1977

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Millamena, Oseni M.

Aquaculture Department, Southeast Asian Fisheries Development Center

Millamena, O. M., Jereos, E. C., & Gorriceta, I. R. (1977). Methods of harvesting and preservation of the diatom Chaetoceros calcitrans. SEAFDEC Aquaculture Department Quarterly Research Report, 1(4), 3-8.

http://hdl.handle.net/10862/2303

Methods of harvesting and preservation of the diatom *Chaetoceros calcitrans*

Oseni M. Millamena, Eve C. Jereos, and Ilda R. Gorriceta

Aquaculture hatchery operations are often hampered by limited supply of natural feeds. Experiments with methods of harvesting and preservation of algae were made to provide supply during periods of scarcity. The single-celled diatom *Chaetoceros calcitrans,* a known suitable feed for *Penaeus monodon* larvae, was used in this experiment. Diatom culture and larval feeding experiments were conducted to test for the viability and acceptability of preserved algal concentrates.

These one-celled diatoms are characterized by the presence of setae which keep them suspended in cultures and make autoflocculation very difficult. Flocculation was induced by the addition of a floc-forming chemical. Inexpensive and local alum and lime were tried as coagulants.

The Jar Test was used to obtain information concerning the effect of coagulation variables as pH, coagulant dose, and type of coagulant that establishes optimum conditions for coagulation. Parameters measured to assess the amount and concentration of algae recovered were suspended solids, percentage light transmittance, and cell count of supernatant.

The optimum pH for coagulation was determined by adjusting the culture pH to levels of pH 5.5 to 8.0 for alum and pH 8.5 to 10 for lime. Samples were then simultaneously treated with a uniform coagulant dose and subjected to the Jar Test.

Optimum pH for algae removal with alum was found to be 6.5 (Fig. 1). At pH 6.0 or lower, algae cells rapidly deteriorated. With lime, algae removals increased with pH and was optimum at pH 9.5 (Fig. 2). Increasing the pH further to nearly 10 caused the precipitation of excess nutrients from solution.

The effect of coagulant dose was determined by first adjusting the samples to their optimum pH for coagulation. Measured additions of coagulant were added to give dosages of 0, 50, 100, 150 and 200 mg/L before subjecting to the Jar Test, The optimum coagulant dose was taken as the point at which maximum return per unit of reagent could be obtained. Highest algal yield was obtained at 50 mg/L dose for both coagulants (Fig. 3 and Fig. 4).

Using the optimum conditions for coagulation, it was possible to harvest the algae within 1-hour settling time with about 84% recovery.

Harvested algal slurry was neutralized to the initial pH, kept in plastic bags, and stored in freezers at 0^{o}C with and without protectants. Simple freezing was found to be a suitable method of preserving *Chaetoceros* for a period of 2 months without any protectant. With glycerol (0.1%) as protectant, frozen algal concentrates have been stored successfully for 3 months.

The viability of frozen *Chaetoceros* was proven by actual cell reproduction. Frozen concentrates were thawed and suspended in equal volumes of fresh water and seawater resulting in a salinity of 16 to 18 ppt. SEAFDEC medium formulated especially for *Chaetoceros* was used for culture studies.

There was difficulty in determining the initial cell count of frozen cultures because the

appearance of the cells was altered and resembled faint outlines. This might be due to the masking effect of flocculants or freezing.

Fig. 5 shows the growth curves for frozen and fresh algal cultures. Fresh diatom culture reached peak growth on the 3rd day and declined thereafter. The cells were taken from a previous culture in the log phase, hence, the absence of a lag phase. Frozen cultures harvested with either alum or lime showed growth after 2 to 3 days lag period. Lime-flocculated cells gave more yield than fresh or alum-flocculated cells. Cell count was done using a hemacytometer.

Preliminary feeding experiments showed that frozen *Chaetoceros* can successfully be used as substitute for fresh diatoms as feed for *P. monodon* larvae.

Simple freezing technique with or without the use of protectants has been found convenient for preserving algal concentrates in small volumes for both feeding and culture purposes.

