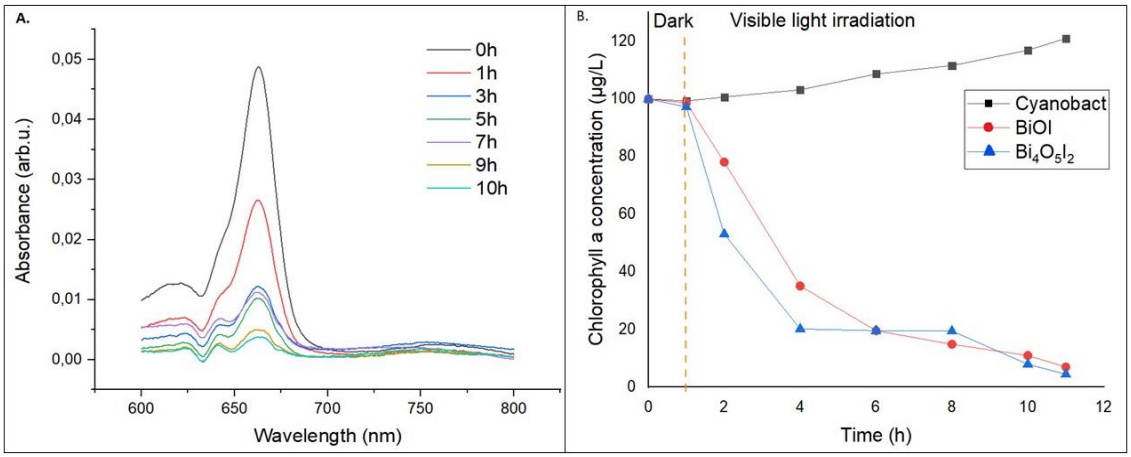


Figure 5. Plots of the inactivation of potentially toxic cyanobacteria during 11 hours. A. Chlorophyll a absorbance curves with $Bi_4O_5I_2$ (best performing microspheres) without the dark step; B. Decrease in chlorophyll a concentration with BiOI, $Bi_4O_5I_2$, and without the microspheres as negative control.
Made by: Rivadeneira-Arias, 2023

The initial concentration of chlorophyll a was 100 µg/L and reduced to 7.02 µg/L (Eq.2) with BiOI, obtaining an inactivation percentage of 92.97%; while with $Bi_4O_5I_2$ the final concentration of 4.51 µg/L, representing an inactivation percentage of 95.49% (Fig. 5B) using equation 3. The photocatalytic process consisted of the rupture of the cyanobacterial cell membrane by activation of reactive oxygen species (ROS), resulting in the depletion of chlorophyll a. Chlorophyll a is an indicator of the cell inactivation that occurs during the photocatalysis process. [31]. The results obtained have higher efficiency compared to previous studies of $Bi_2Mo_3O_{12}$ as a novel photocatalyst for the treatment of *M. aeruginosa*

reporting an efficiency of 84% [39]**.** The variation between the inactivation percentages may be due to the fact that the BiOI microspheres upon undergoing a calcination step and converting to $Bi_4O_5I_2$ acquires better photocatalytic activity of the pure phase bismuth oxyiodides, because of its relatively high charge separation efficiency and large surface area [40].

4. CONCLUSIONS

In conclusion, the striae depletion isolation method showed good colony separation, which allowed mass production of cyanobacteria for molecular and photocatalytic experiments. The molecular technique employed confirmed the presence of potentially toxic cyanobacteria of the species *Microcystis* sp. in the Ikiam WWTP. On the other hand, the rupture of the cell membrane of *Microcystis* sp. was successfully carried out, the BiOI and $Bi_4O_5I_2$ microspheres offered inactivation percentages of more than 90%. Therefore, this type of process can be scaled up and included in a WWTP as a complementary treatment to conventional systems or as a substitute for advanced oxidation technologies using ultraviolet radiation. Furthermore, the application of this photocatalytic process provides a future perspective for the simultaneous removal of potentially toxic cyanobacterial cells and their secondary metabolites (cyanotoxins), since these microspheres have the ability to inactivate cells of pathogenic microorganisms and degrade organic compounds of emerging concern.

REFERENCES

[1] M. J. Harke *et al.*, “A review of the global ecology, genomics, and biogeography of the toxic cyanobacterium, Microcystis spp.,” *Harmful Algae*, vol. 54, pp. 4–20, 2016, doi: 10.1016/j.hal.2015.12.007.

[2] X. He, P. Wu, S. Wang, A. Wang, C. Wang, and P. Ding, “Inactivation of harmful algae using photocatalysts: Mechanisms and performance,” *Journal of Cleaner Production*, vol. 289. Elsevier Ltd, Mar. 20, 2021. doi: 10.1016/j.jclepro.2020.125755.

