Research

Standard Operating Procedure for Microalgae Cultivation, Harvesting and Biomass Processing

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Abstract:
A standard operating procedure (SOP) for the cultivation, harvesting, and processing of microalgae biomass is presented. The aim of this SOP is to optimize biomass production
in the laboratory while preserving valuable bioactive compounds through precise control of growth conditions and efficient harvesting techniques. Microalgae are increasingly recognized as a valuable source of bioactive compounds, including compounds with potential for agricultural, pharmaceutical, and environmental applications thus contributing to bioeconomy development. By following the outlined steps, high biomass yield and quality can be achieved. High biomass quality ensures that all active compounds are contained in the sample and are stable for various further treatments and chemical analyses such as for the determination of phytohormones or antimicrobial compounds when stored properly.

Keywords: Bioactive compounds; Green product; Microalgae biomass; Microalgae sample preparation

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1. Definitions

BBM: Bold's basal medium
DO: concentration of dissolved oxygen
EC: electroconductivity
GC/F: glass microfiber filters grade GF/C
HPLC: high-performance liquid chromatography
LC-MS/MS: liquid chromatography coupled with double mass spectrometry
SOP: standard operating procedure
TSS: total suspended solids
OD: optical density

2. Background

Microalgae are a diverse group of photosynthetic microorganisms, which are capable of thriving in both freshwater and marine environments (Singh and Saxena, 2015). Due to their fast growth rates and the ability to produce a wide variety of valuable biomolecules, they have gained increasing attention across various industries, including cosmetics, food (nutritional supplements, animal feed), biofuel production, as well as in medicine and pharmaceutical industry to produce bioactive compounds (Basheer et al., 2020; Castro et al., 2023; Eze et al., 2023; Zuccaro et al., 2020) or for agricultural application (Gonçalves, 2021; Miranda et al., 2024). The cultivation of microalgae involves providing optimal conditions for their growth, including temperature, access to light, CO_2, pH, essential nutrients such as nitrogen, phosphorus, and trace elements, as well as the selection of appropriate cultivation systems (Amaro et al., 2023); namely, microalgae can be cultivated auto/hetero/mixotrophically either in closed (photo/fermentation) bioreactors or open ponds (Borowiak and Krzywonos, 2022; Muhammad et al., 2020; Tran et al., 2020).
Once microalgae reach their desired growth stage, they need to be separated from the growth medium. Common methods include centrifugation, flocculation, filtration and sedimentation. Centrifugation is highly effective and suitable for lab-scale research (Kumar et al., 2023; Singh and Patidar, 2018).

For a better understanding of the potential of microalgae to produce bioactive molecules, such as phytohormones, it is essential to standardize procedure including microalgae cultivation, harvesting and final processing steps. The procedure should ensure that all target compounds are covered and stable during the process and storage even though some compounds are sensitive to various environmental factors. SOP is therefore essential to fully uncover the bioactive potential of microalgal biomass for further applications (Thiviyanathan et al., 2024). Also, more in-depth research focused on optimizing the production of bioactive substances in selected microalgal species is needed, with an emphasis on manipulating environmental and stress factors in order to achieve the highest production of bioactive compounds (Liang et al., 2009; Magalhães et al., 2024; Mohsenpour et al., 2021). Detailed SOP for microalgae cultivation at the laboratory scale, along with harvesting and biomass processing, is presented to support advancements in this research field.

3. Purpose, Scope and Applicability

Cultivation of microalgae, particularly for biomass with high valuable compounds such as phytohormones, often necessitates optimized growth conditions, appropriate synthetic media and/or complex bioreactors (Amaro et al., 2023; La Bella et al., 2022; Menegazzo and Fonseca, 2019). The presence and the role of bioactive molecules in microalgae are still not fully understood, therefore complete understanding of the biological, chemical, and physical processes taking place during microalgae cultivation, harvesting and biomass processing, is urgently needed (Van Do et al., 2020; Senousy et al., 2023).

