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Culturing Algae for Sustainable Foodstock: A STEAM Education Platform

Resource Guide

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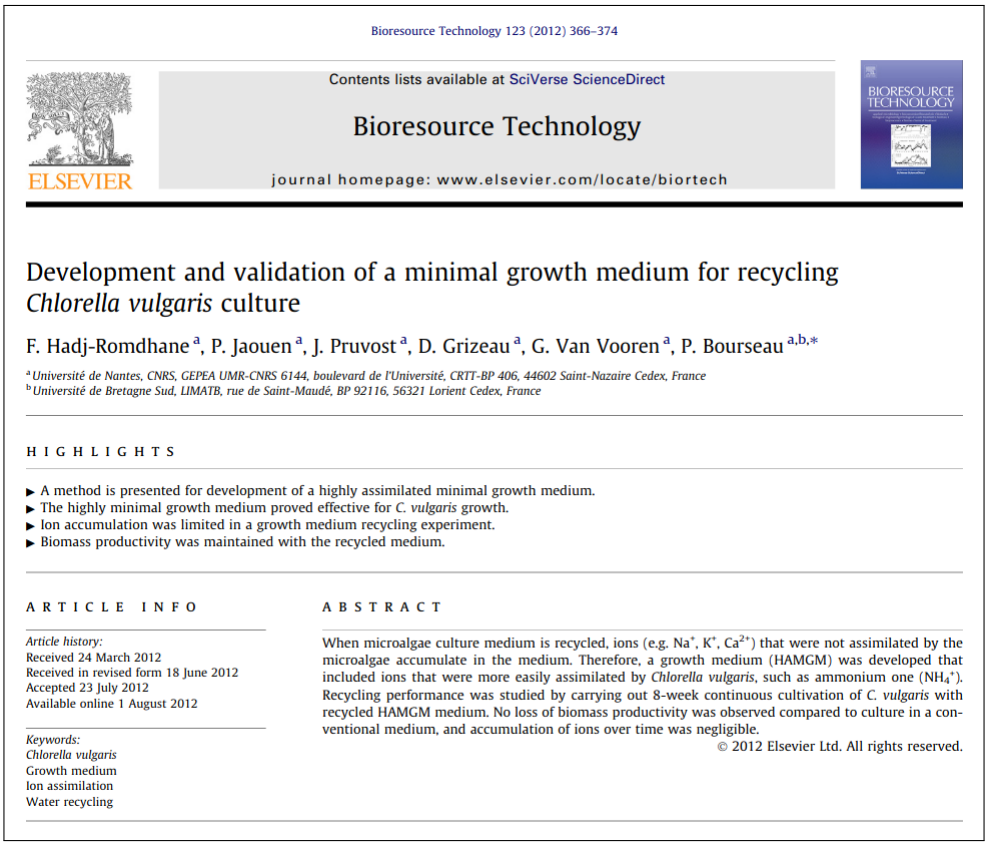
Key Journal Articles

F. Hadj-Romdhane, P. Jaouen, J. Pruvost, D. Grizeau, G. Van Vooren and P. Bourseau, "Development and validation of a minimal growth medium for recycling Chlorella vulgaris culture," *Bioresour. Technol.*, vol. 123, pp. 366-374, Nov., 2012, doi: 10.1016/j.biortech.2012.07.085



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Development and validation of a minimal growth medium for recycling *Chlorella vulgaris* culture

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HIGHLIGHTS

- A method is presented for development of a highly assimilated minimal growth medium.
- The highly minimal growth medium proved effective for *C. vulgaris* growth.
- Ion accumulation was limited in a growth medium recycling experiment.
- Biomass productivity was maintained with the recycled medium.