[3] Y. Xu, J. Yang, M. Ou, Y. Wang, and J. Jia, “Study of Microcystis aeruginosa inhibition by electrochemical method,” *Biochem Eng J*, vol. 36, no. 3, pp. 215–220, Oct. 2007, doi: 10.1016/j.bej.2007.02.022.

[4] C. S. Romanis, L. A. Pearson, and B. A. Neilan, “Cyanobacterial blooms in wastewater treatment facilities: Significance and emerging monitoring strategies,” *Journal of Microbiological Methods*, vol. 180. Elsevier B.V., Jan. 01, 2021. doi: 10.1016/j.mimet.2020.106123.

[5] M. A. Chia, I. Ameh, K. C. George, E. O. Balogun, S. A. Akinyemi, and A. S. Lorenzi, “Genetic Diversity of Microcystin Producers (Cyanobacteria) and Microcystin Congeners in Aquatic Resources across Africa: A Review Paper,” *Toxics*, vol. 10, no. 12. MDPI, Dec. 01, 2022. doi: 10.3390/toxics10120772.

[6] W. Tawong, P. Pongcharoe, and T. Nishimura, “Geosmin–producing Scytonema foetidum sp. nov. (Scytonemataceae, Cyanobacteria): morphology and molecular phylogeny,” *Fottea*, vol. 22, no. 1, pp. 78–92, 2022, doi: 10.5507/fot.2021.017.

[7] J. S. Metcalf and G. A. Codd, “Cyanobacterial Toxins (Cyanotoxins) in Water,” 2014.

[8] I. Gajer, P. Pereira, and F. Skuras, “Desarrollo de un sistema integrado de detección de Microcystis aeruginosay microcistina-LR en muestras de agua mediante qPCRy UHPLC,” Universidad ORT Uruguay, 2020.

[9] J. Wang and R. Zhuan, “Degradation of antibiotics by advanced oxidation processes: An overview,” *Science of the Total Environment*, vol. 701. Elsevier B.V., Jan. 20, 2020. doi: 10.1016/j.scitotenv.2019.135023.

[10] M. E. Simonsen, “Heterogeneous Photocatalysis,” in *Chemistry of Advanced Environmental Purification Processes of Water: Fundamentals and Applications*, Elsevier Inc., 2014, pp. 135–170. doi: 10.1016/B978-0-444-53178-0.00004-3.

[11] P. Y. Ayekoe, D. Robert, and D. L. Goné, “Preparation of effective TiO2/Bi2O3 photocatalysts for water treatment,” *Environ Chem Lett*, vol. 14, no. 3, pp. 387–393, Sep. 2016, doi: 10.1007/s10311-016-0565-3.

[12] Q. Yan, Y. Zhao, M. Xu, and Y. Wang, “Enhanced visible-light photocatalytic performance of various bismuth oxyiodide with 3D hierarchical microspheres architecture,” *J Nanosci Nanotechnol*, vol. 16, no. 7, pp. 7731–7737, Jul. 2016, doi: 10.1166/jnn.2016.12585.

[13] H. Feng, T. Clara, F. Huang, J. Wei, and F. Yang, “Identification and characterization of the dominant Microcystis sp. cyanobacteria detected in Lake Dong Ting, China,” *Journal of Toxicology and Environmental Health - Part A: Current Issues*, vol. 82, no. 22, pp. 1143–1150, Nov. 2019, doi: 10.1080/15287394.2019.1700604.

[14] F. R. Jacinavicius, W. A. Gama Júnior, M. D. P. Azevedo, and C. Santanna, “Manual para el cultivo de cianobacterias,” *São Paulo: Secretaria do Meio Ambiente.*, 2013.

[15] D. M. Cabrera Gavilanes, E. Santos Ordónez, and G. Parra-Riofrío, “Aislamiento y caracterización molecular de cepas fitoplanctónicas obtenidas en Engabao, Guayas-Ecuador,” *Revista Científica de Ciencias Naturales y Ambientales*, vol. 15, no. 2, 2021.

[16] E. Barrios Barcia and C. Coleto Fiaño, “Catálogo de cianobacterias planctónicas potencialmente tóxicas de las aguas continentales españolas,” Ministerio Medio Ambiente y Medio Rural y Marino, 2011.

[17] M. C. Guamán Burneo and N. P. González Romero, “CATÁLOGO DE MICROALGAS Y CIANOBACTERIAS DE AGUA DULCE DEL ECUADOR BIODIVERSIDAD,” 2016.

[18] V. Almanza M., O. O. Parra, and Trama Impresores S.A.), *“Guía para el estudio de cianobacterias en el sistema lacustre del Gran Concepción, Chile”: aspectos taxonómicos, ecológicos, toxicológicos y de control-vigilancia*. Unesco, 2016.

