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ECONOMIC MULTI-STEP METHOD IN THE MASS CULTURE OF MICROALGAE IN ABBASSA-SHARKIA-EGYPT

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Abstract

Microalgae are the natural feeds of many aquaculture species, and are the basis of the natural food chains on which sush species depend in the wild. The algae were grown in batches using successively larger containers. A cell density, chlorophyll and dry biomass were obtained from the final 4-day culture of 6.5 X 10^6 cells/ml, 4.77 mg /l & 0.75 g/l for *Chlorelle vulgaris*, and 5.4 X 10^6 cells/ml, 4.96 mg/l & 0.82 g/l for *Scenedesmus bijuga*, respectively.

The mass production costs for outdoor tanks of microalgae were about LE 66.38/kg dry biomass and of LE 40.53/ton of live algae (US$ 1.00 = 3.35 LE Egypt.). In the present study, the cost of labor (39.48%), electricity (25.94%), nutrients (8.11%) and mixing (26.47%) of the total production costs were studied. The multi-step method used in the mass culture of microalagae in this investigation minimized the production costs.

INTRODUCTION

Microalgae have been investigated as a human animal food for over 40 years (Vonshak, 1991).The use of microalgae in aquaculture has several potential advantages over the production of microalgae for human foods or terrestrial animal feeds such as high conversion efficiencies and no need for harvesting, drying and storage, as the animals or food chains could use the algae as produced. However, the production of microalgae for aquaculture feeds has been relatively neglected, mainly, because the aquacultre systems themselves were generally poorly developed. Understandably, most of the emphasis in aquaculture has focused on developing the animal culture processes (Benemann, 1992).

Microalgae are of great importance to the common culture of bivalves (Larvae, juveniles and adults/, crustaceans (mostly the early stages), zooplankton, and to a lesser degree, finfish (larvae and /or adults), (De Pauw and Pruder, 1986). Large scale intensive production of microlgae suffers from two major problems, i.e expensive and often unreliable.

Chlorella powder for human consmption is sold by the manufacturing in Taiwan for US$ 8.00/kg. The final customer pays for the tablets manufactured therefrom an appreciably higher price. The Japanese customer accepts this price, as *Chlorella* is considered a "super food" with healthy effects (Behr and Soeder, 1979).

Silver carp larva rearing often depends on the production of phytoplankton which serve as feed for the fish larvae. Feeds prodution must be consistent in quality and quantity for the duration of the hatchery cycle if larval rearing is to be successful. Phytoplankton production can occupy the majority of the space and labor allocated to larval rearing. Phytoplankton produuction generally requires the most space.Therefore, any improvement in the production of algae has the potential to improve overall hatchery production (Dawah, 1988).

There are various estimates for the production of microalgae which differ to a considerable degree depending on the basic assumptions, in particular, the assumed production capacity. Some of the estimates are given in Table 1.

Table 1. Estimates of the produuction costs of microalgae according to Behr and Soeder, 1979.

Cultivated alga	Cost estimate US$ per ton powder	Author
Chlorella	520	Tamiya (1956)
Coelastrum	1140	Soeder (1976)

De Pauw *et al.* (1984) estimated that mono-specific algal cultures produced indoors or in a greenhouse range in cost from US$ 120-200/kg dry weight, while, costs may be lower for some operations. There is no question that commercial production costs for phytoplankton are high (James *et al.*, 1988).

Production costs for outdoor ponds of photosynthetically grown algae are in the range of ca. US$ 4-20/kg dry biomass (De Pouw and Persoone 1988).

Heterotrophic production of microalgae can be performed for less than $ 20/kg of dry biomass using large fermentors as culture vessels (Soong 1980).

The economic woes of cultivators stem form the fact that most microalgal culture today is labor intensive and requires a great deal of space (inside and /or outside). Additionally, the cost of energy (for lighting, pumping, aeration / mixing and heating /

cooling) and nutrients is high. De Pauw and Persoone (1988), also Helm *et al.*, (1979) reported the following cost-breakdowns for culturing algae by the bloom induction technique: labor (50-85%), pumping (4-24%), nutrients (4-20%) and mixing (5-8%) of the total production costs.

Some strains of fresh water *Chlorella (C.vulgaris, C. ellipsoides, C.reguralis,* etc.) are heterotrophic or mixotrophic. For this reason, they can be mass produced easily in aspectic, dark conditions, and the production costs are comparatively low. (Maruyama *et al.* 1989).

The production cost of *Chaetoceros calcitrans* growing in batches using multi-step method, was US$ 28.6/ton of cell density of 2.65% X 10^6 cells/ml (Samonte, *et al.*, 1993).

The limitations to greater use of microalgae feeds are both technical and economic, in some cases the problem is how to mass culture desirable species. In others, the cost of production must be reduced . Information of the economics of algal production is very scarce.