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ABSTRACT

When microalgae culture medium is recycled, ions (e.g. Na^+, K^+, Ca^{2+}) that were not assimilated by the microalgae accumulate in the medium. Therefore, a growth medium (HAMGM) was developed that included ions that were more easily assimilated by *Chlorella vulgaris*, such as ammonium one (NH_4^+). Recycling performance was studied by carrying out 8-week continuous cultivation of *C. vulgaris* with recycled HAMGM medium. No loss of biomass productivity was observed compared to culture in a conventional medium, and accumulation of ions over time was negligible.

Notes

This paper was used to establish the feasibility of indefinite cyclic regeneration in the context of producing education-grade *Chlorella vulgaris* culture. The method described is intended primarily for automated industrial processes, but the core concept is adaptable to the static bioreactor presented in this document.

In particular, the ideal growth medium was to contain calcium chloride, dipotassium phosphate, magnesium sulphate, carbonate and ammonium as a compounding ion. Millipore BG-11 NutriSelect Broth was found to be an appropriate approximation of this ideal.

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L. Sena, D. Rojas, E. Montiel, H. González, J. Moret and L. Naranjo, "A strategy to obtain axenic cultures of Arthrospira spp. cyanobacteria," *World J. Microbiol. Biotechnol.*, vol. 27, is. 5, pp. 1045–1053, May, 2011, doi: 10.1007/s11274-010-0549-6

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A strategy to obtain axenic cultures of *Arthrospira* spp. cyanobacteria

Lucia Sena, Diego Rojas, Edie Montiel, Héctor González, Josnell Moret & Leopoldo Naranjo

World Journal of Microbiology and Biotechnology **27**, 1045–1053 (2011) | Cite this article

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Abstract

A strategy to obtain axenic cultures of the cyanobacterium *Arthrospira* sp. (*'platensis'*) Lefevre 1963/M-132-1 strain, consisting of a series of physical and chemical procedures, and the application of an optimized pool of antibiotics, is described in this paper. This strategy, which is an inexpensive and fast way to obtain axenic cultures, can be applied to *Arthrospira* spp. from culture collections or samples from their natural habitats to eliminate a wide spectrum of contaminants. A high alkaline treatment (pH 12, using KOH) of 72 h is a determinant initial procedure applied to eliminate protozoa and *Microcystis* sp.

Notes

The first iteration of the bioreactor used an *Arthrospira* spp. (Spirulina) culture, and it was found that maintaining a stable culture was challenging. In particular, it was not possible to easily source commercial light solutions suitable for growth. The requirement for a brine solution also increased susceptibility to rust and biofouling, and a generally higher pH was required to exclude other algae.

Chlorella was found to be a hardy alternative with many of the same food characteristics as Spirulina. While this bioreactor does not produce a food-grade culture, edible alga provides students with a tangible outcome.

While focussing on isolating *Arthrospira platensis*, the paper provides a comparison of the growth conditions of common contaminants including *Microcystis* sp., *Chroococcus* sp., protozoa and bacteria.