The purpose of the SOP is to define the key parameters and optimize the processes in order to develop an effective product from microalgae biomass. The cultivation of microalgae on laboratory scale is described, and efficient harvesting and biomass processing techniques are presented.

The scope of the SOP is to define the steps of microalgae biomass preparation to obtain bioactive compounds, starting from the cultivation to the point when samples of biomass are used for analyses/application. When developing protocols, care must be taken to maintain the bioactive properties of the biomass, as inappropriate processing techniques may lead to the degradation or loss of valuable compounds. Following the standardized procedures is crucial to ensure the effective preparation of high-quality biomass.

The applicability of the SOP is mainly targeted at the preparing of microalgae biomass for research of its bioactive potentials; however, the samples can also be further processed for other research purposes, e.g., antimicrobial activity, analysis of the main components of interest for biofuel and bioplastic production (Barsanti and Gualtieri, 2018; Van Do et al., 2021; Parmar et al., 2023).

In the framework of this SOP, the prepared samples proved to be suitable for phytohormones quantification using LC-MS/MS and biostimulatory potential screening using a commercial phytotoxicity assay to determine the direct effect of algae biomass on the germination of *Sinapis alba* (*S. alba)* seeds.

4. Health and safety warning

Microalgae biomass used in experiments is not applicable for personal use, nor approved for any other purposes, except for specific scientific research. In the laboratory, it is crucial to have a clean and organized workspace and to regularly calibrate and maintain the laboratory equipment to ensure research credibility and safe operation, respectively.

Sterile material is required for microalgae cultivation in the laboratory. Autoclaving operates under specific pressure and temperature conditions (121°C, 15 min), therefore proper training in safe operation is essential to avoid accidents. Always monitor operating parameters and follow shutdown procedures during maintenance.

The hazards and safety measures regarding the use of the centrifuge are thoroughly described in Centrifuge Safety Guidelines prepared at the University of Stanford (https://ehs.stanford.edu/reference/centrifuge-safety). Prevent leakage into the centrifuge chamber because of damaged or poorly closed tubes, additionally, rotor should be loaded in a balanced way to minimize vibrations (Kralj-Iglič et al., 2024).

Synthetic growth media used in the microalgae cultivation may contain hazardous chemicals. To minimize the risk of exposure, proper handling of the chemicals, as well as the

use of personal protective equipment such as gloves, goggles, and lab coats, is required. All personnel need to be trained in first aid and emergency procedures, including spill response and chemical handling protocols. The solvents used in sample preparation (such as ethanol) are flammable, they should therefore be stored in appropriate flammable storage cabinets.
Fine particulate matter is produced during drying. To avoid respiratory hazards, it has to be ensured that the drying is conducted in well-ventilated areas and the respiratory protection is used when handling powdered biomass. When storing biomass at the – 80 °C appropriate gloves should be used.

5. Cautions

Disposal of all used material should be in accordance with applicable laws and good research and laboratory practices.
Written operating instructions for the equipment used should be available to the users on site. An electronic or printed register for user reservations should be provided as well as the laboratory notebooks (log sheets) for researchers to log and keep track of the use of individual instruments.
Operators should be aware of the potential hazards associated with the cultivation of microorganisms and should follow established safety protocols. All chemicals in the laboratory must be kept in a separate room to which only employees have access.

6. Personnel Qualifications / Responsibilities

The staff should be trained in handling the samples and applying the SOP. Chemical handling instructions and safety precautions (safety data sheets) should be reviewed prior to their use. Staff should be qualified to perform procedures, trained to use specific equipment, such as autoclave, freeze-dryer or centrifuge, and always follow the established protocols. Staff must accurately document all process parameters, observations, and results in laboratory notebooks (log sheets) or digital systems.
Regular equipment maintenance should be conducted as per the manufacturer's instructions and internal guidelines. Repairs and adjustments must only be performed by authorized maintenance personnel with the necessary technical qualifications.
Staff must ensure that all equipment, surfaces, and tools are properly cleaned and sanitized after each use to prevent cross-contamination and comply with operational hygiene standards.
Ensure proper labelling, storage, and tracking of samples to ensure traceability and prevent sample deterioration. Reports should be submitted to supervisors.