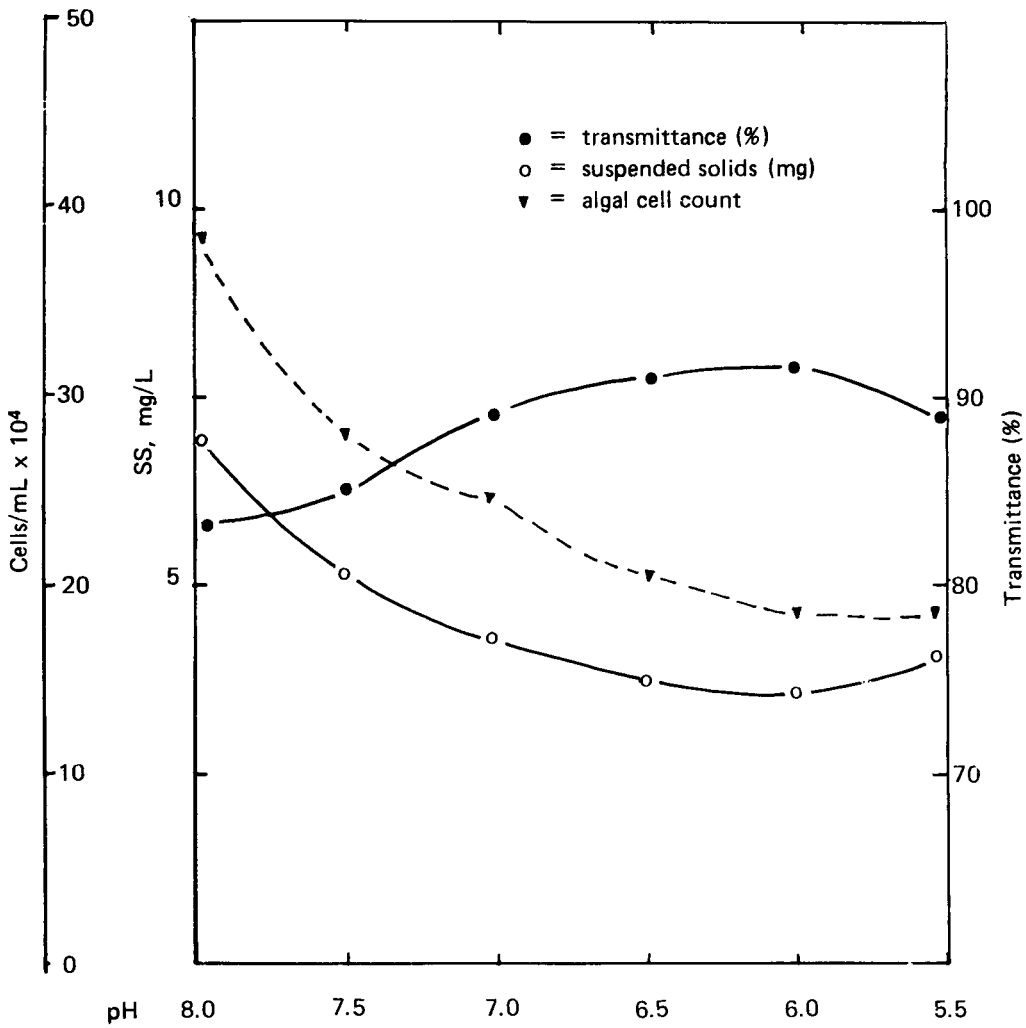

Fig. 1. Effect of pH on coagulation with alum, at an alum dose of 100 mg/L.