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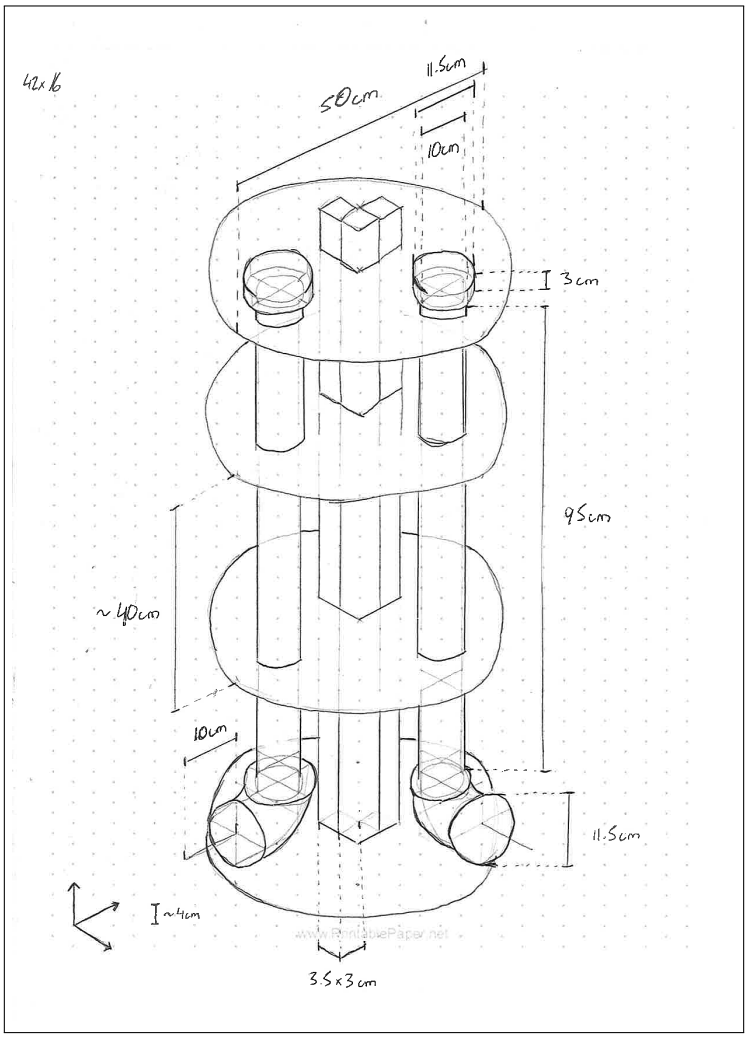
Physical Construction

Approximate Geometry



Tube *excluding base and cap*	Length: 950 mm exposed (1000 mm internal) Outside Diameter: 100 mm
Cap	Depth: 30 mm Outside Diameter: 115 mm
Base	Height: 190 mm Depth: 190 mm Outside Diameter: 115 mm
Assembly *excluding lighting and tubing* *only two tubes illustrated*	Height: 1100 mm Depth: 500 mm Each disk approximately 400 mm separated
Lights	Height: 1290 mm Width: 80 mm Length: 70 mm
Pumps	Height: 150 mm Width: 950 mm Length: 550 mm

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Bill of Materials

Please note that this bill of materials was compiled retrospectively. While every effort has been made to ensure the accuracy and completeness of the information, this list may not be reflective or exhaustive of the exact components used in the original construction.

Item Name	Material Type	Source	Units
Holman 100 mm ID × 88° Elbow *DWVF0146*	PVC	Bunnings Warehouse	6
Holman 100 mm ID Push-On Cap *DWVF0198*	PVC	Bunnings Warehouse	12
1000 mm × 100 mm OD Tube	Clear Acrylic		6
1000 mm x 600 mm Board	Acrylic		2
Kinetic 15 mm Ball Valve *0135531*	Brass	Bunnings Warehouse	6
Pope 13 mm × 15 mm Director *1011060B*	Polypropylene	Bunnings Warehouse	6
Brasshards 15 mm Threaded Hexagon Nipple *5NP015B*	Brass	Bunnings Warehouse	6
M10 1200 mm Threaded Rod *63SG1012*	Galvanised Steel	Bunnings Warehouse	6
M10 Lock Nut *SFA359*	Galvanised Nylon	Bunnings Warehouse	48 *5 × 10*
M10 Flat Washer *CDE1010*	Zinc Plated Steel	Bunnings Warehouse	48 *1 × 50*
Arlec 40W Weatherproof Batten *EL340S*	Polycarbonate	Bunnings Warehouse	3
Aqua One Precision 9500 Twin Outlet Air Pump *10047*		Petbarn	3
Aqua One 25W Glass Heater *11301*	Glass	Petbarn	6
Aqua One Airline Tubing 2.5 m *10403*	PVC	Petbarn	12
Aqua One Air Stone 2.5 cm *10143*	Sand	Petbarn	6
Simplepure Syringe Filter 0.1 μm	Polyethersulfone		6

