Introduction to Microalgae Biomass Production (4 sessions)

In this section, students will dive into the essentials of producing microalgae biomass, covering the full process from cultivation to harvesting. The course begins with an overview of biomass production, including the culture media, bioreactor systems, and environmental factors required for optimal growth. Through hands-on lab work, students gain skills in preparing culture media, sterilizing equipment, and monitoring important parameters like biomass concentration. Facility visits to research labs and associated groups will deepen their understanding of professional microalgae cultivation practices.

Moving from growth to harvest, the course then addresses the challenge of separating microalgae cells from their liquid medium, focusing on efficient, cost-effective techniques. Lab sessions will allow them to practice transitioning from batch to continuous processes, with hands-on work in cell separation techniques both in lab and semi-pilot scales. This section emphasizes understanding and optimizing separation techniques to facilitate scalable and sustainable production.

Learning Outcomes: By the end of this section, students will be proficient in microalgae cultivation terminology and methods, understand the influence of environmental conditions on biomass productivity, and be familiar with essential lab skills for setting up and maintaining cultures. They will also gain insight into key harvesting techniques, the challenges of concentrating cultures, and practical experience in separating microalgae in both lab and semi-pilot scales.

PREVIOUS KNOWLEDGE:

- Preparing medium solutions by dissolving solids and liquids.
- Calculating solute amounts for solution preparation.
- Accurately preparing aqueous solutions from solids and liquids.

OBJECTIVES:

- To acquire best practices in preparing sterile materials for inoculum and small bioreactors.
- To learn proper techniques for handling sterile materials using laminar flow and flame sterilization.
- To prepare stock solutions of nutrients.
- To inoculate and maintain axenic microalgae cultures.
- To expand technical Spanish vocabulary relevant to laboratory procedures.

DYNAMIC:

Students will work in teams of 2-3, completing the practice over three sessions, as sterilization, cooling, and microalgae growth require time. The teaching team will demonstrate essential techniques, which students will then apply to inoculate bioreactors of varying sizes and monitor biomass concentrations over time. Upon completion, students will harvest the cultures, clean the equipment, and safely dispose of biological material.

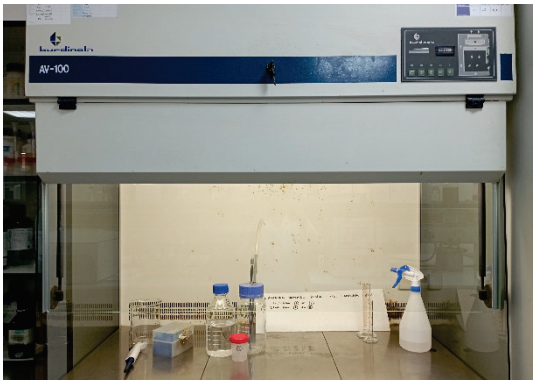
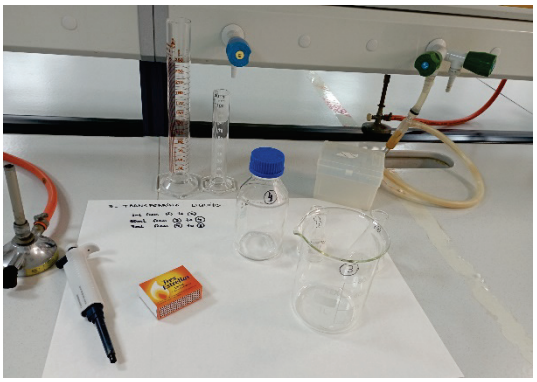
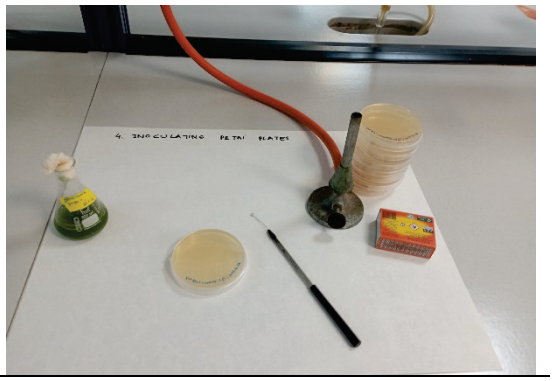
Session 1:
Learning sterile techniques and preparing sterile material