[19] M. A. Nienaber and M. Steinitz-Kannan, *A Guide to Cyanobacteria*. 2018.

[20] S. S. Baek *et al.*, “Identification and enumeration of cyanobacteria species using a deep neural network,” *Ecol Indic*, vol. 115, Aug. 2020, doi: 10.1016/j.ecolind.2020.106395.

[21] S. Singh, S. Patidar, S. Goyal, and A. Mohammed Saleh, “Microbial Profiling Of Cyanobacteria From VIT Lake,” *INTERNATIONAL JOURNAL OF SCIENTIFIC & TECHNOLOGY RESEARCH*, vol. 5, p. 1, 2016, [Online]. Available: www.ijstr.org

[22] M. Yilmaz *et al.*, “Comprehensive multi-technique approach reveals the high diversity of microcystins in field collections and an associated isolate of Microcystis aeruginosa from a Turkish lake,” *Toxicon*, vol. 167, pp. 87–100, Sep. 2019, doi: 10.1016/j.toxicon.2019.06.006.

[23] I. A. Guedes *et al.*, “Intraspecific variability in response to phosphorus depleted conditions in the cyanobacteria Microcystis aeruginosa and Raphidiopsis raciborskii,” *Harmful Algae*, vol. 86, pp. 96–105, Jun. 2019, doi: 10.1016/j.hal.2019.03.006.

[24] Y. L. Seo, S. E. Jeong, H. M. Jin, and C. O. Jeon, “Flavobacterium microcysteis sp. Nov., isolated from a culture of microcystis aeruginosa,” *Int J Syst Evol Microbiol*, vol. 70, no. 2, pp. 1037–1041, 2020, doi: 10.1099/ijsem.0.003867.

[25] M. Yilmaz, E. J. Phlips, and D. Tillett, “Improved methods for the isolation of cyanobacterial dna from environmental samples,” *J Phycol*, vol. 45, no. 2, pp. 517–521, Apr. 2009, doi: 10.1111/j.1529-8817.2009.00651.x.

[26] M. L. Saker, J. Fastner, E. Dittmann, G. Christiansen, and V. M. Vasconcelos, “Variation between strains of the cyanobacterium Microcystis aeruginosa isolated from a Portuguese river,” *J Appl Microbiol*, vol. 99, no. 4, pp. 749–757, 2005, doi: 10.1111/j.1365-2672.2005.02687.x.

[27] B. A. Neilan, D. Jacobs, and A. E. Goodman, “Genetic Diversity and Phylogeny of Toxic Cyanobacteria Determined by DNA Polymorphisms within the Phycocyanin Locus,” 1995. [Online]. Available: https://journals.asm.org/journal/aem

[28] L. Biessy, S. A. Wood, M. Chinain, M. Roué, and K. F. Smith, “Exploring benthic cyanobacterial diversity and co-occurring potentially harmful dinoflagellates in six islands of the South Pacific,” *Hydrobiologia*, vol. 848, no. 11, pp. 2815–2829, Jun. 2021, doi: 10.1007/s10750-021-04599-6.

[29] M. Zuarez-Chamba, D. Tuba-Guamán, M. Quishpe, K. Vizuete, A. Debut, and M. Herrera-Robledo, “Photocatalytic degradation of bisphenol A on BiOI nanostructured films under visible LED light irradiation,” *J Photochem Photobiol A Chem*, vol. 431, p. 114021, Oct. 2022, doi: 10.1016/j.jphotochem.2022.114021.

[30] J. Song *et al.*, “Removal of Microcystis aeruginosa and Microcystin-LR using a graphitic-C3N4/TiO2 floating photocatalyst under visible light irradiation,” *Chemical Engineering Journal*, vol. 348, pp. 380–388, Sep. 2018, doi: 10.1016/j.cej.2018.04.182.

[31] G. Fan *et al.*, “Photocatalytic inactivation of harmful algae and degradation of cyanotoxins microcystin-LR using GO-based Z-scheme nanocatalysts under visible light,” *Chemical Engineering Journal*, vol. 392, Jul. 2020, doi: 10.1016/j.cej.2019.123767.

[32] G. Fan *et al.*, “Z-scheme Ag3PO4@polyaniline core-shell nanocomposite with high visible light photocatalytic performance for Microcystis aeruginosa inactivation,” *Chemical Engineering Journal*, vol. 427, Jan. 2022, doi: 10.1016/j.cej.2021.132005.

[33] H. Cai, H. Cui, Y. Zeng, M. An, and H. Jiang, “Sandarakinorhabdus cyanobacteriorum sp. Nov., a novel bacterium isolated from cyanobacterial aggregates in a eutrophic lake,” *Int J Syst Evol Microbiol*, vol. 68, no. 3, pp. 730–735, Mar. 2018, doi: 10.1099/ijsem.0.002571.