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Consumables

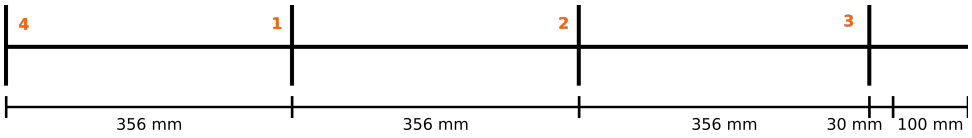
Item Name	Source	Frequency
100% Silicone *e.g. Sellys Clear Glass Silicone*	Bunnings Warehouse	As required for resealing
100X NutriSelect® BG-11 Broth	Sigma-Aldrich Millipore	10mL per litre *Approx. 25 L per 250 mL bottle*
Live Chlorella 6 mL	Southern Biological	Single-use unless bioreactor is completely drained and sterilised

Procedure

This procedure will be easiest to follow if all materials are available at once. Construction will require access to a laser cutter or CNC with sufficient precision to bore M10 holes. Alternatively, pieces could be pre-fabricated and bored using power tools.

Support structure

1. Laser cut one "01 - Algae stand" (base and centre), and one "02 - Algae stand" (two centre) pieces on 1000 mm x 600 mm acrylic board.
2. Using the drill method detailed in the notes or otherwise, place a lock nut and washer on each M10 threaded rod at the point illustrated as "1" in the diagram below. Measurements are provided for reference only and are not exact. Ensure a gap of at least 130 mm between "3" and the end of the threaded rod is maintained.
3. Slide a centre laser cut disk onto the M10 threaded rods at the point illustrated as "1".
4. Place a washer and then lock nut on each threaded rod to secure the disk.
5. Repeat steps 2-4 for points "2" and "3". *Do not* place the base disk at point "4" yet.



Algae tubes

6. Optionally spray-paint the PVC components (elbows, caps) to the desired colour. Directions depend on the paint used.
7. Screw the Pope director to the Kinetic ball valve. If desired, apply Loctite or another thread glue to secure the connection.
8. Bore an M15 hole through the end of one push-on cap.
9. Screw the Kinetic ball valve to the Brasshards nipple to ensure that the bored hole is sufficient. If desired, apply Loctite.
10. Use silicone or an appropriate plumber's glue to waterproof either side of the push-on cap.

11. Use silicone to waterproof the push-on cap and acrylic tube to the elbow. This joint is the weakest point of the construction, so take care. It may help to use wedges to make the seal more secure or to add additional screws around the connections.
12. Repeat steps 6–11 for the remaining five tubes.
13. Perform a leak test on each tube if desired. This could potentially save many hours of frustration later.

Final construction

14. Once completely dried or cured, slide the six algae tubes into the support structure from the bottom end (from "4" towards "3" in the diagram above).
15. Insert the Arlec battens into the centre of the support structure with the cable leads facing upwards. These will fit in the frame without modification when arranged in a pyramid.
16. Place the base disk at point "4" using the process described in steps 2–4. Stand the structure upright.
17. Insert one syringe filter between each pair of 2.5 m airline tubes. There will be six composite tubes in total.
18. Secure one air stone at the end of each composite tube. This will be immersed in the fluid and can be placed in the tubes during this step.
19. Secure the opposite end of each composite tube to one of the three twin-outlet air pumps.
20. Set each glass heater to the optimal temperature for the algae culture. For *C. vulgaris*, this is approximately 28°C to 30°C. Then insert one heater into each tube.

Care

The algae tubes are now ready to be seeded. The exact directions for this depend on the species of algae used. Contact the supplier for more detailed information on how to grow the Chlorella starter to a larger volume. Do not seed one of the algae tubes until you have at least 2 L of concentrated algae culture, which may take several weeks to obtain.

When seeding between tubes, use vinyl tubing to split the volume in one tube across another. Approximately 10% of the tube volume should be added in dilute BG-11 solution daily, if possible. Chlorella is more resistant to missed dosing than other species of algae.