MATERIALS (MATERIALES):

- Ethanol for disinfection (etanol para desinfección)
- Permanent marker (rotulador permanente)
- Automatic pipette and tips (1mL) (pipeta automática y puntas)
- Balance, aluminum foil, spatula, and spoon (balanza, papel de aluminio, espátula y cuchara)
- Lab coat and gloves (bata de laboratorio y guantes)
- Magnetic stirrer and bar (agitador magnético e imán)
- Beaker (500mL), volumetric flask (1L), and Duran bottle (1L) (vaso de precipitado, matraz aforado y frasco Duran)
- Glass rod (varilla de vidrio)
- Chemicals (see appendix 1) (reactivos: ver apéndice 1)
- Incubator (incubador)
- Gauze, cotton, tape, and scissors (gasas, algodón, cinta adhesiva y tijeras)
- Spectrophotometer and cuvettes (espectrofotómetro y cubetas pequeñas)
- Microscope (microscopio)
- Inoculation loop (asa de siembra)
- Bunsen burner or lighter (mechero Bunsen o similar)
- Sterile material (e.g., 100mL Erlenmeyer with cotton cap, media stock solutions, inoculum). (Material estéril e.g. Erlenmeyer de 100mL con torunda de algodón, soluciones stock de medio, inóculo)

STUDENT TASKS IN THE LAB:

1. Training in sterile and disinfection techniques.
2. Handling materials in stations for each task, ensuring proper sterile practices for accurate and contamination-free results. Students will rotate through stations, as illustrated in Figure A1, performing the tasks with appropriate materials and sterile techniques.

	Task	Picture of the station
1	**Aseptically transfer 1mL of liquid** from Vessel Nº1 to Vessel Nº2 using sterile techniques. **Aseptically transfer 21mL of liquid** from Vessel Nº2 to Vessel Nº3, ensuring sterility is maintained throughout the process.	
2	**Prepare a cotton cap** for a 100mL Erlenmeyer flask, ensuring proper fit and sterility. **Prepare a cotton cap** for a 500mL Erlenmeyer flask, following the same procedure to maintain sterility.	
3	**Aseptically transfer 50mL of liquid** from Vessel Nº3 to Vessel Nº4, using appropriate sterile handling techniques. **Aseptically transfer 3mL of liquid** from Vessel Nº4 back to Vessel Nº3, ensuring no contamination occurs.	
4	**Inoculate a Petri plate** using the contents from the 100mL Erlenmeyer flask, following aseptic techniques to prevent contamination.	

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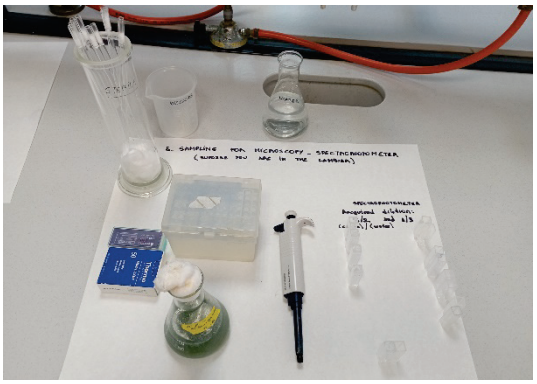
	Task	Picture of the station
5	**Take a sample** from the 100mL Erlenmeyer flask for observation under the microscope, using sterile handling. **Take a sample** from the 100mL Erlenmeyer flask and measure its absorbance at 710nm. Ensure the sample is diluted appropriately before measuring absorbance.	
6	**Prepare a series of dilutions** of the sample and measure the absorbance of each. **Evaluate the results** (yours and your classmates') to validate the Lambert-Beer law.	
7	**Verify the chemicals** required to prepare stock solutions according to the BG11 recipe (refer to Appendix 1, Tables 1 and 2). **Check and adjust** the amount of nutrients based on the availability of chemicals. **Prepare stock solutions** following steps 1 to 3 (Appendix 1, part C, Tables 1.1 and 1.2) for liquid medium preparation only.	

Figure A1: illustration of the different working stations to practice sterile techniques.

FORMATIVE ASSESSMENT

a. Upon completion of the laboratory activities, each group will be assigned to a specific station. Using a mobile phone, the group will record a 1-3 minute video capturing only the key steps of the technique demonstrated. This video will then be shared with the other groups via the online platform or application designated by the teaching team.
b. Each group will review and assess the videos produced by their peers using the provided rubric (Table A1).
c. Bring questions and comments to the next class for discussion.

