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Medium recycling for *Nannochloropsis gaditana* cultures for aquaculture

C.V. González-López [a], M.C. Cerón-García [a,*], J.M. Fernández-Sevilla [a], A.M. González-Céspedes [b], J. Camacho-Rodríguez [a], E. Molina-Grima [a]

[a] Department of Chemical Engineering, University of Almería, E04120 Almería, Spain
[b] Department of Greenhouse Technology, Estación Experimental Fundación Cajamar, Almería E04710, Spain

HIGHLIGHTS

- A methodology to recirculate medium in microalgae cultures is developed.
- The most adequate sterilization method is established.
- The recirculation reduces the demand of nutrients cutting costs in the process.
- *N. gaditana* continuous cultures were maintained using the recirculated medium.
- The biomass biochemical composition resulted of high interest for aquaculture.

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ABSTRACT

Nannochloropsis gaditana is a good producer of proteins and valuable fatty acids for aquaculture. Recycling of culture medium is interesting for microalgae commercial production as it cuts costs and prevents environmental contamination. The recycled medium must be sterilized to prevent the buildup of unwanted metabolites and microorganisms. We tested several sterilization methods: filtration, ozonation, chlorination, addition of hydrogen peroxide and heating. Results showed that the most successful method is ozonation lowering the bacterial load to 1.9 10^3 CFUs/mL, which is 1000-fold and 10-fold lower than the supernatant obtained after harvesting and the initial filtered medium, respectively. Continuous cultures of *N. gaditana* were grown using this recirculated supernatant. A maximum biomass productivity of 0.8 g/L/d composed of ~50% proteins and 40% lipids with more than 3% d.w. EPA was obtained making this biomass very interesting for aquaculture.

1. Introduction

The production of microalgae is essential for the commercial cultivation of larvae of mollusks, crustaceans and fish. Microalgae can be used directly as live food (for mollusks and crustaceans) or indirectly as food for zooplankton species, such as the rotifer *Brachiomus plicatilis* and *nauplii* of Artemia, used as prey for fish larvae (Benemann, 1992). The microalgae used as food in the early larval stages of fish should ensure the nutritional requirements of the species, particularly in essential compounds like amino acids or polyunsaturated fatty acids. The microalgal diet must contain high levels of polyunsaturated fatty acids as docosahexanoic acid (DHA 22:6n3), eicosapentanoicacid (EPA 20:5n3) and araquidonic acid (AA 20:4n6) to ensure a good larvae growth and high levels of survival (Navarro and Villanueva, 2003; Morais et al., 2005).

The maintenance of axenic mass cultures of microalgae requires the sterilization of large volumes of seawater and thus of a method that can be implemented in the large scale. Kawachi and Nöel (2005) reviewed several techniques to sterilize seawater. Among the methods discussed by these authors are filtration, application of ultraviolet radiation (UV), addition of sodium hypochlorite and pasteurization. Other techniques such as membrane-based separation methods have been suggested (Rathore and Shirke, 2011) but these are more difficult to implement in large-scale culture systems and especially in seawater.

Many aquaculture facilities use filtration, UV irradiation or a combination of both methods to sterilize seawater. Ozonation and ultraviolet irradiation (UV) are the most frequently used methods for viral control in land based aquacultural systems. These two methods can be used to eliminate pathogens in the inlet seawater culture medium, in the supernatant and in the recirculated water (the supernatant obtained after harvesting). Disinfection by ozonation and UV irradiation are also used in other aquacultural applications, e. g., to reduce or eliminate the presence of potential

* Corresponding author. Tel.: +34 950 015981; fax: +34 950 015484.
E-mail address: mcceron@ual.es (M.C. Cerón-García).

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