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ECONOMIZATION INTENSIVE OUTDOOR MASS PRODUCTION OF THE GREEN ALGA *SCENEDESMUS* SP.

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Abstract

The green alga *Scenedesmus* sp. was out door scaled-up within three cement open ponds with a final capacity of $15m^3$ for each one. The used nutrient solution was composed of tap water enriched by macronutrients (NPK) from commercial sources. Dilution of cultures was done according to growth rate and nutrients supplementation as growth reached their maximum dry weight. Continuous centrifugation was carried out at the end of each batch and the obtained biomass was calculated. A comparison was performed; during long term successive season; as growth was employed under the recommended amount of NPK. It was found that electric power represented the maximum cost and the net cost was found in contrast with the volume of growth unit. In addition, enriched growth medium by extra amount of urea and micronutrients led to yield maximization. Maximum cost was observed during inoculums preparation and the lowest cost was found with cultures grown in large scale.

Introduction

Microalgae combine properties typical of higher plants with biotechnological attributes proper of microbial cells. This particular combination supports the use of these microorganisms for applied processes and represents the basis of microalgal biotechnology. The potential of microalgae for practical uses is certainly great. Besides the use as feed for aquatic and terrestrial animals with many chemicals, biochemicals, and pharmaceuticals are generated by microalgae, which could be mass-produced outdoors. The food, feed, pharmaceutical, cosmetic, and chemical industries benefit from microalgal products. Systems based on these photosynthetic microorganisms also offer a great potential for sunlight-driven biofuel generation, reclamation of wastewater, and other bioremediation solutions, such as the arrestment of carbon dioxide accumulation in the environment (Pulz and Gross, 2004; Richmond, 2004).

Microalgae are expensive to produce, although many efforts are under way addressed to achieve cost-efficient modes for mass cultivation of these organisms. Different systems have been designed for the growth and handling of microalgae on a large scale (Gudin and Chaumont, 1980; Weissman *et al*., 1988; Borowitzka, 1999; Molina-Grima *et al*., 1999; Pulz, 2001; Richmond, 2004; Tredici, 2004). Within the open systems, the best choice seems to be the open shallow pond, made of leveled raceways 2–10 m wide and 15–30 cm deep,

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running as simple loops or as meandering systems. Each unit covers an area of several hundred to a few thousand square meters. Turbulence is usually provided by rotating paddle wheels, which create a flow of the algal suspensions along the channels at a rate of 0.2–0.5 $m.s^{-1}$. The adequate supply of carbon dioxide is very critical, and it is usually controlled through a pH-stat, so warranting both provision of carbon and optimum pH of the culture simultaneously.

Mineral nutrition, carbon dioxide and man power were considered to be the major factors which in turn determine the world wide of algae production. High production costs could be minimized through out the downing of mineral nutrition costs using some commercial sources instead of those, which traditionally used. Of these, urea as a sole source of nitrogen; beside their buffering action against the rise of pH; and supporting growth medium by extra amount of carbon dioxide was early recommended. Phosphoric acid, on a commercial grade (84%) and potassium sulfate were also used in the same concern (El-Sayed, 1999; El-Fouly *et al*., 2001 and El. Sayed *et al*., 2001). Electric power; the second item of production; consumed by steering, illumination, pumping, harvesting and drying represented the maximum costs (Borowitzika, 1992). In contrast, Zaborsky (1985) suggested that about 70% of total costs return to nutrition especially carbon source. Such costs could be minimized by changing some technical aspects including chemicals nutrition and drying (El-Sayed, 2005).

Costs could be downed by intensive volume of cultivation per growth unite as well as the directly use of algal biomass without the common drying methods, *i.e.*; drum, sun, freeze and steam–drying. Such technique allows the safe of biologically active components including phyto-hormones (Abdel-Maguied *et al*., 2005). In comparison with other cultivation systems mainly bioreactors, open ponds characterized by batch or semi-continuous operating regime, low area volume ratio, low population density and poor light utilization efficiency. Otherwise, such system allow the easy scaling up purposes with low operating system and low investment (Del Campo *et al*., 2007).The present work was conducted to evaluate the production costs of the pre-isolated green alga *Scenedesmus* sp. (El-Sayed, 2004 a and b) under the out door conditions.