3. Preparation of stock solutions.

To prepare stock solutions and subsequently use them for algae cultivation medium, follow these steps:

- **Dissolve Solids:** Dissolve each solid component individually in a portion of distilled water (dH_2O), usually about 90% of the final volume, while stirring continuously.
- **Adjust Volume:** Bring the total volume of each solution to the desired final volume with dH_2O in a volumetric flask.
- **Autoclave:** Transfer the solution to a suitable container and autoclave to sterilize.
- **Cool and Store:** Allow the stock solutions to cool and then transfer them to sterile containers (1 small for normal use and 1 large). Label and store the containers at refrigerator temperature.

In Appendix 1 you will find the complete indications for preparing BG11 medium with a full description of components and concentrations. Remember in all the steps, to label the content (name and mass of components) of each of the vessels, including your name and date of preparation.
Read the material necessary for session 2 and check that you prepared all he sterile material in advance: sterile pipette tips, sterile stock solutions, approximately 100mL of sterile sea/fresh water (with the appropriate dose of solid components required by recipe), 100mL Erlenmeyer with a cotton cap, sterile volumetric cylinder to measure 20-50mL.

Table A1: Assessment of Sterile Techniques Demonstrated in Videos. Assess the videos with a scale of 0 (extremely bad) to 5 (perfect) and share.

Video Station 1 Name of the Operators:		
	0..5?	Comments (free description of the observations)
Correct use of materials (pipettes, volumetric flasks, etc)		
Correct handling to prevent contamination		
Video quality		
Video Station 2 Name of the Operators:		
	0..5?	Comments (free description of the observations)
Correct use of materials (pipettes, volumetric flasks, etc)		
Correct handling to prevent contamination		
Video quality		

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Video Station 3 Name of the Operators:		
	0..5?	Comments (free description of the observations)
Correct use of materials (pipettes, volumetric flasks, etc)		
Correct handling to prevent contamination		
Video quality		
Video Station 4 Name of the Operators:		
	0..5?	Comments (free description of the observations)
Correct use of materials (pipettes, volumetric flasks, etc)		
Correct handling to prevent contamination		
Video quality		
Video Station 5 Name of the Operators:		
	0..5?	Comments (free description of the observations)
Correct use of materials (pipettes, volumetric flasks, etc)		
Correct handling to prevent contamination		
Video quality		
Video Station 6 Name of the Operators:		
	0..5?	Comments (free description of the observations)
Correct use of materials (pipettes, volumetric flasks, etc)		
Correct handling to prevent contamination		
Video quality		
Video Station 7 Name of the Operators:		
	0..5?	Comments (free description of the observations)
Correct use of materials (pipettes, volumetric flasks, etc)		
Correct handling to prevent contamination		
Video quality		

Session 2:
Inoculum preparation and sampling

MATERIALS (MATERIALES):

- Ethanol for disinfection (etanol para desinfección)
- Permanent marker (rotulador permanente)
- Sterile material for demonstration preparated by the tutor:
  - ✓ 2L Duran flask, with cap, tubes and vents for bubbling air. (Frasco Duran de 2L con tapa, venteo y conducciones para aireación)
  - ✓ Sterile pipette tips of 1-5mL (puntas de pipeta estériles de 1-5mL)
  - ✓ 500mL volumetric cylinder (probeta de 500mL)
- Sterile material previously prepared by the students (per group)
  - ✓ Sterile 100mL Erlenmeyer
  - ✓ Sterile tips (1ml and 5ml) (puntas estériles)
  - ✓ Sterile stock solutions (soluciones stock estériles)
  - ✓ Sterile cotton caps (torundas estériles)
  - ✓ -Sterile material for sampling (Pasteur pipettes with cotton filter)
  - ✓ -Sterile volumentric cylinder (20mL and 50mL) (Probeta estéril)
- Spectrophotometer and cuvettes (espectrofotómetro y cubetas pequeñas)
- Automatic pipettes (1mL and 5mL) (pipetas automáticas)
- Non-sterile materials: 1L-2L Duran flasks, caps, tubes, conductions that fit the tubes and filters, vent filters, seawater, distilled water, chemicals (sodium nitrate and sodium bicarbonate)
- Microscope (microscopio)
- Bunsen burner or lighter (mechero Bunsen o similar)
- Lab coat and gloves (bata de laboratorio y guantes)
- Incubator (incubador) or agitator placed in the inoculum camera

