MEMORIA DE 6 MOVILIDADES inter-grupos 2023

Persona	Grupo de procedencia	Grupo de destino	Duración	Objetivos
GOVINDA GUEVARA	UCM	LNEG	12-24 FEBRERO	Estudio de la microalga D14 (germinación, crecimiento en purines, etc.)
ANA SANCHEZ ZURANO	UAL	LNEG	3 MESES 8 abril-8 JULIO	Conserving synthetic nitrogen and enhancing the production of *Chlorella vulgaris* using nitrogen-fixing bacteria
ALICE FERREIRA	LNEG	UAL	1 sept- 30 nov	Colaboración con el grupo del Dr. Gabriel Acién ya que la tesis es a medias entre los dos grupos LNEg-UAL
KARLA Z GUARNEROS	UPMP MEXICO	UCM	11 NOV-14 DIC	Aprender técnicas microbiológicas y genéticas en cianobacterias
XAVIER ALVAREZ	ECUADOR	UAL	26 Nov-10 dic	I Recognize the operation of open and closed systems (photobioreactors) of microalgae cultivation. II. Analysis of the operation of photobioreactor systems for wastewater treatment. III. Definition of cell disruption processes and subsequent formulation of microalgae biomass
IGNACIO RUIGOMEZ	Univ LA LAGUNA	PORTUGAL	4 nov-11 dic	Participate in the design and construction of a microalgae biomass electrocoagulation's harvesting prototype. Familiarization with the functional unit, workflows, and tools of Life Cycle Assessment (LCA).

RED CYTED RENUWAL 320RT0005

MEMORIA DE LA ESTANCIA DE MOVILIDAD

Movility report

Any details provided on this form, except contact information, may be published on the website or social networks. Esta ficha será de dominio de los miembros de la Red Renuwal y se podrá publicar en los canales de la misma.

Name	**Govinda Guevara**
Organisation	**UCM, Madrid, España**
Contact Address (inc. country)	**Facultad de Biológicas, c/Jose Antonio Novais 12, 28040 Madrid**
Telephone Number	**+34913944145**
Email	**fguevara@ucm.es**
Center visited and country	**LNEG, Portugal, Grupo de la Dra. Luisa Gouveia**
Dates of Visit	**12-24 de febrero de 2023**

Portrait Photograph: *Please supply a portrait photograph of yourself as a separate jpeg file at a resolution of at least 300 dpi or 500MB*

RED CYTED RENUWAL 320RT0005

Project title

Estudios de bioestimulación y crecimiento en purines de la microalga D14

A brief overview of the activities done

Objetives

Se ha utilizado esta estancia para terminar experimentos que lleven a una publicación conjunta de la microalga D14 entre la UCM y LNEG.

Methodology, work schedule, results

Se ha llevado a cabo estudios de germinación y en purines siguiendo los protocolos estandarizados en el LNEG que tiene experiencia en estas técnicas. Además de la caracterización de la biomasa y ensayos de biopesticidas con *Fusarium* sp.

Describe the benefits of the stay

RED CYTED RENUWAL 320RT0005

MEMORIA DE LA ESTANCIA DE MOVILIDAD

Movility report

Any details provided on this form, except contact information, may be published on the website or social networks. Esta ficha será de dominio de los miembros de la Red Renuwal y se podrá publicar en los canales de la misma.

Name	**Ana**
Organisation	**University of Almeria**
Contact Address (inc. country)	**Carr. Sacramento, s/n, 04120 La Cañada, Almería**
Telephone Number	**+34 699656745**
Email	**asz563@ual.es**
Center visited and country	**LNEG**
Dates of Visit	**08/06/2023-08/07/2023**

Portrait Photograph:

Project title

Conserving synthetic nitrogen and enhancing the production of *Chlorella vulgaris* using nitrogen-fixing bacteria