7. Materials, Equipment and Supplies

For microalgae cultivation and harvesting: *Chlorella vulgaris* can be isolated from the environment or acquired from various sources such as culture collections, commercial suppliers, and research institutions. Controlled laboratory conditions and sterile materials are essential for axenic cultivation of microalgal culture. Use appropriate growth media, such as agar plates or liquid media, to support algal growth, and equipment as**:** Erlenmeyer flasks with stoppers facilitating gas exchange, laboratory shaker (e.g. Phoenix OS 20), appropriate room temperature (T; 20-25 °C) and light intensity (e.g. 4000 K, fluoro or LED lights; 16:8 light:dark period), centrifuge tubes, tubes' holders and sterile dH_2O.
Growth medium: Bold's Basal Medium (BBM; prepared from commercially available solid mixtures (e.g. PhytoTech Lab) or in the laboratory from primary chemicals.
Cultivation conditions are monitored using a portable multiparameter meter (e.g. Multi 3610 IDS), by measuring T, pH values, dissolved oxygen concentration (DO), and electrical conductivity (EC) in the growth medium.
Culture fitness / cell number is determined with optical microscopy (e.g. Olympus CX21) by counting in a Neubauer counting chamber, and the growth of microbes on nutrient agar is checked. Optical density at 680 nm (e.g. spectrophotometer Nanocolor, VIS, Macherey-Nagel) and Chlorophyll A concentration is measured using extraction with ethanol, as we described in Proceedings 12th Socratic Lectures 2025 Part I, and calculated according to Lichtenhaler and Buschmann (2001).

The total suspended solids (TSS) in the sample are determined by filtration using glass Whatman glass microfiber filters (GF/C) according to the standard method ISO 11923:1997 or APHA (2540 D).

Nutrients (nitrates; N, phosphates; P) are analysed spectrophotometrically according to standard methods (e.g. ISO 6878:2004 for phosphate, ISO 7150-1:1996 for ammonia, ISO 7890-3:1996 for nitrate, EN 26777:1996 for nitrite), using cuvette tests (e.g. Nanocolor, Macherey-Nagel) or by high-performance liquid chromatography (HPLC) (e.g. Agilent Technologies 1100 Series).

For biomass processing: centrifuge tubes and sterile dH_2O for washing biomass, oven for drying and freeze-dryer for lyophilization, the fridge/freezer for biomass storing.

Devices and other equipment used: autoclave, centrifuge, oven, spectrophotometer, microscope, Neubauer counting chamber, portable meter for physio-chemical parameters, cuvette tests, HPLC (e.g. Agilent Technologies 1100 Series).

8. Computer Hardware and Software

Hardware: PC; software: photo editing software e.g. ImageJ, data management and word processing software such as Microsoft Office (Word, Excel); data saving and sharing insfrastructure: hard drive or cloud-based services, communication platforms and software: e.g. Microsoft Teams.

9. Step by Step Procedure

9.1. Chlorella vulgaris cultivation in controlled laboratory conditions

Microalgae cultivation in the laboratory involves several carefully controlled steps to optimize algal growth and produce quality biomass:

- A *Chlorella vulgaris* sample obtained from AlgEn, algal technology centre Ltd. was maintained in sterile laboratory conditions in a liquid medium.
- Bold's Basal Medium (BBM) liquid and agar plates are prepared, sterilized in an autoclave and allowed to cool to room T.
- Culture is isolated from BBM liquid to the BBM agar plates: the isolates are purified by streak plating; individual colonies are then transferred from BBM with agar (15 g/L) plates into liquid BBM.
- Aseptic techniques assure maintenance of culture purity during transfer. The flasks are covered with sterile foam stoppers to prevent contamination and ensure gas exchange, and afterwards placed on an orbital shaker (160 rpm) to ensure gentle mixing and avoid sedimentation.
- This process typically takes several weeks, as it involves multiple transfer and incubation steps to ensure the purity of the isolated culture (Alam et al., 2019).