STUDENT TASKS IN THE LAB:

1. Each student group will inoculate a 100mL Erlenmeyer flask using a prepared inoculum and pre-sterilized materials.
2. The instructor will demonstrate the inoculation of a 2L microalgae culture, explaining the procedure for monitoring cell concentration over time. Each group of students will take turns measuring and recording the concentration on subsequent days after inoculation. The data will be recorded in a table like Table A2, which will be placed near the spectrophotometer for easy access.

Table A2: Absorbance Data for the 2L Culture

Day	Hour	Operator	mL of Culture	mL of Water	Absorbance (710nm)	Observations
.....						

3. Each student group will plan the required material to inoculate a 2L flask. Think of the culture container, provide all the proper mechanisms to bubble sterile air, prepare the material for transferring the liquids and the required medium. Share your list of materials with the other groups and check you don't forget anything. Autoclave.

Session 3:
Scaling Up Microalgae Culture: Preparation of Materials, Inoculum and Culture Medium for Inoculating a 2L Bioreactor

MATERIALS (MATERIALES):

- Ethanol for disinfection (etanol para desinfección)
- Permanent marker (rotulador permanente)
- Sterile material previously prepared by the students (per group)
    - ✓ Sterile Duran flask
    - ✓ Sterile tips (1ml and 5ml) (puntas estériles)
    - ✓ Sterile stock solutions (soluciones stock estériles)
    - ✓ Sterile cotton caps (torundas estériles)
    - ✓ Sterile material for sampling (Pasteur pipettes with cotton filter)
    - ✓ Sterile volumentric cylinders if necessary (Probetas estériles si se necesitaran)
- Spectrophotometer and cuvettes (espectrofotómetro y cubetas pequeñas)
- Automatic pipettes (1mL and 5mL) (pipetas automáticas)
- Non-sterile materials: 1L-2L Duran flasks, caps, tubes, conductions that fit the tubes and filters and the inlet port of the bioreactor, vent filters, seawater, distilled water, chemicals (sodium nitrate and sodium bicarbonate)
- Microscope (microscopio)
- Bunsen burner or lighter (mechero Bunsen o similar)
- Lab coat and gloves (bata de laboratorio y guantes)
- Incubator (incubador) or agitator placed in the inoculum camera

STUDENT TASKS IN THE LAB:

1. Puzzle: relate the images a) b) and c) from **Figure A2** to their potential use in the process of culturing microalgae. Each configuration can be related to more than one words.
2. Decide on the schedule and proceed on the following activities.
   The total number of groups will be divided into an even number of subgroups. Two groups at a time will attend the lab, simultaneously, for a 20-30-minute session. During the alternate ~half-hour, the students will take their normal break. This scheduling minimizes waiting time for the use of the laminar flow hood. Decide on the schedule for each of the groups to fit in the class schedule and start. During this part of the session, two key tasks must be completed:
   i. Medium Preparation and Inoculation: Students will prepare fresh medium (using sterile water and stock solutions) and inoculate a 500mL Erlenmeyer flask with the inoculum from their previous 100mL culture. Assure that the inoculum is well conserved by observing cells under the microscopy.
   ii. Collaborative preparation for Inoculation of Bubble Column: Each group will prepare a portion of the medium and a cap provided with connections for inoculating the sterile bubble column collaboratively. For example, if there are 4 groups and the total volume of the bubble column is 4L, each group will be responsible for preparing 1L of medium.