RED CYTED RENUWAL 320RT0005

A brief overview of the activities done

Objetives

The objective of the stay at LNEG (National Laboratory for Energy and Geology) with Dr. Luisa Gouveia is to evaluate the interactions between microalgae and nitrogen-fixing bacteria using flow cytometry with specific markers. This will also involve characterizing the biomass produced (lipids, proteins, carbohydrates, and pigments) and utilizing this produced biomass for applications of interest, such as in agriculture. It is anticipated that this study will result in a collaborative research article between the two institutions. The study and optimization of these interactions are of great significance in the field of microalgae biotechnology, as avoiding the need to supply nitrogen in the form of fertilizers for microalgae growth represents a substantial cost-saving in their production. This also opens up an important avenue of industrial interest for companies in the sector. Additionally, the application of microalgae biomass as biofertilizers and bio-stimulants can be enhanced through interaction with nitrogen-fixing bacteria.

Methodology, work schedule, results

To achieve the proposed objective, various materials and methods will be employed.

- First, the microorganisms utilized include *Chlorella vulgaris* UAL-1. This microalga is available at the culture collection of the SABANA Demonstration Plant in Almería, Spain, and was used in this study. Additionally, the N2-fixing bacteria examined in this research are *Sphingobacterium canadense* (strain TPY5), *Microbacterium maritypicum* (strain KUDC1778), and *Endophytic bacterium* (strain CR1b). These bacteria belong to the Laboratory of Strain-producers of BAS and Biosynthesis Culture Collection, SPC "Armbiotechnology," SNPO NAS RA, Armenia.
- For the experiments, five conditions were tested. The condition "A" contained *Chlorella vulgaris* UAL-1 at a final concentrations OD540 0.2 and the culture medium described below with NaNO3 (Positive Control). The condition "B" was prepared with *Chlorella vulgaris* UAL-1 at a final concentrations OD540 0.2 and the culture medium described below without NaNO3 (Negative Control). The condition "C" consisted on *Chlorella vulgaris* UAL-1 at a final concentrations OD540 0.2, strain TPY5 at a final concentrations OD540 0.2 and the culture medium described below

RED CYTED RENUWAL 320RT0005

without NaNO3. The condition "D" was prepared with *Chlorella vulgaris* UAL-1 at a final concentrations OD540 0.2, strain KUDC1778 at a final concentrations OD540 0.2 and the culture medium described below without NaNO3. The condition "E" was prepared with *Chlorella vulgaris* UAL-1 at a final concentrations OD540 0.2, strain CR1b at a final concentrations OD540 0.2 and the culture medium described below without NaNO3.

- The trials were performed in ten illuminated 1 L photobioreactors. The reactors were then filled with the culture media until a final working volume of 600 mL and the corresponding inoculums. They were operated in batch mode for 8 days.
- The cultures´ growth were evaluated for 7 days by measuring optical density (OD) at 540, 680 and 750 nm wavelength applying the Hitachi U-2000 spectrophotometer, and by dry weight. Also the Fv/Fm was evaluated daily.
- The microbial dynamics were analyzed using the flow cytometer CytoFLEX (Beckman Coulter Life Sciences, Brea, CA, USA) equipped with a 488 nm argon laser. Forward (FSC) and Side Scatter (SSC) detectors were used to distinguish cells with different sizes and internal complexities (respectively). Fluorescence channel FITC (green) was used to collect data on the esterase enzymatic activity of microalgal cells using the viability dye carboxyfluorescein diacetate succinimidyl ester (CFDA). Data from FITC was also used in samples stained with fluorescent dye SYTO9 for identifying bacterial populations. The fluorescence channel PC5.5 (red) was used to identify microalgal communities since it collects fluorescence by chlorophyll emission.
- The biomass composition was characterized using appropriate methods. Proteins were determined by the Lowry method. Fat content was determined by Soxhlet, according to the Portuguese standard method NP4168 (1991). The carbohydrate content was calculated using phenol sulphuric method. Moreover, the pigments were determined by spectrophotometric methods.
- The germination tests were performed in Petri dishes using lettuce seeds. Distilled water was used as the negative control and the hormones gibberellic acid was used as the positive controls. The microalgal extracts were assessed at a biomass concentration of 0.2 and 0.5 g·L−1 in triplicate. Also, the supernatants (after removing the cells) were tested. Seeds were incubated at 20 °C in the dark for 5 days in a growing chamber (FITOCLIMA S600 PL, Aralab, Portugal). The

