Journal of Applied Phycology **6**: 423–425, 1994.
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Note

A laboratory-scale system for mass culture of freshwater microalgae in polyethylene bags

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Received 14 June 1993; revised 2 May 1994; accepted 12 May 1994

Key words: Ankistrodesmus falcatus, Scenedesmus incrassatulus, Chlorophyceae, mass production, microalgae

Abstract

A laboratory-scale system for mass culture of microalgae in 8-, 20- and 40-L polyethylene bags, was designed. Bags are 16.8 cm diameter and 52 cm (8-L bags), 112 cm (20-L) or 224 cm (40-L) length. The system was tested successfully with two freshwater microalgae, *Ankistrodesmus falcatus* and *Scenedesmus incrassatulus*, cultured in Bold's Basal medium (prepared with either deionized or tap water). The procedure described is simple, reliable and practical, and enables a very cost-effective production of freshwater microalgae to satisfy any laboratory requirements, and when quantities demanded for special applications can not be met by the standard laboratory culture procedures.

Most of the procedures currently available for mass culture of microalgae are designed for large-scale production (de la Noüe & de Pauw, 1988; Huntley, 1989; Tredici & Materassi, 1992). At the laboratory scale it is often necessary to produce microalgae as food for stock or experimental cultures of filter-feeder organisms, or as a supply of massive- live-, fresh- fucal material for physiological, ecological, toxicological, and nutritional studies. Laboratory-scale mass culture of microalgae is usually carried out in carboys and other big glass containers (10 to 20 L), under aseptic conditions. The use of these vessels is disadvantageous as they are expensive, their cleaning is laborious and time-consuming, sterilisation is difficult to achieve and sustain during the culture, and the growth of algae can be impaired by the light extinction usually occurring in large-diameter containers.

Laing and Ayala (1990) and Laing (1991) described a simple procedure for the mass production of marine microalgae in a 60-L semi-continuous culture in polyethylene bags. Other examples of polyethylene containers involve the use of sophisticated frameworks (Laing, 1991).

We designed a system for the mass culture of freshwater microalgae in disposable polyethylene bags, and the system was tested successfully with the green microalgae *Ankistrodesmus falcatus* and *Scenedesmus incrassatulus*.

Inoculum was produced in 2-L flasks containing autoclaved Bold's Basal medium (Stein, 1973). Mass production was carried out in non-sterilised Bold's Basal medium (prepared with dechlorinated tap water), in 8-, 20- and 40-L polyethylene bags, constructed from 25-cm width 'layflat' tube. The bags were 16.8 cm diameter and 52, 112 or 224 cm length, respectively; they were hung in a tubular framework fitted with vertical walls. On both sides of the walls, 40-Watt-'daylight'-fluorescent 112.5- cm length tubes were installed vertically with a 10 cm distance between lamps. The containers of 8- and 20-L had their bottom heat-sealed in the form of a truncated cone to prevent settling; 40-L containers were hung folded by the middle. For hanging the bags, 16.4-cm diameter PVC rings were constructed according to the design shown in Fig. 1; the tube fits exactly to the PVC ring and is fastened with a metallic endless clasp. Each PVC

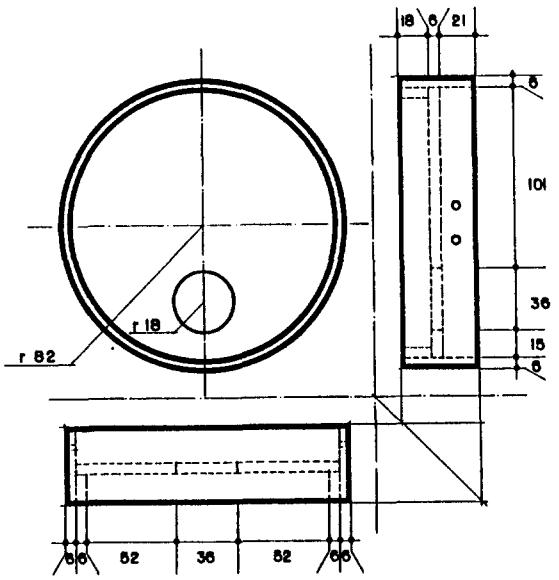

Fig. 1. Dimensions (in mm) of the PVC ring used as support for 8-, 20- and 40-L polyethylene bags, for the culture of microalgae.

ring has a 3.6-cm hole, fitted with a two-hole rubber plug; each hole held a glass tube (for aeration) topped with a cotton plug to prevent microbial contamination. The biomass produced in one two-L flask was used as inoculum for one 8-L bag; one of these 8-L bags was used as inoculum for each 20-L bag; two 8-L bags were applied in the case of the 40-L containers. Compressed air was supplied through air stones. Cultures were kept at room temperature (25 to 27 ° C).