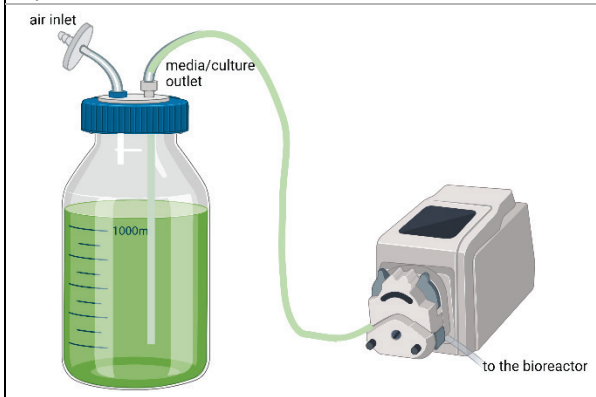
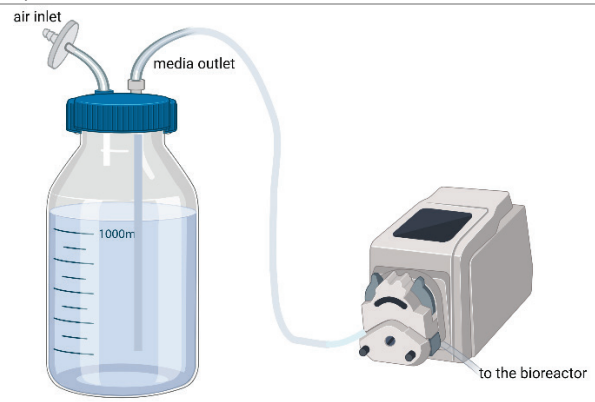
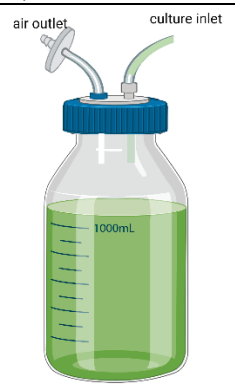
a)	b)	c)
		
INNOCULATION-DILUTION-HARVESTING Place the corresponding words and write the description in the next row for each of the figures (a to c)		

Figure A2: Material for activity 1) Relate the figures a) b) and c) to the potential uses during inoculation and continuous culture. Briefly describe the use.

Session 4:
Preparing and Setting Up a Bubble Column Bioreactor and a raceway for Inoculation

MATERIALS (MATERIALES):

- Ethanol for disinfection (etanol para desinfección)
- Permanent marker (rotulador permanente)
- Sterile material previously prepared by the students to inoculate and clean (per group)
- Spectrophotometer and cuvettes (espectrofotómetro y cubetas pequeñas)
- Automatic pipettes (1mL and 5mL) (pipetas automáticas)
- Non-sterile materials: 1L-2L Duran flasks, caps, tubes, conductions that fit the tubes and filters and the inlet port of the bioreactor, vent filters, seawater, distilled water, chemicals (sodium nitrate and sodium bicarbonate)
- Microscope (microscopio)
- Bunsen burner or lighter (mechero Bunsen o similar)
- Lab coat and gloves (bata de laboratorio y guantes)
- Incubator (incubador) or agitator placed in the innoculum camera

STUDENT TASKS IN THE LAB:

To set up and inoculate a bioreactor a logical series of operations must be performed. In Table A3 you can find a series of steps and in Table A4 a series of description of those steps for either a small indoor bubble column or a small indoor raceway. You have to order it chronologically according to headings indicated in Table A5, where you can see an example solved.

Table A3: Titles of the steps ordered alphabetically to chronologically order in the second column of Table A5. The step used as example is underlined.

STEP
Final checking
Inoculation. Initiating the culture
Inoculation. Inoculum Preparation
Select the proper bioreactor for your application
Setting Up the Culture Vessel. Autoclaving Components
Setting Up the Culture Vessel. Checking the Culture Vessel
Setting Up the Culture Vessel. Chemical sterilization
Setting Up the Culture Vessel. Cleaning the Bioreactor:
Setting Up the Culture Vessel. Equilibrate
Setting Up the Culture Vessel. Filling with media and Preparing for Cultivation
Setting Up the Culture Vessel. Final Rinse

Table A4: Titles of the steps ordered alphabetically to order chronologically in the second column of Table A5. The items used in the example are underlined.