RED CYTED RENUWAL 320RT0005

number of germinated seeds in each Petri dish was counted, and the root and shoot lengths were measured. The germination index (GI) was determined according to Zucconi et al. (1981).

This methodology was followed during the time of the stay in order to fulfil the proposed objective. As a result, a similar biomass productivity between the positive control culture and the cultures with bacterial involvement. Nevertheless, a lower Fv/Fm ratio suggested the potential presence of a stress condition. The stress could be due to the low values of nitrogen that were detected in the co-cultures, indicating a low level of nitrogen fixation by the bacteria, which in turn could be limited by carbon. To address this, future investigations could involve the introduction of a carbon source to maintain organic carbon levels for bacterial growth. Related the microbial dynamics, growth of microalgae and bacteria was monitored by flow cytometry. The results showed that from day 3 of culture, the number of cells in the negative control was significantly lower than the other treatments ($p < 0.05$). In addition, the number of microalgae cells at the end of the experiments was lower in the co-cultures with bacteria than in the positive control. Regarding the produced biomass, significant differences were observed in the negative control, where lipid levels increased while protein levels decreased. The biomass of the positive control and the co-cultures had similar carbohydrate content, with differences in protein content. All produced biomasses exhibited a higher germination capacity compared to the control with water.

Describe the benefits of the stay

The results of this study demonstrated the potential to produce microalgae biomass without the need to add a nitrogen source, using nitrogen-fixing bacteria. This enhances the sustainability of the process and reduces its economic cost. Further studies on a larger scale are necessary to validate this technology. In addition to the research results, the stay has been very rewarding due to everything learned alongside Dr. Luisa Gouveia and her team. They have been a great support and a valuable reference during this time, and we have established a highly beneficial collaboration network for both research groups.

Date and signature

SANCHEZ ZURANO ANA - 76636955N

Firmado digitalmente por SANCHEZ ZURANO ANA - 76636955N
Fecha: 2023.09.01 13:44:41 +02'00'

RED CYTED RENUWAL 320RT0005

MEMORIA DE LA ESTANCIA DE MOVILIDAD

Movility report

Any details provided on this form, except contact information, may be published on the website or social networks.
Esta ficha será de dominio de los miembros de la Red Renuwal y se podrá publicar en los canales de la misma.

Name	**Alice Ferreira**
Organisation	**LNEG**
Contact Address (inc. country)	**Estrada Paço do Lumiar, 22 1649-038 Lisboa, Portugal**
Telephone Number	**+351916944675**
Email	**alice.ferreira@lneg.pt**
Center visited and country	**University of Almeria, Spain**
Dates of Visit	**01/09/2023 – 31/11/2023**

Project title

OPTIMIZATION OF SOLAR PHOTO-FENTON AS A PRE-TREATMENT FOR MICROALGAE-BASED PIGGERY WASTEWATER TO REDUCE WATER INPUTS

A brief overview of the activities done

Objetives
To study a solar photo-Fenton process as a pre-treatment of piggery wastewater in order to reduce the need for dilution before microalgae cultivation and optimize de concentrations of iron and hydrogen peroxide, reducing energy and reagents costs.