This study presents aims to describe the multi-step method of culturing live microalgae, *Chlorella vulgaris and Scenedesmus bijuga* to estimate the costs involved using this method.

MATERIALS AND METHODS

Culturing of the algae was carried out at Central Lab. for Aqaculture Research (CLAR) at Abbassa, Abou-Hammad, Sharkia, Egypt. A set of collected Nile water samples was incubated at 25^{o}C±2 and 14/10 light-dark cycle (5000 lux by two units of 20 watt fluorescent lamps) after addition of sterile nutrient solution using Bold's basal medium (BBM) (Bischoff and Bold, 1963), for flourishing of algal organisms. *Chlorella vulgaris and Scenedesmus bijuga* were the dominant species in the Nile water collected samples. They were isolated using standard sterile microbiological techniques according to Guillard (1973) and identified as, *Chlorella vulgaris and Scenedesmus bijuga* according to Pascher (1915). By the help of microscopic examination, unialgal organisms were isolated and passed into a sterile culure solution. Continuous dilution and examination of unialgal organisms was done until we obtained stock from each organism. Monthly, two or three slants of the species were subcultuered. Media was autoclaved in the test tubes before hand, and aliquots from each selected tube were transferred to four new tubes. After all the transfers have been completed, the test tubes were set in racks beneath fluorescent lamps. For the next month, each new culture was inverted

once each day. Growth in the new culture test could usually be observed within a week and was allowed to continue for month before the next set of transfers took place. At this time, two or three were selected for transferring, while, test tubes which showed no growth were discarded. Cultures for starting production flasks were selected from the remaining culture test tubes. The cost of inoculum was LE 6/L. Fig 1 shows the flow chart of microalgae production from primary stock to large-scale culture. Inoculum for the algae culture came from a pure algae stock collection at the plankton laboratory.

Fiber glass tanks were used in the outdoor system whose capacity was about 1.5 ton. The tap water was used and OI Algae Culture Medium for outdoor culture was based on Miquel's enrichment solution as modified by Allen and Nelson (1910). However, agricultural-grade fertilizers available through commercial sources, were used instead of laboratory-grade reagents. A complete trace metal mixutre was also added.

Moderate aeration was provided. The culture period was 4 days, as detected from growth curve experiment. The temperature was 25-30^{o}C. The cultures in the tanks were usually inoculated with 2.2 x 10^5 cells/ml (0.2 g dry weight/litre and 1.19 mg/l pigment content for *C.vulgaris* and with 2.6 x 10^5 cells/l (0.27 g dry weight/litre and 1.66 mg/l chlorophyll a content) for *S.bijuga* and brought up to a maximum density of (6.5 x 10^6 cells/ml, 0.75 dry weigth/litre and 4.77 mg/l pigment content) and (5.4 x 6.5 x 10^6 cells/ml, 0.75g dry weight/litre and 4.77 mg/l pigment content) and (5.4 x 10^6 cells/ml), (0.82g dry weight/litre 4.96 mg/l chlorophyll a content) for *C.vulgaris* and *S.bijuga*, respectively. Cultures were nutrient enriched with ammonium sulfate, urea, monopotassium phosphate and ferric EDTA.

Equipment and materials were depreciated using the straight-line method (Shang, 1981).

RESULTS AND DISCUSSION

Inoculum was prepared in the laboratory using single-cell techinque. After reaching adequate concentration in indoor culture, algal suspension was then transferred to outdoor cultures using glass aquaria of 100-litres, then transferred to the outdoor cultivation tank after a retention time of 4 days. The yield of the biomass of *C.vulgaris* obtained was 5.5 g/m2 /day and for *S.bijuga* was 5.7 g/m2/day. Initial and final population densities of *C.vulgaris* and *S.bijuga* are shown in Table 2.

Asset requirement for the production of microalgae was LE 14905.8 (Table 4). Equipment was 81.6% of the total asset cost.

Fig. 1. Flow chart of microalgae mass production.

Culture	Types of algal cultures							
Conditions	Primary stock cultures		Secondary stock cutures		Large-scale cultures			
Culture container (volume)	Test tube Agar slant (15 ml)	Broth (15 ml)	Erlenmeyer Flask (125 ml)	Glass bottle (1 litre)	Galon Jars (2.5 litres)	Glass Carboys (20 litres)	Glass aquaria (100 litres)	Fiberglass Tank (1.5 ton)
Aeration/ Agitation	none	manual shaking	manual shaking	aerated	aerated	aerated	aerated	aerated
Illumination	2 units 20 watt Fluorescent lamps (5000 lux)							Sunlight
Temperature	20-25 °C							25-30 °C
Volume of inoculum	1 loopful	1 ml	10 ml	50 ml	400 ml	1800 ml	19 litres	100 litres
Grade of chemica	Analyzed reagent grade					Technical grade		Agricultural fertilizers
Culture media	(BM) Bold's basal medium					(OI) Oceanic Institute algae culture medium		ammonium sulfate urea 46 Monopotassium phosphate
Water treatment	Autoclaved			Boiled (20 minutes)		Aqua pure filters		Sand filters
Sterilization of culture vessels	Autoclaved			Oven-rying		Clorox desinfection (48 hours) (5.25% solution of NaCl)		
Culture period	5-7 days		4-5 days			4 days		