DESCRIPTION	
CLOSE BIORREACTOR: BUBBLE COLUMN	OPEN BIOREACTOR: RACEWAY
Add the inoculum to the bioreactor	After chemical sterilization, rinse the bioreactor, accessories, and tubing with sterile water using sufficient pressure to ensure all surfaces are clean and free of contaminants.
Autoclave any bioreactor components that are compatible with this sterilization method, such as venting filters and conduits.	Check the correct work of sensors, spargers, and lights
Check the correct work of sensors, spargers, lights and paddles.	Dispense the culture medium into the bioreactor by pouring it inside.
Dispense the culture medium into the bioreactor using a sterile filling process. Any modifications to the medium must be performed carefully within a sterile environment, such as a laminar flow cabinet.	Ensure the vessel is undamaged and all O-rings and vessel seals are intact and correctly positioned to avoid contamination during inoculation. Verify the functionality of all sensors, gas inputs, ports for corrective agents, sampling, venting, dilution, and harvesting according to process requirements. Adjust the height of any immersion pipes or adjustable fittings, ensuring they are positioned correctly. Calibrate all sensors as required for the specific process.
Ensure the vessel is undamaged and correctly positioned. Verify the functionality of all sensors, gas inputs, ports for corrective agents, sampling, venting, dilution, and harvesting according to process requirements. Adjust the height of any immersion pipes or adjustable fittings, ensuring they are positioned correctly. Calibrate all sensors as required for the specific process.	Introduce the inoculum via a sterile tubing connection. This connection can either be made in a sterile cabinet or created directly at the bioreactor using a tube welding machine. Sterilize the risky parts during “in-situ” handling with an alcohol solution, and consider working near a flame to reduce contamination risks.
Not necessary	Not necessary
Not necessary	Not necessary
Not necessary	Prepare the inoculum (e.g., from a shake flask pre-culture) and transfer it into a suitable vessel for inoculation. Sterilize the risky parts during “in-situ” handling with an alcohol solution, and consider working near a flame to reduce contamination risks.

DESCRIPTION	
CLOSE BIORREACTOR: BUBBLE COLUMN	OPEN BIOREACTOR: RACEWAY
Removing Salts: Rinse the bioreactor thoroughly with tap water and hydrochloric acid (HCl) to eliminate any residual salts. Rinsing the Bioreactor: Use adequate water and pressure to clean all surfaces and corners of the bioreactor. Ensure that all accessories and tubing are also rinsed thoroughly with tap water until the system is free of any chemical residues or contaminants.	Removing Salts: Rinse the bioreactor thoroughly with tap water and hydrochloric acid (HCl) to eliminate any residual salts. Rinsing the Bioreactor: Use adequate water and pressure to clean all surfaces and corners of the bioreactor. Ensure that all accessories and tubing are also rinsed thoroughly with tap water until the system is free of any chemical residues or contaminants.
Run a "blank" culture medium (containing no cells) for up to 24 hours to allow the vessel conditions to equilibrate before inoculation.	Sterilize components that cannot be autoclaved using chemical agents like sodium hypochlorite. Ensure all chemicals are thoroughly rinsed out after sterilization.
When the culture requires axenic conditions	When the most important is the process of depuration of the water or the quality of the cells are not so important

Table A5: Heading for ordering the items of tables A4 and A3, including an example of the step number 8 solved.

ORDER	STEP	DESCRIPTION	
		CLOSE BIORREACTOR: BUBBLE COLUMN	OPEN BIOREACTOR: RACEWAY
1	----------	----------	----------

8	**Setting Up the Culture Vessel** Equilibrate	Run a "blank" culture medium (containing no cells) for up to 24 hours to allow the vessel conditions to equilibrate before inoculation.	Not necessary
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Once agreed on the order, collaboratively inoculate two bioreactors of 2L (a raceway and a bubble column) by using the material prepared in the previous session. Simultaneously, the students who are not engaged in the practical tasks will be working in the data treatment of the data obtained from previous cultures

1. Data Presentation: Copy and paste an image of the absorbance data collected over the previous days into your report.

2. Correction of Absorbance Values: Calculate and report the corrected absorbance values for the diluted samples. To adjust the absorbance values, account for the dilution factor by multiplying the measured absorbance by this factor. The dilution factor is determined by the ratio of the total volume of the diluted sample to the volume of the original sample used. For example, if a sample was diluted by adding 1mL of the original sample to 9mL of solvent (totalling 10mL), the dilution factor is 10. Multiply the absorbance measured from the spectrophotometer by 10 to obtain the absorbance of the undiluted sample.
3. Plotting the Growth Curve: Construct a growth curve for the microalgae culture inoculated in the 2L bioreactor by plotting absorbance against time.
4. Identification of Growth Phases: Analyze the growth curve to identify and describe the different growth phases observed in the microalgae culture.

Assessment:

Teaching Team Observations: The teaching team will conduct observations during the laboratory session to assess performance and adherence to protocols.