Methodology, work schedule, results

RED CYTED RENUWAL 320RT0005

The work schedule was according to the work I had at that day. However, since it was only 3 months in order to take advantage of all the remaining time, by the final month I worked very intensively usually from 8 am to 7 pm. I performed several solar photo-Fenton runs using raceway ponds and did all the physico-chemical (turbidity, nitrogen, phosphorus, COD, organic carbon) and microbiological analysis to assess the treatment performance. In the microalgae trials, I did the monitoring of the cultures (OD, pH, quantum yield). As results, I was able to select a set of optimized conditions for the solar photo-Fenton process and reduce process costs reaching an adequate pre-treated effluent that I could use to grow the microalga reducing the water for dilution.

Describe the benefits of the stay

This stay at the University of Almeria was an amazing opportunity for me not only for my PhD but for my personal and professional growth. In my PhD plan, I was able to perform a more in-depth study of the photo-Fenton process in CIESOL group since this is their area of expertise. I was able to scale up this pre-treatment process using 19L raceway ponds and replace artificial light with sunlight, doing several experimental runs of solar photo-Fenton to optimize the concentrations of Fe and H_2O_2.

It was also a very rich experience since I was able to work in a different environment and group and learn new methods and ways of thinking and working. I was very fortunate to work not only in the microalgae group at IFAPA, but also at CIESOL so I learned many diverse techniques and skills. I was also able to participate in different activities and congresses promoted by the university and I was even selected for an oral presentation and awarded second place in the category of Biotechnology with the work developed during my stay.

In terms of personal growth, being my first stay outside of Portugal, it was very beneficial for the development of my autonomy and a way to overcome my fears. I was very blessed to get to know so many amazing people not only from Almeria but from other countries which will be forever in my heart, and I would love to keep collaborating with them. It was such an enriching experience, and I am very grateful for the opportunity.

11 de enero de 2024

Alice Ferreira

Photos

Figure 1	My first day at the University of Almeria.

RED CYTED RENUWAL 320RT0005

Figure 2	Setting up the solar photo-Fenton experiments at CIESOL.
Figure 3	Taking samples during the solar photo-Fenton experiments at CIESOL.
Figure 4	My raceway ponds during the solar photo-Fenton experiments.
Figure 5	Pre-treated piggery wastewater after neutralization and settling.
Figure 6	Microalga cultivation trials using the pre-treated piggery wastewater.
Figure 7	Me and my colleagues at the Simposio de Investigación en Ciencias Experimentales.
Figure 8	My poster at the Simposio de Investigación en Ciencias Experimentales.
Figure 9	My oral presentation at the Simposio de Investigación en Ciencias Experimentales.
Figure 10	Dinner with the work group.
Figure 11	One of many "desayunos".
Figure 12	Me and my colleague Luigi from Brazil with our Almeria t-shirts given by our friends and colleagues.
Figure 13	Arriving back in Lisbon with my University jumper (given by my Almeria colleagues) without bags, but happy.