Culture growth is maintained in Erlenmeyer flasks with liquid BBM:

- Growth conditions are 16:8 light/dark cycle and an ambient temperature of 25 °C ± 2 °C with continuous mixing.
- The batch is harvested when the culture reaches the desired growth phase (Section 9.2.). Once the growth rate stabilizes, the culture enters the stationary phase (> 1.0×10^8 cells/mL), indicating that fresh inoculum should be prepared for a new batch.
- A part of the grown culture is removed for the preparation of fresh inoculum; the culture in the exponential growth phase (on the third day, when the inoculum is prepared in 100 - 250 mL BBM; OD 680 < 1, approximately 10^7 cells/mL) is harvested by centrifugation, washed, and concentrated in fresh BBM (OD 680 = 2).
- 10 % of the inoculum is transferred into sterile Erlenmeyer flasks (volume 250 - 2000 mL) containing BBM (100 – 1000 mL; added inoculum 10 - 100 mL).
- The growth of *C. vulgaris* typically stabilizes after approximately two weeks of culturing in 100 - 250 mL BBM under the specified conditions. However, the optimal time for harvesting should be determined through preliminary tests, as growth rates may vary based on specific experimental conditions; consequently, the duration of the experiment may be influenced (Section 9.6).
- In general, subculturing is performed every few weeks when the culture reaches the stationary phase to prevent aging and sustain optimal growth (Sharma et al., 2012).

- To ensure reliable and reproducible results, it is essential to maintain consistent experimental conditions and optimized biomass processing methods as described here.

9.2. Microalgae biomass harvesting

Laboratory-grown cultures are separated from the growth medium with centrifugation. Samples should be subsequently washed in sterile buffer or dH_2O before further processing by centrifuging in 50 mL tubes for 10 min, 6500 rpm, removing the supernatant and repeating process until all biomass is harvested.

For biomass washing the process is repeated 3 times:

- 25 mL of sterile dH_2O is added, vortexed 10 seconds and centrifuged (10 min, 6500 rpm).
- Supernatant is removed and concentrated biomass is prepared in buffer/fresh medium, based on target use: cultivation/analysis/application.

9.3. Biomass processing

Possible ways to process washed/unwashed biomass for further application or storage are various, including using fresh biomass, freezing and thawing, drying (in the oven at the T < 45 °C for few hours, freeze-drying for 24 h). The selection of the processing procedure affects the activity and abundance of bioactive compounds. In terms of phytohormones research, processed biomass is stored at – 80 °C till further use, but immediate use of biomass is recommended. Otherwise, storage time should be further considered.

The main steps of SOP, including microalgae cultivation, harvesting and biomass processing are presented in **Figure 1**.; The recommended analyses are described in the section below.

