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Culturing *Chlorella Vulgaris* and *Desmodesmus Quadricauda*

Jakub Nedbal[1]

[1]King's College London

1 Works for me dx.doi.org/10.17504/protocols.io.bd6vi9e6

Jakub Nedbal
King's College London

ABSTRACT

This protocol describes the steps required for maintaining a healthy culture of *Chlorella vulgaris* and *Desmodesmus quadricauda*. It uses ½ SŠ Growth Medium (0.5 g/l). The cells are grown until saturation or when a new split is needed. At the lowest light level, the cell culture split should take place at least once every two weeks.

EXTERNAL LINK

https://app.labstep.com/sharelink/5ac9f965-791b-4333-b17b-f57e5e1dc42d

GUIDELINES

Culturing the microalgae is a process of replenishing fresh growth medium and diluting the culture suspension density.

The microalgaeculure involves the following steps:

- Preparing medium
- Dissociating clumped cells
- Counting the cell culture density
- Calculating the fresh medium and inoculation culture volumes
- Counting cells
- Calculating cell culture density

MATERIALS

NAME	CATALOG #	VENDOR
Virkon powder	148-0201	VWR international Ltd
1/2 SŠ medium (0.5 g/l)	View	Homemade
Sterile Erlenmeyer flasks with cotton wool plug and aluminium foil seal	View	Homemade

MATERIALS TEXT

Sterile flow hood

Watchmaker tweezers

Serological pipettes and pipette filler

SAFETY WARNINGS

The main hazards involved in culturing cells include handling microalgae, liquid media containing a range of chemical substances, chemical bleach agents, fragile glass flasks, repetitive strain injury due to pipetting and using a tally counter for counting cells. Each of these hazards needs to be assessed according to local rules and regulations and appropriate measures need to be taken.

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Use personal protective equipment. Take breaks between repetitive tasks. Clean working areas and wipe down spills using appropriate equipment. Do not release live microalgae into the environment, use prescribed bleach or microbicides to destroy the cultures and appropriate biohazard disposal routes for contaminated consumables.

BEFORE STARTING

Make sure the ½ SŠ Growth Medium is prewarmed to room temperature. Leave it standing for at least an hour outside the fridge for it to warm up.

Start and wipe down the laminar flow cabinet with 70 % denatured ethanol solution.

Make sure you have enough sterile culture flasks. You can use disposable flasks, like T75, or prepare glass Erlenmeyer flasks as described in Autoclaving Erlenmeyer Flasks for Sterile Algal Cultures.

Have all equipment ready for counting cells, this includes a hemocytometer and a tally counter, as described in Counting Microalgae Culture Density.

Peparatory Steps

1 Warm-up ½ SŠ Growth Medium (0.5g/l) to room temperature.

Leave it outside of a fridge for at least an hour, or leave it in a 37 °C water bath for 10 minutes or so.

2 Pre-label Erlenmeyer flasks with the culture type, date, own initials, passage number or other pieces of information as required.

For sterilizing the Erlenmeyer culture flasks, see Autoclaving Erlenmeyer Flasks for Sterile Microalgae Cultures

3 Turn on and wipe clean the sterile hood.

4 Take out previous culture passage from the shaker and bring it to the tissue culture room.

5 Dissociate cell aggregates on a shaker.

Use low power setting to prevent the suspension splashing onto the cotton wool plug..

Desmodesmus quadricauda cells do not require as much vortexing. Five seconds is enough.

Chlorella vulgaris, when overgrown, equires much more vortexing. The more shaking the better. Long vortexing is especially important when cells are deposited along the flask sides and thus form clumps. A minute or two of shaking is not excessive!

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Inoculating Fresh Culture Medium

6 **Count the microalgae cell culture density**

Handle the cultures inside sterile hood. Pipette **10 µl** of the dissociated cell culture suspension into the hemocytometer.

For detailed protocol see Counting Microalgae Culture Density.