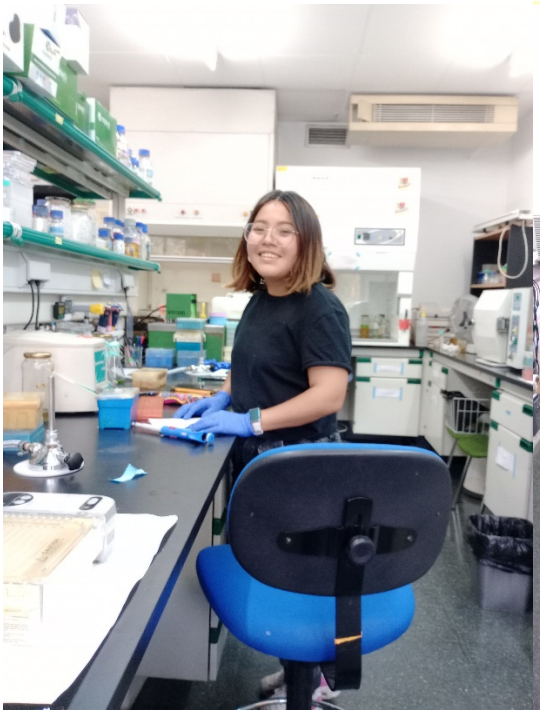
MEMORIA DE LA ESTANCIA DE MOVILIDAD

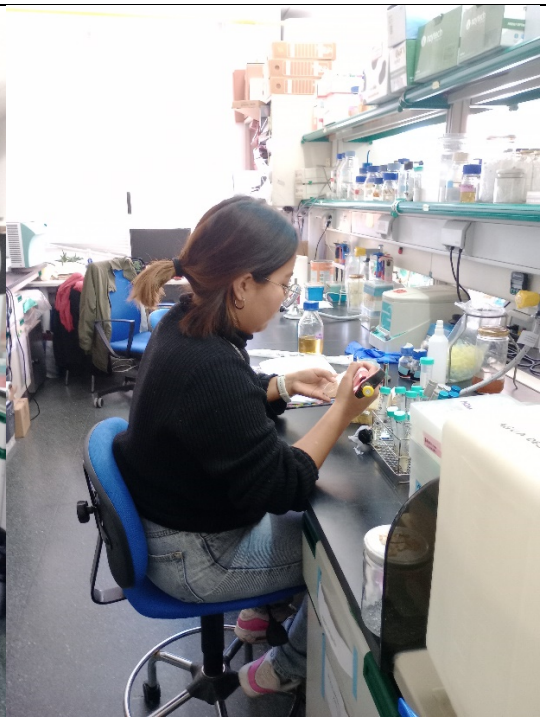
Movility report

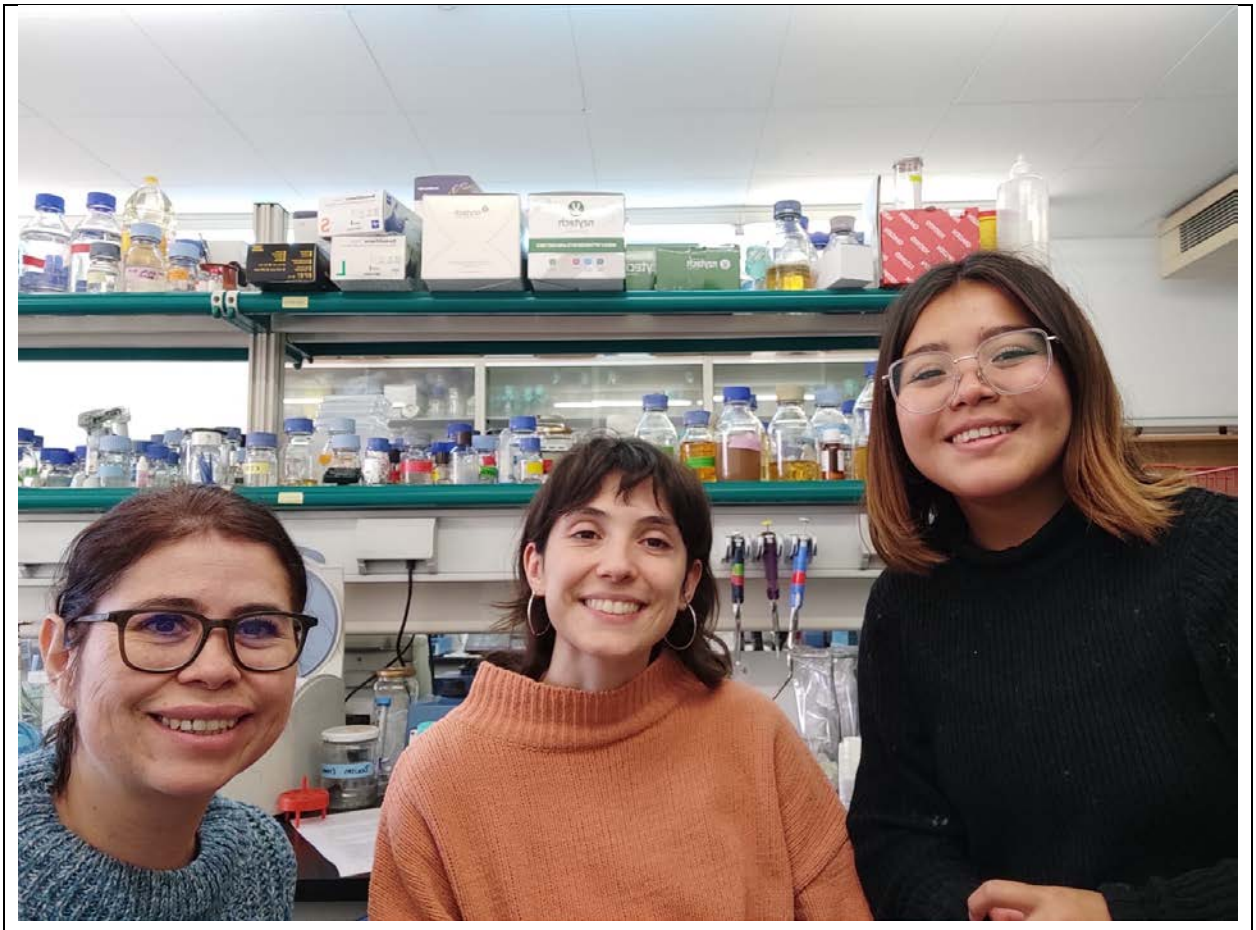
Any details provided on this form, except contact information, may be published on the website or social networks. Esta ficha será de dominio de los miembros de la Red Renuwal y se podrá publicar en los canales de la misma.