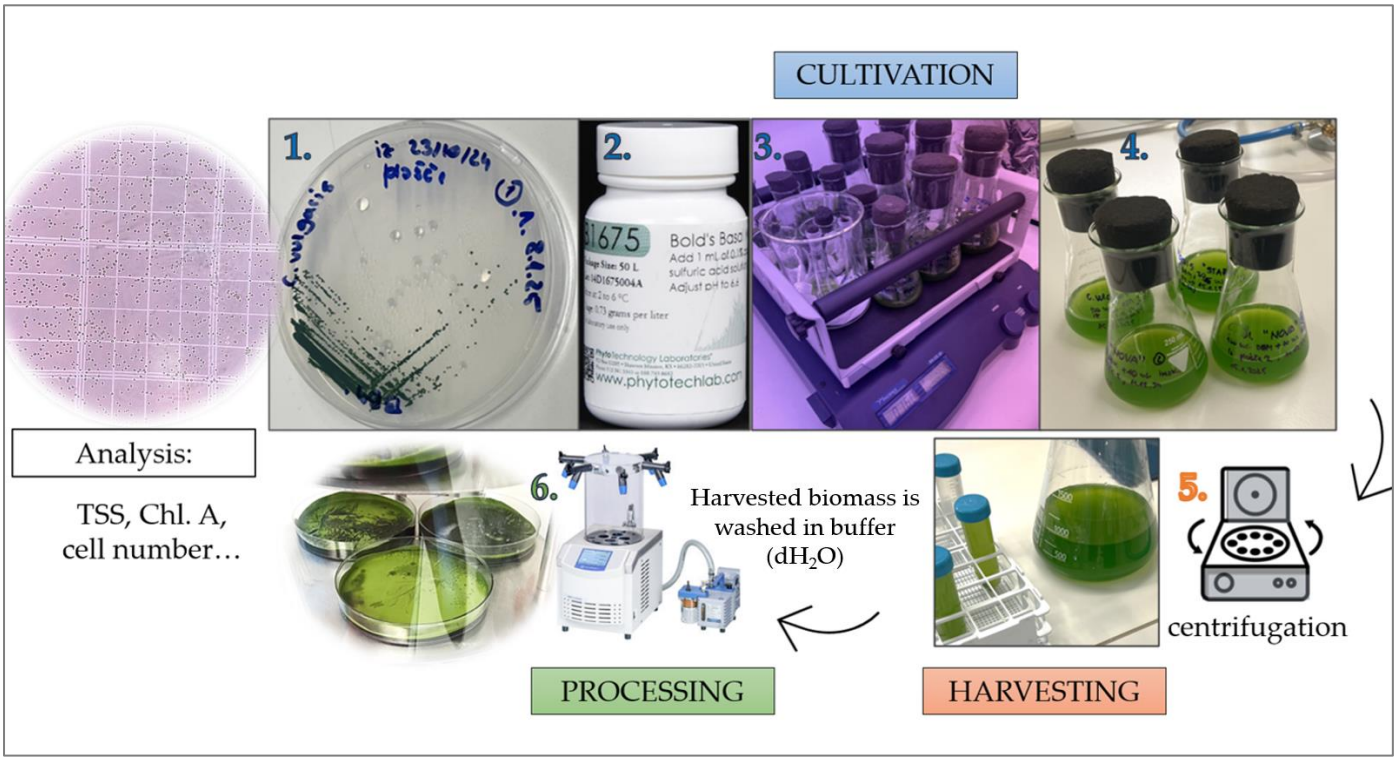

Figure 1. Cultivation of *Chlorella vulgaris* in controlled laboratory conditions; total suspended solids (TSS), chlorophyll a (Chl. A), cell counting, biomass harvesting and processing: 1. *C. vulgaris* purification on BBM + 15 g/L agar; 2. BBM medium; 3., 4. maintaining culture growth in liquid BBM; 5. harvesting; 6. processing: freeze-drying for application/analysis or storing at the – 80 °C until further use.

9.4. Recommended analysis

Cultivation conditions are daily monitored using a portable multiparameter meter (e.g. Multi 3610 IDS), by measuring T, and physicochemical parameters in the growth medium (pH, DO, EC). *Chlorella* sp. can grow in a wide pH range (4-10), but it is recommended by a supplier PhytoTech Lab to adjust the pH of BBM to 6.6. Measure initial DO, pH and EC in the medium, before and after microalgae inoculation. Parameters can vary depending on mixing, biomass density, and metabolic activity (Section 9.6.).

Periodically measure and adjust nutrient levels with controlled nutrient supplementation/diluting medium to maintain optimal levels. Namely, the appropriate NO_3 concentration at the beginning of the experiment is 250-500 mg/L, but nitrates may become limiting in late growth phases (< 5 mg/L); therefore, appropriate supplements should be added. Additionally, culture composition, OD, Chl. A, and TSS should be daily determined.