Self-Assessment: Upon completion of the laboratory work, each student should evaluate their own understanding and performance by indicating their level of agreement with the statements in Table A6. Assign a score from 0 (totally disagree) to 5 (completely agree) for each statement based on the acquired knowledge

Table A6: Self-Assessment of Laboratory Practice

STATEMENT	MARK
I know how to prepare media stock solutions.	
I know how to disinfect working surfaces.	
I know how to monitor microalgae growth in a bioreactor.	
I know how to produce and maintain small inoculums of microalgae in the lab.	
I attended all the sessions punctually.	
I clean my workspace after completing the tasks.	
I can name the materials in Spanish.	

Comments:

APPENDIX 1: BG-11 Medium Recipe

a- Directions to prepare the medium

For 1 liter Total Volume Liquid Medium

1. Add the first 8 components of Table 1 (in the specified order) to, approximately, 900mL of dH2O while stirring continuously.
2. Bring the total volume to 1 liter with dH2O.
3. Cover the medium and autoclave to sterilize.
4. Allow the medium to cool then store at refrigerator temperature.

b- For the Agar Medium:

1. Add the first 8 components of Table 1 (in the specified order) to, approximately, 400mL of dH2O while stirring continuously.
2. Bring the total volume to 500mL with dH2O.
3. In a separate container add 15g of agar to 500mL of dH2O (final 1.5% w/v).
4. Cover and autoclave both solutions separately.
5. In a water bath allow both solutions to cool to 45-50 °C.
6. Add sterile Sodium Thiosulfate (Stock 10 of Table 1) to the agar solution and mix well.
7. Combine both agar and liquid solutions, mix well.

c- Directions to prepare the stock solutions

1. Dissolve each of the solids individually in 0,9 times the final volume of dH2O while stirring continuously.
2. Bring to total volume with dH2O in a volumetric flask.
3. Transfer to a Duran bottle.
4. Autoclave.
5. Allow to cool.
6. Transfer part of the stocks to small sterile containers to handle. Then store both, the small and the big flasks at refrigerator temperature.

Table 1: Description of components and concentration of the different stocks required to prepare BG11 medium, with indication of the proportion to be added for medium preparation and final concentration of the components.

	Component	Amount	Stock Solution Concentration	Final Concentration
1	$NaNO_3$	1 mL/L	15 g/100 mL	17.6 mM
2	K_2HPO_4	1 mL/L	4 g/100 mL	0.22 mM
3	$MgSO_4 \cdot 7H_2O$	1 mL/L	7.5 g/100 mL	0.3 mM
4	$CaCl_2 \cdot 2H_2O$	1 mL/L	3.6 g/100 mL	0.24 mM
5	Citric Acid$\cdot H_2O$	1 mL/L	0.6 g/100 mL	0.012 mM
6	Ferric Ammonium Citrate	1 mL/L	0.6 g/100 mL	0.02 mM
7	$Na_2EDTA \cdot 2H_2O$	1 mL/L	0.1 g/100 mL	0.002 mM

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	Component	Amount	Stock Solution Concentration	Final Concentration
8	Na_2CO_3	1 mL/L	2 g/100 mL	0.18 mM
9	BG-11 Trace Metals Solution	1 mL/L	*See recipe (Table 2)**	*See recipe (Table 2)**
10	Sodium Thiosulfate Pentahydrate (agarmedia only; sterile)	1 mL/L	24.8 g/100 mL	1 mM

***Directions to prepare the stock of Trace Metals Solution (Table 2)**

For 1 liter Total Volume

1. To approximately 900mL of dH2O add the T components in the specified order while stirring continuously.
2. Bring total volume to 1 liter with dH2O.
3. Store at refrigerator temperature.

Table 2: Components of the trace metal stock solution and final concentration.

	Component	Amount	Final Concentration
T1	H_3BO_3	2.86 g/L	46.00 mM
T2	$MnCL_2 \bullet 4H_2O$	1.81 g/L	9.00 mM
T3	$ZnSO_4 \bullet 7H_2O$	0.22 g/L	0.77 mM
T4	$Na_2MoO_4 \bullet 2H_2O$	0.39 g/L	1.60 mM
T5	$CuSO_4 \bullet 5H_2O$	0.079 g/L	0.30 mM
T6	$Co(NO_3)_2 \bullet 6H_2O$	49.4 mg/L	0.17 mM