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Notes

- Take care not to expose any PVC surface to bleach, sodium or hydroxides. For general cleaning, use warm soapy water and a brush. If you need to sterilise the tank, use an alcohol-based agent.
- Generic plumber's glue may be used to seal joints. Alternatively, aquarium-safe silicone such as *GE Silicone One* may be used. Ensure the sealant is suitable for permanent water immersion and does not contain fungicides or antiseptic agents ("mildew-resistant", "mold-free", "bacteria protection", "anti-microbial").
- Distilled or demineralised water may be used in conjunction with BG-11 broth, but not alone as it lacks the nutrients for growth. Treated tap water may contain chemicals that are harmful for algae growth. Check your local water distribution service (e.g. Melbourne Water) for information on additives or water conditions.
- 15 mm is equivalent to ½" BSP. Metric units have been used where possible in this document.
- Air pumps have replaceable diaphragms for extended use. These can be purchased from the same supplier as item 41208.
- Glass heater has an automatic thermostat but requires manual adjustment and cannot be controlled remotely. 25W is suitable for up to 25L, with each tube containing no more than 8L in practice.
- For organising lock nuts on the threaded rod, it may be helpful to stabilise the nut and use a drill to push the rod through the nut. This has the potential to save a significant amount of time.

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Next Steps

The design presented in this report is the result of at least two major iterations. There is clear potential for this design to be expanded. The paper presented by Hadj-Romdhane, et al. provides some insight into what a fully automatic, industrial-scale design might look like. In particular:

- Pumping of CO_2 rather than ambient air.
- Automated harvesting of algae from tubes.
- Automated recycling of nutrient broth.
- Automated refilling and draining of tubes.

At least one item was attempted with some success. A "babysitter" monitoring system was developed to automatically pump pre-prepared growth medium into each of the tubes according to measured volume. This system would not automatically drain the tubes nor recycle or prepare the growth medium from nutrient broth itself. For further information, contact the Monash Tech School.

Additionally, a different material could be found for the air stone. The air stone used in this design rusts over time with exposure to the brine fluid. Different materials (particularly ceramic or plastic) could resist this but would need to be dense enough to sink the air line. This is more difficult to achieve than in standing water since active aeration dramatically reduces the relative density of the fluid.

During the Monash Tech School Superfoods program, the algae culture was used for microscopy demonstrations and algae balls. The design presented offers numerous potential STEAM lessons and could be considered a platform for further programs. For example, chemicals readily found in high school laboratories can be used to break down algae cell walls to produce biofuels. There are procedures using *Saccharomyces cerevisiae* in literature for this exact lesson design. This could be a low-cost, practical exploration of concepts in both biology and chemistry.

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Resources

Files
PDF file for laser cutting the acrylic board base and centre https://drive.google.com/file/d/1O1QboveDsTxdn-_Lp_1ep4tLs2mQbi_y/view?usp=sharing
PDF file for laser cutting the acrylic board remaining centre pieces https://drive.google.com/file/d/1Lykg-PykSPLTTnQ3tUAgoiXam05mlkX4/view?usp=sharing

Consumables
BG-11 100X concentrate from Sigma-Aldrich https://www.sigmaaldrich.com/AU/en/product/sial/73816
Chlorella 6 mL starter culture from Southern Biological https://www.southernbiological.com/biology/specimens/living-specimens/algae/l1-20-chlorella-live-approx-6ml/

Babysitter Inspiration
Demonstration of GUI interfacing with a peristaltic pump https://drive.google.com/file/d/1TKltsFIfhHVIy5pwjyFVxY5rhQOl2tn6/view?usp=sharing
Experimental flow rate of Adafruit 12V/5000RPM peristaltic pump https://drive.google.com/file/d/1Xc1gNDDzMWToR-If6v-w8WETpr1gZqoP/view?usp=sharing