Materials and Methods

Alga and growth conditions

The pre-isolated green alga; *Scenedesmus* sp. (El-Sayed, 2004 b) was out door scaled-up within three cement open ponds in a final capacity of $15m^3$ for each one. Concerning macro nutrients, growth medium was composed of tap water enriched by certain commercial fertilizer compounds (0.3g urea, 0.1ml phosphoric acid and 0.05g K_2SO_4) in a concentration varied due to growth rate and dilution (El-Sayed *et al*., 2001).

As for micronutrients, Wuxal micro containing 2.8% of Fe, Zn and Mn for each, with 0.014% Cu, 3.3% S and 14% N was used aiming at the enhancement of vegetative growth as well as minimizing the production costs. The trail was achieved after the recommended results were obtained from the indoor cultivation, where the superior concentration of urea; determined as μ; was employed.

A serial of dilution was started from 3000L to reach $15m^3$ through different dilution times four days apart and stirring was performed by PVC paddle wheel at the rate of 16 r.p.m.

Inoculums preparation

Preparation of inoculums was carried out by the universal method, where algal cells were incubated with the recommended NPK doses (El-Sayed *et al*., 2001 and El-Sayed, 2005). The initial was prepared by polyethylene bottles of one liter and dilution was achieved according to growth rate as determined as dry weight. The net obtained volume was about 25L, followed by vertical poly carbonate sheet; as growth reached the maximum; with a final capacity of 200 L. The consumed nutrients, water and power concerning aeration and illumination during these periods listed below in (Table 2).

Scaling up of outdoor cultivation

Following to the indoor cultivation including flasks and vertical sheet, the obtained inoculums were transferred to the open plate containing 1000L and then to the modified open plate 2000L (El-Fouly *et al.*, 2001) when growth reached their maximum dry weight ($g.L^{-1}$). The net obtained volume was about 3000L for two units. All of the whole volumes (3000L) were pumped to the first open pond to reach about 6000 L of growth volume by tap water dilution. As growth was progress, dilution took place till the net volume of about 15000 L. A part of this volume was used to inoculate the second pond. By the same the third pond was cultivated and all of the three ponds are equal in volume and approximately equal in their initial dry weight. The used nutritional technique including dilution and nutrients addition was as described above using tap water.

Measurements:

Daily measurement of dry weight ($g.L^{-1}$) was routinely carried out by filtering a define volume of algal slurry (5-10ml) over pre weighted dried membrane filter (0.45 μm). Filters were dried (105^oC/30min.), kept over anhydrous calcium chloride till room temperature and then re-weighted. The differences between weights represented the obtained algal biomass. Dry weight was also determined following all the dilution time to achieve dilution and nutrients supplementation.

As for growth evaluation, growth rate on the maximum ($\mu_{max.}$) was calculated as ($\mu = \ln_I - \ln_o / t$).

Harvesting and preservation

At the end of cultivation period; as open pond cultures reached their maximum growth determined as dry weight; cultures were pumped to continuous centrifugation (50001.h^{-1}). The precipitated biomass was kept on deep freezer till it use.

Result and Discussion

Indoor growth

During indoor growth, no variable results were obtained as they compared with the previous data (El-Fouly *et al*., 2001, El-Sayed *et al*., 2001 and El-Sayed, 2004 a and b). Growth was progress associated to the advantage age. All of the different urea concentrations (0.1-0.5 g.L^{-1}) increased growth rate of the incubated alga *Scenedesmus* sp., however the obtained dry weight was found to be corresponded to the given concentrations (Fig. 1). Increasing of urea led to the dry weight increases, but represented variable growth rate (µ) due to nitrogen concentrations (Table1). In this connection, algae can utilize both ammonium and nitrate salts as well as urea (Syrett, 1963 and Khalil *et al*., 1979) and urea is the best source for mass *Chlorella* sp. cultures as maintains a constant pH of the culture and limit the development of accompanying micro-flora. With better growth rate than other nitrogen source, high concentration does not inhibit the development of these organisms. Furthermore, the enzymatic degradation of urea to CO_2 and NH_3 prior to its incorporation to the cell (Allison *et al*., 1954). Concentrations required for growth of *Chlorella* ranged from 100 to 300 mg N.l^{-1}, whereas cell division is fastest at concentration from 200 to 600 mg N.L^{-1} (Pribil, 1970).