9.5. Troubleshooting

- Cultivation system not operating as usual. The problem should be defined and addressed appropriately. The natural increase in pH during microalgae cultivation can affect nutrient availability and, consequently, EC levels. When observing a decrease in EC with an increase in pH in a microalgal culture, this may indicate the precipitation of nutrients, which reduces their availability to the algae (Beltrán-Rocha et al., 2021). If key nutrients (N, P) become depleted, photosynthetic activity may drop, leading to lower oxygen production and decreased DO (Wan Hee et al., 2021). For new experiments, consider appropriate addition of nutrients.
- Variability in experimental factors; variations in flask volume, inoculum composition, or cultivation parameters such as temperature, light intensity and nutrients availability can significantly alter the growth patterns and overall performance of the culture. Therefore, controlling these factors throughout the experiment is critical for accurately monitoring growth phases and optimizing culture conditions.
- Microalgae cultures are contaminated. The culture should be discarded immediately (autoclaved before disposal). For new experiments, fresh non-contaminated culture should be used.
- Changed turbidity of (stock) solutions/reagents. The solutions/reagents should be discarded and freshly prepared.
- For better efficiency of chlorophyll extraction small volumes of microalgae culture should be used. Additionally, pellets of biomass will be more stable after centrifugation.

10. Data and records management

All experimental details should be carefully documented in the lab journal. Both raw and processed data should be securely stored in electronic format with appropriate backups. Pictures should be taken during experiments, and afterwards stored on hard drives or uploaded on cloud storage services.

11. Waste management

Disposal material used by the staff should be disposed of in appropriate waste fractions, according to applicable laws and good research laboratory practice. Chemicals should be disposed of in appropriate containers.

12. Related protocols or SOPS

This SOP includes a combination of optimized known procedures. Optimizing conditions and processing methods on the production and activity of compounds as phytohormones in microalgae can guide the development of high-quality biomass for agricultural use.

13. Quality control and quality assurance section

13.1 Instrument calibration

Calibration records should be securely stored and readily accessible for audits or quality assessments. If any of the instruments falls outside acceptable calibration limits, it must be adjusted or serviced before further use. Instruments should be maintained regularly.

13.2 Critical processes parameters and checkpoints

All material needed for SOP performance should be prepared ahead and sterile. Work in aseptic laboratory conditions to help prevent contamination of cultures or samples; only the intended microorganisms or materials should be involved in the experiment to maintain the integrity of the research.

The procedure from harvesting to the end of the process should be performed in the shortest time possible and follow the steps described, to obtain products with consistent quality and reduced variability. Prevent light and air exposure of biomass after drying (wrap flasks in aluminium foil), and immediately store it at – 80 °C, if not used. Ensure that there's minimal air exposure to prevent oxidation, check for properly sealed tubes. Prepared samples of biomass should be stored in small tubes, if not used at once, to avoid multiple opening-closing the tube containing entire gathered biomass. Avoid repeated freezing and thawing of samples as this can degrade or alter their integrity. Maintain detailed records of all stored samples, including storage conditions, date of storage, and any associated data.

Periodically check T conditions, instruments performance, and tube integrity to ensure samples are properly preserved. Samples should be used in the shortest time possible. Repeat chosen tests after few months of sample storage to control the quality.

14. Data on procedures and samples

Data on procedures and samples is given in **Table 2**.

Table 2. Data on procedures and samples.

Description of the yield	Processed (freeze-dried) biomass of *C. vulgaris* culture; immediate use of samples is recommended
Total volume of the culture yield	e.g., 500 mL
Total mass of the microalgae yield	0.05 g
Time required to obtain the yield	2 weeks
Estimated cost without manpower	Cost of equipment and maintenance
Contact person	Eva Cepec, eva.cepec@zf.uni-lj.si

Conclusions

This SOP describes laboratory procedures for *C. vulgaris* cultivation, harvesting and biomass processing to provide quality biomass for further analysis or application. Strict adherence to sterile conditions, proper cultivation of microalgae cultures, biomass processing and sample handling are essential to maintain the integrity of cultures, prevent contamination and obtain quality microalgae biomass. By following these critical process parameters and checkpoints, researchers can enhance the reliability of experimental outcomes and improve the overall efficiency of laboratory workflows.

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