The acquisition of equipment specifically for phytoplankton culture was a major cost incurred during initial investment in shrimp hatcheries (Israel *et al.*, 1986). Samonte *et al.* (1993) recommenced that the equipment was 84% of the total asset cost. Table 4 shows that the costs of producing Bold's basal medium (BBM) were LE 38.65/l and Ol algae culture medium was LE 5.38/l as in Table 5. The operating costs of producing 1 ton of micoalgae using the multi-step method were LE 48.53/ton live algae (Table 6). The harvesting of ton live algae and oven dried gave 700 g dry biomass. The 1.43 ton live algae produced 1 kg biomass. Table 7 shows the costs of 1kg ton live algae and oven dried gave 700 dry microalgae. The 1.43 ton live algae produced 1 kg dry biomass. Tale 7 shows the costs of 1 kg dry microalgae.

Various artificial feeds such as freeze-dried or processed natural products have been developed to substituute, if not eliminate, the use of microalgae in the hatcheries. Sun-dried and frozen algae were tested with *Penaeus monodon* larvae (Millamena *et al.*, 1990) but the technique was not adopted by hatchery operators due to the additional skill and equipment needed. Biedenbach *et al.* (1990) reported that spray-dried *Teteraselmis sueciaca* may be able to partially replace live agae. Chen *et al.* (1985) introduced *Isochrysis galbana* an excellent feed for larvae of scallops. Hatchery operators are convinced that natural feeding with microalgae has inherent advantages (Pantastico, 1989). Hence, they are still depending on the production and use of microalgae as live food for commercially important fish, mollusks and crustaceans during at last part of their life cycle (De Pouw *et al.*, 1984).

The multi-step method is an efficient, method for the scaling-up operation of microalgae production (Samonte *et al.*, 1993). It is a simple technique and can easily be adopted by fish hatchery operators. The minimal costs involved using this method make it an applicable technique for the mass culture of microalgae.

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Table 2. Density of *C.vulagaris* and *S.bijuga* using the multi-step method.

Culture container	Inoculum	Culture condition	Culture period	Density (x 10^6 cell/ml)			
				Initial		Final	
			(days)	*C. vulgaris*	*S. bijuga*	*C. vulgaris*	*S. bijuga*
Glass bottle	50 ml	indoor	4-5	0.12	0.13	5.65	4.95
Gallon jar	400 ml	indoor	4-5	0.15	0.15	5.81	5
Carboy	1800 ml	outdoor	4	0.18	0.19	5.85	5.15
Glass aquarium	19 l	outdoor	4	0.21	0.23	6.2	5.25
Fiberglass tank	100 l	outdoor	4	0.22	0.26	6.5	5.4

Table 3. Inventory of equipment, materials and depreciation schedule (pound).

Item	Quantity	Unit cost (LE)	Total cost (LE)	Economic life (years)
Equipment				
Air compressor (100l)	1 unit	1500	1500	10
Air conditioner (2 hp)	1 unit	3000	3000	10
Dry oven	1 unit	500	500	15
Haemacytometer cell	1 unit	150	150	5
Mettler balance	1 unit	4000	4000	20
Microscope compound	1 unit	800	800	20
Refrigerator (8 cu. Ft.)	1 unit	800	800	20
Water pump (1.5 hp)	1 unit	1500	1500	10
Sub-total			12250	
Materials				
Air hose	25 m	2	50	2
Air stone	8 stones	3	24	2
Carboys (20-1 units)	6 units 2 pcs	35	210	5
Cover slips	3 units	0.4	0.8	2
Fiberglass tamks (1.5-tons)	2 units	400	1200	10
Fluorescent lamps (20 watt)	4 jars	200	400	2
Gallon Jar	2 units	3	12	7
Glass aquaria (100 l)	4 bottles	200	400	5
Glass bottle	2 pcs	2	8	5
Glass tubings	2 units	3	6	3
Microfilter	3 pcs	137	274	5
Pipet	1 pc	5	15	2
Plastic tubing (1/4 inch)	2 pcs	20	20	2
Slide glass	6 meters	1	2	3
Tank cover	10 pcs	4	24	5
Test tubes		1	10	2
Sub-total			2655.8	
Total			14905.8	

Table 4. Cost of Bold's basal medium (analyzed grade fertilizer), Pound/litre.