Count the culture density using hemocytometer and keep the tally.

With *Desmodesmus quadricauda*, count each cell, not just the coenobia, i.e. one coenobium with four cells counts as four cells, one coenobium with eight cells count as eight cells.

Calculate the cell culture density:

- *D. quadricauda* $\rho^{Ddq}\ \left[\mathrm{ml}^{-1}\right]$
- *C. vulgaris* $\rho^{Chl}\ \left[\mathrm{ml}^{-1}\right]$

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7 Calculate the dilution factors for inoculating fresh medium.

The seed density in the freshly inoculated medium is:

- $\rho_{Ddq}^{seed} = 2 \times 10^5\ \mathrm{ml}^{-1}$ for *D. quadricauda*
- $\rho_{Chl}^{seed} = 1 \times 10^6\ \mathrm{ml}^{-1}$ for *C. vulgaris*

The volume of culture $V = 20\ \mathrm{ml}$ is same for boths species.

The cell density of the existing culture has been calculated in the above step.

- $\rho_{Ddq}\ \left[\mathrm{ml}^{-1}\right]$ is the existing cell culture density for *D. quadricauda*
- $\rho_{Chl}\ \left[\mathrm{ml}^{-1}\right]$ is the existing cell culture density for *C. vulgaris*

The inoculation volume of the existing culture is calculated as follows:

- $V_{Ddq}^{culture} = V \times \frac{\rho_{Ddq}^{seed}}{\rho_{Ddq}} = 20\ \mathrm{ml} \times \frac{2 \times 10^5\ \mathrm{ml}^{-1}}{\rho_{Ddq}}$ inoculation volume of existing *D. quadricauda* culture
- $V_{Chl}^{culture} = V \times \frac{\rho_{Chl}^{seed}}{\rho_{Chl}} = 20\ \mathrm{ml} \times \frac{10^6\ \mathrm{ml}^{-1}}{\rho_{Chl}}$ inoculation volume of existing C. *vulgaris* culture

The volume of the fresh medium is then calculated by subtracting the inoculation volume (calculated above) from the total volume of the culture V .

- $V_{Ddq}^{medium} = V - V_{Ddq}^{culture} = 20\ \mathrm{ml} - V_{Ddq}^{culture}$ volume of fresh medium for *D. quadricauda* inoculation.
- $V_{Chl}^{medium} = V - V_{Chl}^{culture} = 20\ \mathrm{ml} - V_{Chl}^{culture}$ volume of fresh medium for *D. quadricauda* inoculation.

8 Dispense the right volumes of the fresh medium into the pre-labeled Erlenmeyer flasks:

- V_{Ddq}^{medium} volume of fresh medium dispensed into the flask for *D. quadricauda* culture
- V_{Chl}^{medium} volume of fresh medium dispensed into the flask for *C. vulgaris* culture

8.1 Check that the fresh medium is not too cold, if it was taken out of the fridge only a short while ago. If it is cold, leave it standing for a few minutes, it will warm up quite quickly in the small volume standing on the metal surface of the laminar flow hood.

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9 Inoculate the medium in the Erlenmeyer flasks with the existing cultures:

- $V_{Ddq}^{culture}$ volume of fresh medium dispensed into the flask for *D. quadricauda* culture
- V_{Chl}^{medium} volume of fresh medium dispensed into the flask for *C. vulgaris* culture

10 Place the cells back on the algal shaker.

Microalgae cultures on the illuminated orbital shaker.

11 Clean up the sterile hood and the lab space. Sterilize old cultures by adding bleach or Virkon overnight.

12 ## References

Counting Microalgae Culture Density protocol

Autoclaving Erlenmeyer Flasks for Sterile Microalgae Culture protocol

Preparing 1/2 SŠ Algal Inorganic Nutrient Medium

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1/2 SŠ inorganic nutrient medium: Recipe from the Culture Collection of Autotrophic Organisms

Zachleder & Šetlík, 1982: The original article introducing this medium

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