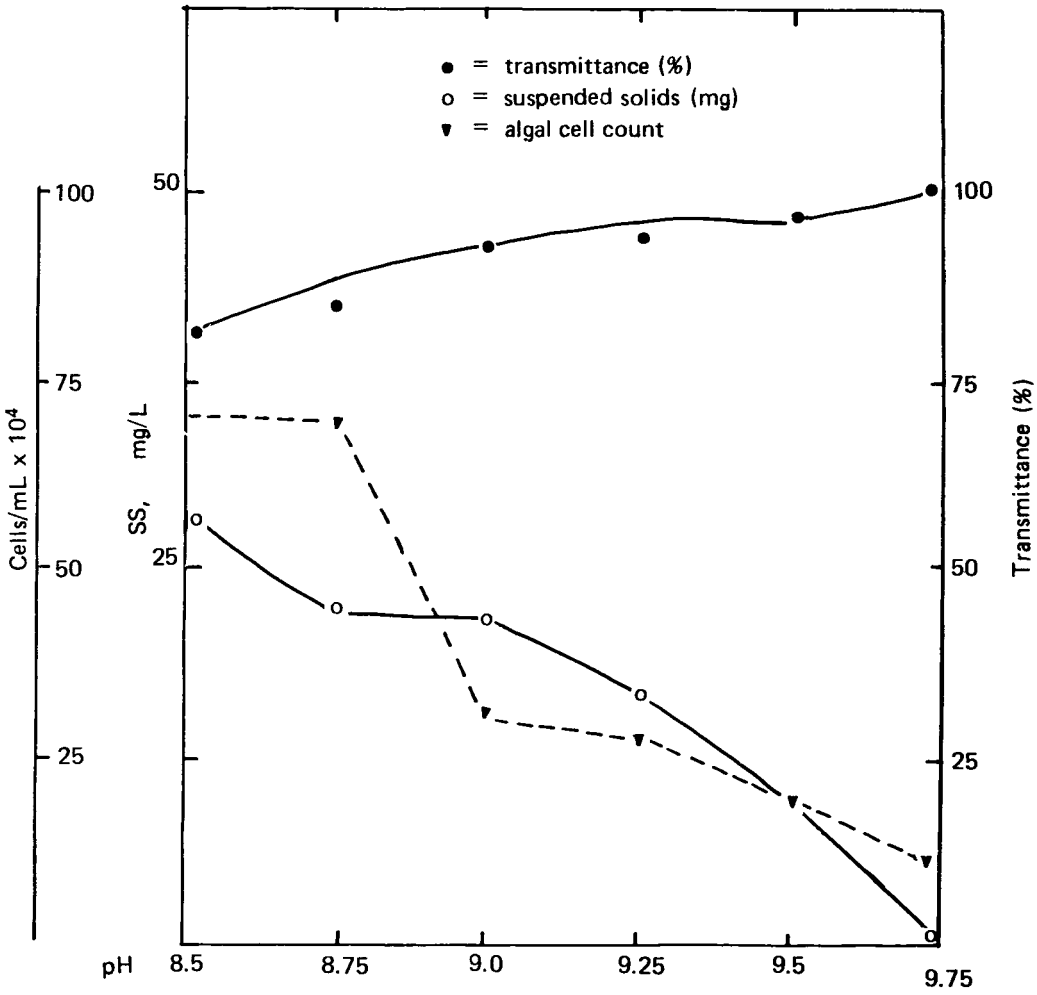

Fig. 2. Effect of pH on coagulation with lime, at a lime dose of 100 mg/L.