Name	**KARLA ZULEM GUARNEROS ANDRADE**
Organisation	**UPMP (GRUPO DEL DR. CUNILL, PUEBLA, MEXICO)**
Contact adress	**64 poniente 918, Guadalupe Victoria, Puebla, Mexico**
Email	**karla.2021120037@metropoli.edu.mx** **tel 222395808**
Center visited and country	**UCM, MADRID, ESPAÑA**
Dates of Visit	**11 nov-14 dic 2023**

Portrait Photograph: *Please supply a portrait photograph of yourself as a separate jpeg file at a resolution of at least 300 dpi or 500MB*







Project title

Introducción al manejo genético de microalgas

A brief overview of the activities done

Objetives

Aprender las técnicas básicas de ingeniería genética aplicada a las microalgas

Methodology, work schedule, results

Se aprendieron técnicas de genética molecular (Extracción de DNA, miniprep, PCR, geles de agarosa, extracción de DNA a partir de geles) y microbiológica (cultivo de cianobacterias, preparación de medios, autoclavado etc.)

Describe the benefits of the stay

Aunque me faltaba algo de base para poder entender todos los conceptos, he aprendido mucho de esta estancia.

9 de enero de 2024

Date and signatura

Image Content: *Please supply photos and diagrams to evidence your experience (e.g. a photo of you working in the lab, equipment, work in progress and staff). List all images in a table below. Please ensure images are high resolution jpegs files (e.g. 300 dpi) and* ***attached as separate files.***

RED CYTED RENUWAL 320RT0005

MEMORIA DE LA ESTANCIA DE MOVILIDAD

Mobility report

Any details provided on this form, except contact information, may be published on the website or social networks. Esta ficha será de dominio de los miembros de la Red Renuwal y se podrá publicar en los canales de la misma.

Name	**Xavier Álvarez Montero**
Organization	**Universidad de las Fuerzas Armadas-ESPE-Sede Santo Domingo**
Contact Address (inc. country)	**Vía Quevedo**
Telephone Number	**+593994776081**
Email	**xgalvarez@espe.edu.ec**
Center visited and country	**Universidad de Almería-España**
Dates of Visit	**26 November to 10 December**

Portrait Photograph: *Please supply a portrait photograph of yourself as a separate jpeg file at a resolution of at least 300 dpi or 500MB*

Project title

Demonstration of a pilot system for the treatment of wastewater in High- Rate Algal Ponds (HRAPs) to produce water and biomass reusable in agricultural applications

A brief overview of the activities done.