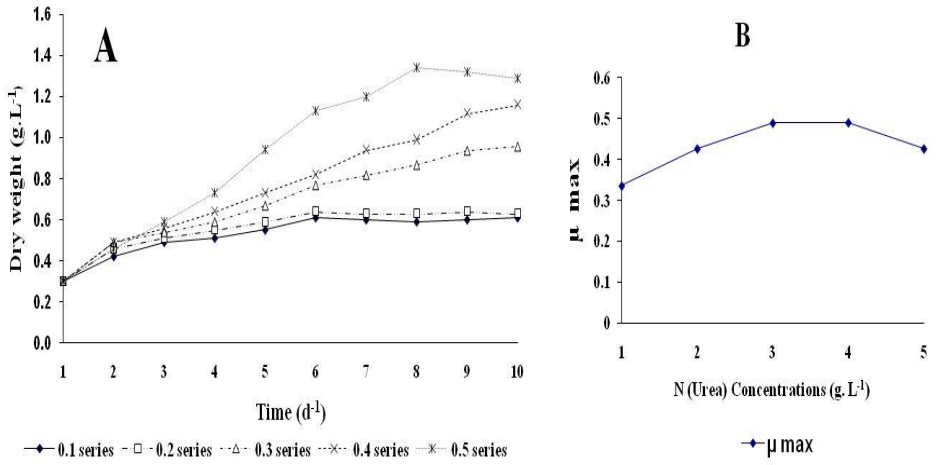

Figure (1): a) Growth and (b) maximum growth rate ($\mu_{max.}$) of *Scenedesmus* sp. under different concentrations of urea.

Table (1): Growth characteristics of *Scenedesmus sp.* as affected by urea concentrations.

Growth parameter	Urea concentrations ($g.L^{-1}$)				
	0.1	0.2	0.3	0.4	0.5
$\mu_{max.}$	0.336	0.427	0.49	0.491	0.427
$\mu_{avr.}$	0.142	0.152	0.143	0.165	0.214

As the cultures of poly ethylene bottles reached the maximum dry weight, the whole volume was transferred to the open sheet by the same technique; however growth was more enhanced during the cultivation period within open sheet (Fig.1). This could be attributed to the changing of the ambient environmental conditions mainly sufficient aeration and illumination (El-Sayed *et al*., 2005).

As shown in Table 2, it may be concluded that one sheet containing 200 L of inoculums (ca200g of algal biomass) consumed about 94.051 L.E suggested that 0.47 L.E required for one liter containing about 1.0 g of dried biomass, however such costs are very low and must be put out of calculation in comparison with the expected volume and also must be calculated as fixed or capital costs.

Table (2): Individual costs during in-door cultivation.

Element	Amount		Total	Local price L.E
	Flasks	Sheet		
N (Urea)	25L x 0.5g =12.5g	175 x 0.5g =87.5	100g	00.180
P (H_3PO_4)	25 x 0.1ml =2.5 ml	175 x 0.1ml =17.5 ml	20ml	00.175
K (K_2SO_4)	25 x 0.01g =0.25g	175 x 0.01g =1.75g	2.0g	00.016
Light	10l x 24h x 10d x 40w = 96kw	20l x 24h x 10d x 40w =192	288kw	72.000
Compressed air	24h x 10d x 150w = 36kw	24h x 10d x 150w =36kw	72kw	21.600
Water	25L	175L	200L	00.080
Total				94.051

g = gram; kw = kilowatt; l = lamp; ml = milliliter; w = watt and L = liter

Outdoor cultivation

I- Open plate

By the same technique occupied during indoor cultivation growth and dilution were performed. Differences on growth rate might goes back to the differences on growth conditions including night illumination, high light irradiation during sunny days and/or low night temperature. Any way, growth reached their maximum after 14 days of cultivation (Fig. 2) and about 0.9 $g.L^{-1}$ of dried biomass was obtained with the first unit (open plate) and about 1.2 $g.L^{-1}$ of the second unit (modified open plate). As mentioned previously, the differences

on growth between tow units return back to the unit shape and design (El-Fouly *et al*., 2001).