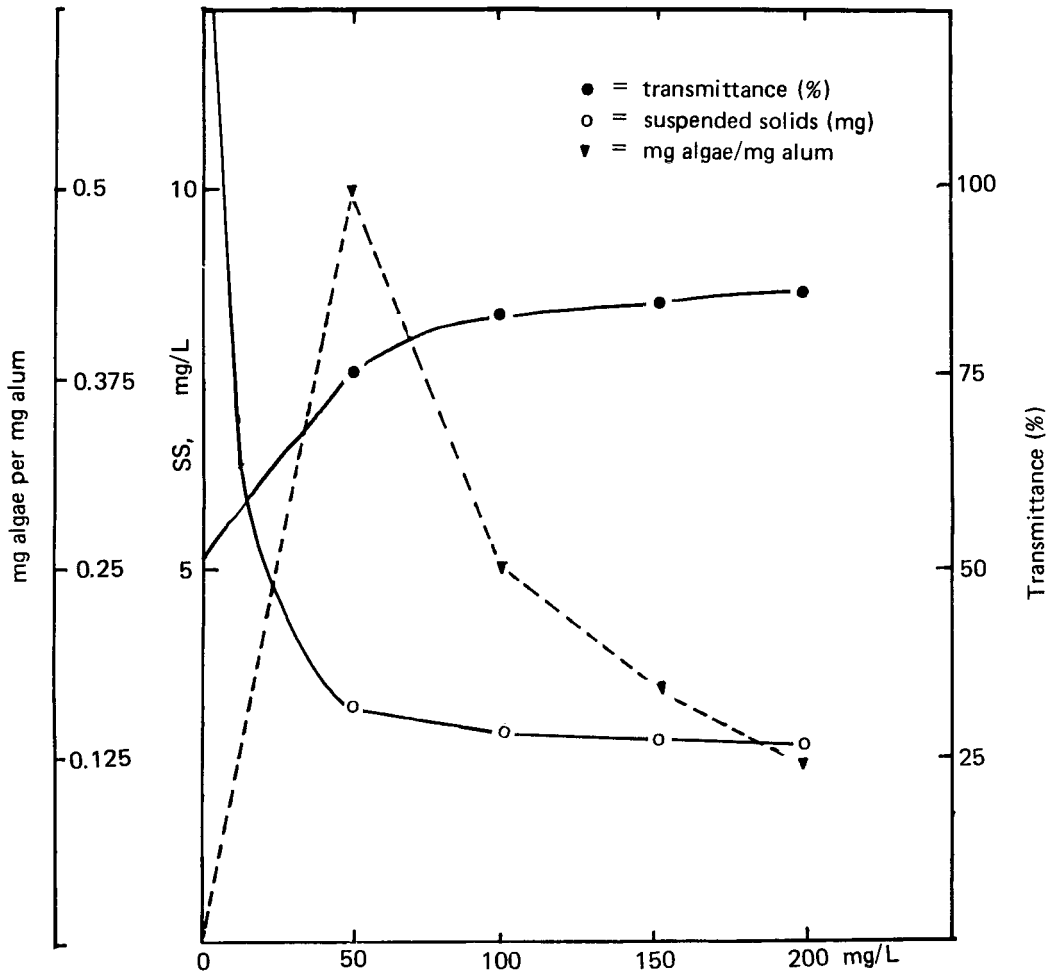

Fig. 3. Optimum alum dose in mg/L at optimum pH (6.5).

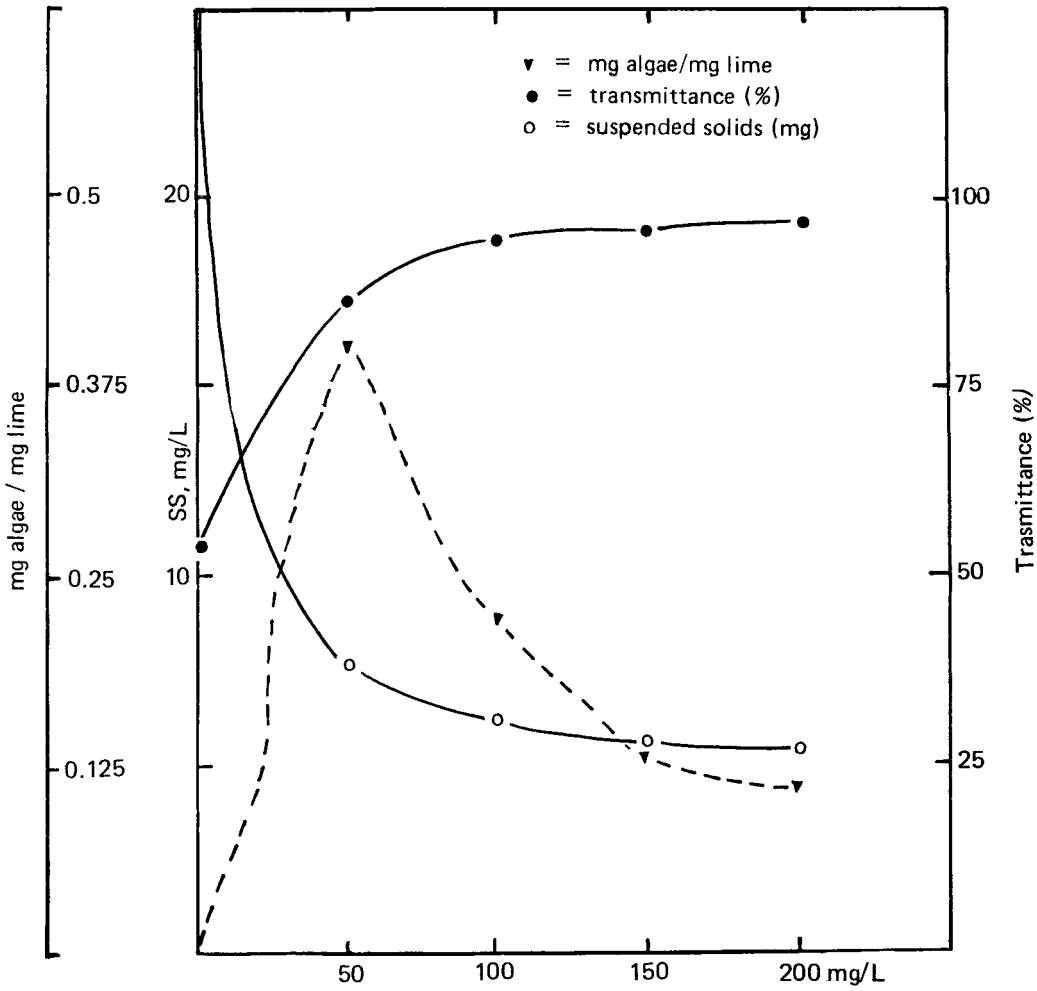

Fig. 4. Optimum lime dose in mg/L at optimum pH (9.5).