Figure 2 shows population growth curves, in terms of optical density, and estimated cell density for the mass culture of *A. falcatus* (average of 150 and 45 replicates, for 8- and 20-L, respectively), and *S. incrassatulus* (average of 196, 150 and 40 replicates, for 8-, 20- and 40-L cultures, respectively). The observed data were described well by the logistic population growth model (determination coefficient values ranged from 0.920 to 0.980). Culture experiments in 40-L bags were only carried out for *S. incrassatulus* due to the frequent protozoal contamination of the *A. falcatus* cultures.

Laing and Ayala (1990) and Laing (1991) used 3.4 or 3 7 m long 25 cm wide polyethylene tubes folded in half, as culture containers; these were secured at both ends with twines, to hang from a framework support. A 'small' hole was bored at the top of each half of the bag for pumping-in sea water, culture medium and the alga inoculum; this was then used for introducing an air line. Although their system is simpler than ours, it has some problems: (a) if the ties of the bags are not tight, these can fall down; (b) it is difficult to control the proper size of the hole and it cannot be sealed (as it is the only exit for the air bubbled into the culture), so it might allow the entrance of microbial contaminants; (c) it also represents a point of weakness of the container, through which the bag can be torn off due to the culture weight (*ca* 60 kg). The ring we designed provides a more reliable support for the culture container,

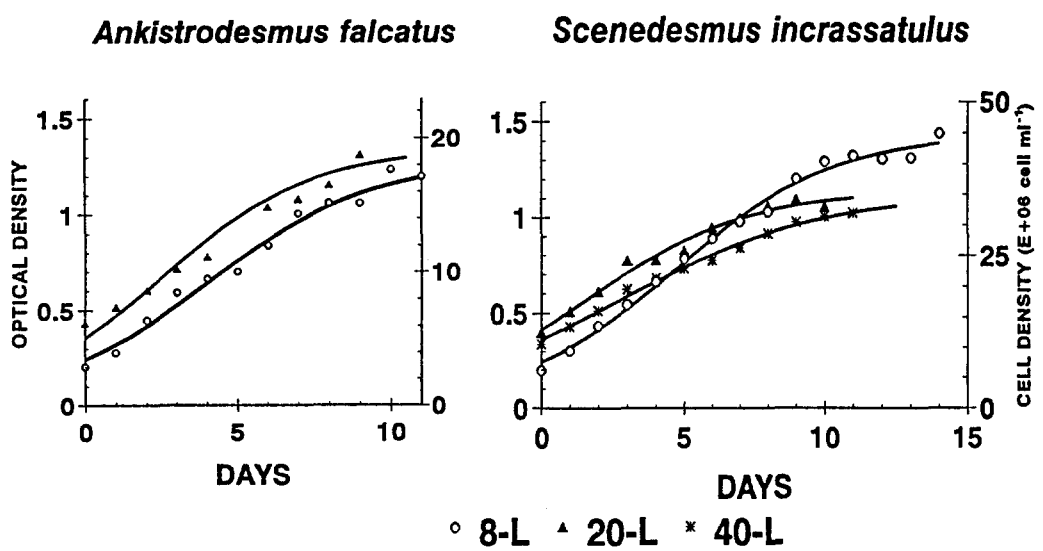

Fig. 2. Observed data and fitted logistic population growth curves for *Ankistrodesmus falcatus* and *Scenedesmus incrassatulus* cultured in polyethylene bags.

and enables an easier and better control of the culture procedures (*in situ* culture medium preparation, alga inoculation, sampling and monitoring of the quality and quantity of fucal material, partial and total harvest, and so on). The stoppers, plastic and glass tubes and the rings, can be autoclaved safely, reducing the chance of contamination.

Acknowledgments

Thanks to Mr Abel Córdova for manufacturing the components, installations and devices used, and to Dr Salvador S. Colón for kindly reviewing the manuscript.

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