[34] A. Banco, “PROGRAMA CONTROL DE CALIDAD DE MUESTRAS DE ADN Y ARN,” 2020. [Online]. Available: https://cit.ligue-cancer.net/CIT_Public/images/stories/CIT/pdf/WebSite%20CIT-%20QC%20PF%20Saint-

[35] N. K. Thu, Y. Tanabe, H. Matsuura, and M. M. Watanabe, “Morphological, biochemical, and molecular characterization of Oscillatoria kawamurae (Oscillatoriales, Cyanobacteria) isolated from different geographical regions,” *Phycological Res*, vol. 68, no. 3, pp. 216–226, Jul. 2020, doi: 10.1111/pre.12419.

[36] A. Cornejo Romero, R. Serrato Díaz, B. Rendón Aguilar, and M. G. Rocha Munive, *Herramientas moleculares aplicadas en ecología: aspectos teóricos y prácticos*. Secretaria de Medio Ambiente y Recursos Naturales, 2014.

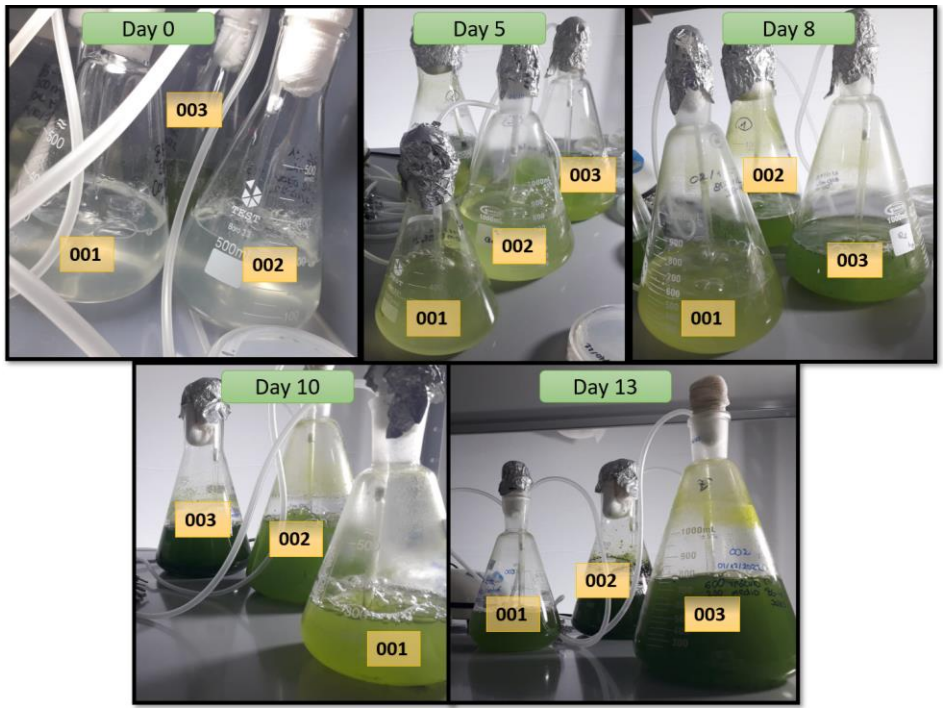
[37] X. Du *et al.*, “The diversity of cyanobacterial toxins on structural characterization, distribution and identification: A systematic review,” *Toxins*, vol. 11, no. 9. MDPI AG, 2019. doi: 10.3390/toxins11090530.

[38] N. M. Khong, Y. S. Khaw, M. F. Nazarudin, and F. Md. Yusoff, “Microcystis aeruginosa grown in different defined media leads to different cultivable heterotrophic bacteria composition that could influence cyanobacterial morphological characteristics and growth properties,” *bioRxiv*, 2019, doi: 10.1101/721175.

[39] P. Li, L. Wang, H. Liu, R. Li, M. Xue, and G. Zhu, “Facile sol-gel foaming synthesized nano foam Bi2Mo3O12 as novel photocatalysts for Microcystis aeruginosa treatment,” *Mater Res Bull*, vol. 107, pp. 8–13, Nov. 2018, doi: 10.1016/j.materresbull.2018.07.008.

[40] H. Huang, K. Xiao, T. Zhang, F. Dong, and Y. Zhang, “Rational design on 3D hierarchical bismuth oxyiodides via in situ self-template phase transformation and phase-junction construction for optimizing photocatalysis against diverse contaminants,” *Appl Catal B*, vol. 203, pp. 879–888, Apr. 2017, doi: 10.1016/j.apcatb.2016.10.082.

APPENDIX



Appendix 1. Cyanobacterial biomass production in a lab-scale photobioreactor system over a 13-day period.
Made by: Rivadeneira V., 2023