Ingredient	Quantity	Unit cost	Total
Sodium nitrate	25.00 g	160/kg	4.00
Calcium chloride	2.50 g	30/kg	0.075
Magnesium sulfate	7.50 g	40/kg	0.30
Dipotassium hydrogen phosphate	7.50 g	55/kg	0.41
Potassium dihydrogen phosphate	17.50 g	40/lg	0.70
Sodium chloride	2.50 g	10.00/kg	0.025
EDTA disodium salt	50.00 g	250.00/kg	12.50
Potassium hydroxide	31.00 g	14.00/kg	0.43
Ferrous sulfate	4.98 g	160.00/kg	0.80
Boric acid	11.42 g	30.00/kg	0.34
Zinc sulfate	8.82 g	350.00/kg	3.09
Manganous chloride	1.44 g	250.00/kg	0.04
Molybdeunum trioxide	0.71 g	600.00/kg	0.43
Copper sulfate	1.57 g	200.00/kg	0.31
Cobalt nitrate	0.49 g	400.00/kg	0.20
Labor	5.00h	3.00/h	15.00
Total cost/litre			38.65

Table 5. Cost of OI enrichment (technical grade fertilizer), Pound/litre.

Ingredient	Quantity	Unit cost	Total
Potassium nitrate	202.00 g	0.32 kg	0.065
Sodium phosphate	39.60 g	0.40 kg	0.016
Hydrochloric cid	28.00 ml	30.00/l	0.84
Calcium chloride	50.60 g	1.00 kg	0.051
Iron-EDTA	30.00 g	7.00/kg	0.21
OI Trace metal mix.	4.00 g	50.00/kg	0.2
Labor	2.00 h	2.00/h	4.00
Total cost/litre			5.382

Table 6. Cost of producing live microalgae (Pound/ton).

Item	Quantity	Unit cost	Total	% of total
Inoculum	50 ml	0.006/ml	0.30	
Bold's baal medium	10 ml	0.039/ml	0.39	←
Agar	5 g	0.125 g	0.625	Nutrients
Ol medium	400 ml	0.0054/ml	2.160	= 8.11
Ammonium sulfate	100 g	0.001/g	0.10	
Monopotassium phosphate	30 g	0.0003/g	0.009	
Urea 46	5 g	0.0005/g	0.0025	←
Clorox	1 l	1.50/l	1.50	
Electricity	150 kwh	0.07/kwh	10.50	25.94
Labor	16 h	1.0/h	16	39.48
Depreciatopn			8.94	
Total operating cost/ton			40.53	

Table7. Cost of operating 1 kg oven dry biomass of microalgae.

Item	Quantity	Unit cost	Total
Live algae	1.43 ton	48.53/ton	57.96
Electricity	6 kwh	0.07/kwh	0.42
Labor	8 h	1.0/h	8
Total oprating cost/kg			66.38

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اقتصاديات طريقة الخطوات المتعددة فى الأستزراع المكثف للطحالب

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المعمل المركزى لبحوث الثروة السمكية - مركز البحوث الزراعية - وزارة الزراعة - جيزة - مصر.

تعتبر الطحالب الدقيقة الغذاء الطبيعى لعديد من أنواع الأستزراع السمكى واساسيات سلاسل التغذية الطبيعية لهذة الأنواع وتجعلها تستطيع الحياة فى البرية . وتنمى الطحالب فى المعامل باستخدام أوعية كبيرة نسبيا . ويمكن الحصول على عدد الخلايا ، محتوى الكلوروفيل أ، والكتلة الجافة فى نهاية اليوم الرابع من الأستزراع وهى ٦,٥ x ١٠^{٦} خلايا /مللى، ٧٧ .٤ ملليجم /لتر ، و ٥٧و. ملليجم / لتر لطحلب الكلوريلا فواجارس وكذلك ٥,٤ x ١٠^{٦} خلايا / مللى ، ٤,٩٦ ملليجم / لتر و٨٢ و. ملليجم / لتر لطحلب السينيدسمس بيجيوجا ، على التوالى.

وكانت تكلفة الانتاج المكثف فى تنكات الإستزراع الخارجى للطحالب حوالى ٦٦,٣٨ جنيه مصرى / كجم كتلة جافة و ٤٠,٥٣ جنيه مصرى / طن من الطحالب الحية (١ دولار = ٣,٣٥ جنية مصرى). وشملت هذه الدراسة تكلفة العمالة (٣٩,٤٨٪) ، والطاقة الكهربائية (٩٤و٢٥٪) ، المغذيات (١١و٨٪) والمتبقيات (٧٤ و ٢٦٪) من تكلفة الانتاج الكمى . لذلك فأن طريقة الخطوات المتعددة فى الأستزراع المكثف المستخدمة فى هذا البحث تقلل تكلفة الانتاج.