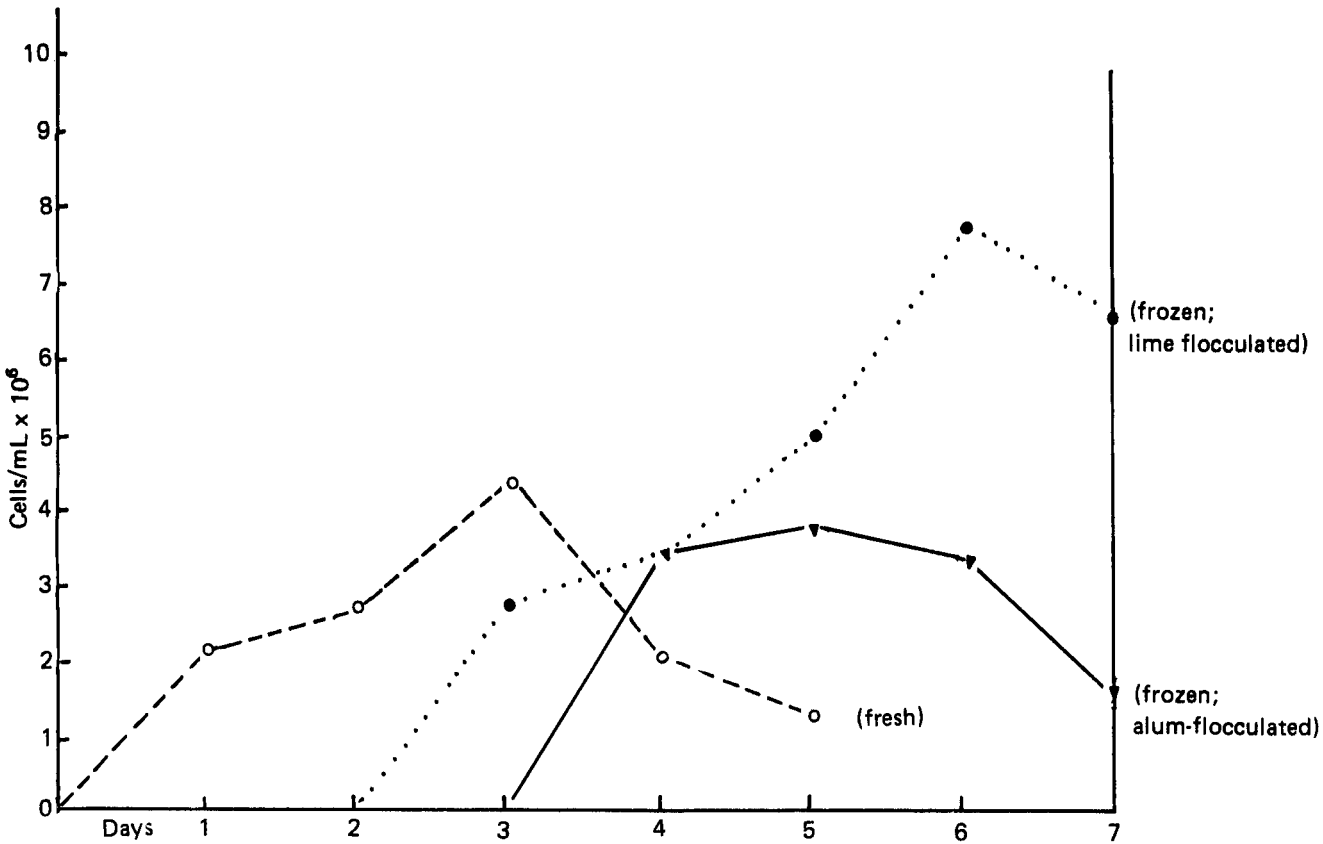

Fig. 5. Growth curves of frozen and fresh cultures of *Chaetoceros* sp.