Objectives

RED CYTED RENUWAL 320RT0005

I. Recognize the operation of open and closed systems (photobioreactors) of microalgae cultivation.
II. Analysis of the operation of photobioreactor systems for wastewater treatment.
III. Definition of cell disruption processes and subsequent formulation of microalgae biomass.

Methodology, work schedule, results

Tests were carried out in both types of cultivation systems, open and closed, in processes such as air bubbling to eliminate excess O2, CO2 bubbling to regulate pH, nutrient addition, harvesting systems, and the cultivation systems were analyzed. automation.

a) November 28 2023, Recognition of open and closed systems of microalgae production.
b) November 29 2023, Analysis of closed systems (photobioreactors) for microalgae production (design, control, operation, and scaling).
c) November 30, 2023 Analysis of closed systems (photobioreactors) for microalgae production (biomass harvesting and processing).
d) December 1, 2023 Analysis of open systems (raceways) for microalgae production (design, control, operation, and scaling).
e) December 4, 2023 Analysis of open systems (raceways) for microalgae production (design, control, operation, and scaling).
f) December 5, 2023 Analysis of open systems (raceways) for microalgae production (biomass harvesting and processing).
g) December 6, 2023 Analysis of open systems (raceways) for microalgae production (biomass harvesting and processing).
h) December 7, 2023 Analysis of wastewater treatment processes.
i) December 8, 2023 Analysis of cell disruption processes.

We acquired in-depth knowledge about the operation of open and closed microalgae cultivation systems (photobioreactors), as well as their automation systems, as well as how the modeling of these automation systems was carried out.

Describe the benefits of the stay

Having been able to visit one of the most relevant centers in the research and development of microalgae cultivation gives me the necessary knowledge to plan the development of pilot systems for the cultivation of these microorganisms in my country (Ecuador), because this does not exist. type of systems, microalgae biotechnology is not yet developed in Ecuador, even though we have a wide diversity of species.

4 de enero de 2024

Date and signature

Firmado electrónicamente por:
XAVIER GUILLERMO
ALVAREZ MONTERO

Blgo. Xavier Álvarez Montero, Ph.D.

RED CYTED RENUWAL 320RT0005

MEMORIA DE LA ESTANCIA DE MOVILIDAD

Movility report

Any details provided on this form, except contact information, may be published on the website or social networks. Esta ficha será de dominio de los miembros de la Red Renuwal y se podrá publicar en los canales de la misma.

Name	Ignacio Ruigómez Sempere
Organisation	Universidad de La Laguna
Contact Address (inc. country)	Departamento de Ingeniería Química- Facultad de Ciencias. Avenida Astrofísico Francisco Sánchez, s/n. Código postal 38200. San Cristóbal de La Laguna. S/C de Tenerife. Spain.
Telephone Number	+34 922318077
Email	isempere@ull.es
Center visited and country	GreenCoLab, Portugal
Dates of Visit	4/11/2023 – 11/12/2023

Portrait Photograph: *Please supply a portrait photograph of yourself as a separate jpeg file at a resolution of at least 300 dpi or 500MB*

Project title

PID2021-125404OB-I00: Biorreactores de membrana no integrada con cultivo de microalgas indígenas para regeneración y recuperación de recursos de aguas residuales domésticas (MainMBR) del programa Generación de Conocimientos financiado por Ministerio de Ciencia e Innovación, Agencia Estatal de Investigación y Fondos FEDER.

RED CYTED RENUWAL 320RT0005

A brief overview of the activities done

Objectives

- Participate in the design and construction of a microalgae biomass electrocoagulation's harvesting prototype.
- Familiarization with the functional unit, workflows, and tools of Life Cycle Assessment (LCA).

Methodology, work schedule, results

The experimental work carried out during the period of the mobility can be divided into two main blocks. In the primary block, I was involved in the conception, design, and construction of a microalgae biomass harvesting by electrocoagulation (EC) module designed to operate continuously with a feed flow rate of 150 L/h. Approximately three out of the four weeks of the mobility was dedicated to this primary block. The remaining week was allocated to the secondary block, which consisted of an initial introduction to the methodology and tools of Life Cycle Assessment (LCA).

For the construction and hydraulic testing of the EC pilot unit, I had to familiarize and work with specific 3D design software such as SketchUp and Autodesk Fusion. Using these computer tools, different configurations were projected, primarily focusing on the placement of electrodes (cathode and anode) in the EC tank. Subsequently, for each of the different configurations, fluid dynamics were evaluated using the Computational Fluid Dynamics (CFD) tool Autodesk CFD. These tools allowed for determining the best configuration by identifying and reducing dead zones of fluid movement in the pilot prototype. Finally, the construction and hydraulic testing of the design were carried out.

Furthermore, concerning the LCA tool, functional unit, workflows, and data organization were addressed to begin using the open-source software tool openLCA. This second phase remains open to future collaborations.

Describe the benefits of the stay

The research stay has provided me with the opportunity to work and familiarize myself with microalgae biomass harvesting techniques, specifically electrocoagulation (EC). In this context, the stay has proven to be highly beneficial, as it has allowed me to learn and work on aspects related to one of the tasks of the project PID2021-125404OB-I00: "Task No. 6: Improvement of the valorization of biomass produced in membrane photobioreactors (MPBR) through harvesting and concentration techniques, using rotary ultrafiltration and electrocoagulation."

RED CYTED RENUWAL 320RT0005

Researchers from GreenCoLab-LNEG in Portugal have recognized expertise in the electrocoagulation of algal biomass, and one of the objectives of the stay was to acquire knowledge related to this technique. Furthermore, the stay lays the groundwork for future collaboration in the calculation and analysis of environmental impacts and the life cycle assessment of MPBR processes carried out in Tenerife.

20/12/2023.

Date and signature

Image Content: *Please supply photos and diagrams to evidence your experience (e.g. a photo of you working in the lab, equipment, work in progress and staff). List all images in a table below. Please ensure images are high resolution jpegs files (e.g. 300 dpi) and* ***attached as separate files.***

RED CYTED RENUWAL 320RT0005

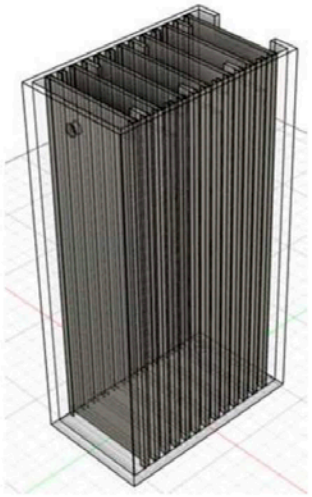


Figure 1. Prototype of the EC module design in Autodesk Fusion.

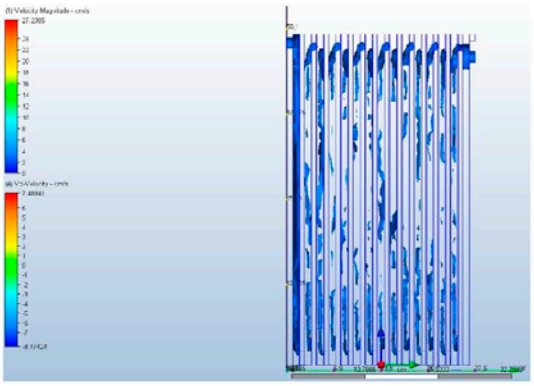


Figure 2. Study of the fluid dynamics in "Autodesk CFD".

RED CYTED RENUWAL 320RT0005

Figure 3. Construction phase of the EC prototype.

Figure 4. Construction phase and hydraulic test of the EC prototype.