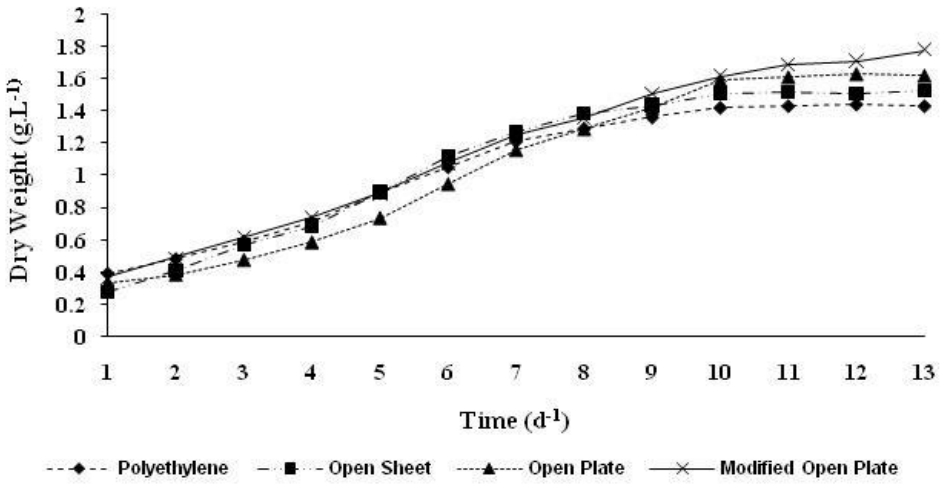

Figure (2): Growth dries weight (g.L^{-1}) of *Scenedesmus* sp. under different growth unit.

Accordingly, the individual costs could be calculated as present in Table (3) and showed that about 244.765 L.E are required o obtain 3000L of the inoculums containing about 3300 g of dried biomass meaning that 0.08156 of L.E are required to produce one liter of the inoclum containing about 1.1 g of dried biomass. It may be concluded that the most consumed costs return to the electric power required for aeration. The actual costs were found to be included 94.051 from the indoor cultivation. So, the net costs are 150.714 L.E.

Table (3): Individual costs during out-door cultivation

Element	Amount		Total	Total market price L.E
	Open plate	Modified open plate		
N (urea)	1000L x 0.5g =500g	2000L x 0.5g = 1000g	1500g	002.700
P (H_3PO_4)	1000L x 0.1ml =100 ml	2000L x 0.1ml = 200 ml	300ml	002.625
K (K_2SO_4)	1000L x 0.01g =10g	2000L x 0.01g = 20g	30g	000.240
Light	4l x 12h x 14d x 40w =26kw	4 x 12h x 14d x 40w = 26kw	52kw	013.000
Aeration Electric pump	2kw x 4h x 4d = 672kw	2.5kw x 4h x 4d = 840kw	902kw	225.500
Water	1000L	2000L	3m^3	001.200
Sub-total				244.765
Inoculums costs				94.054
Actual costs				150.714

II- Open ponds

As the obtained volume (3000 L) was transferred to the first open pond, the same nutritional regime was performed and the pond reached their maximum volume and growth (*ca.*1.0g.L^{-1}) after 17 days of cultivation (Fig.3). Here, dilution took place to inoculate the second pond till the next 31 days of the first incubation. After 58 days of cultivation all of the three cultivated ponds reached their maximum growth determined as dry weight (g.L^{-1}) and volume (15m^3).

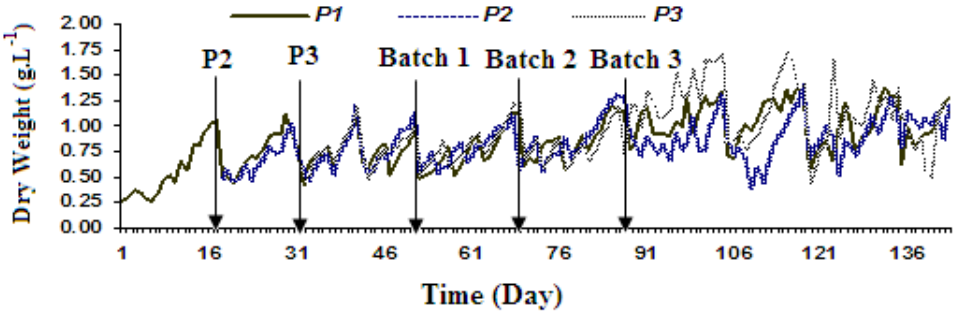

Figure (3): Dry weight of out-door cultivated *Scenedesmus* sp.

Thus, costs of the first batch could be account about 282.825 L.E as 75 kg of fresh algal bulk containing 75% of moisture was obtained. Accordingly, 3.77 L.E are required to obtain one kilogram of wet algae and 15.08 L.E are required to produce to obtain one dried kilogram of the produced alga without additional costs of drying which account as 48 L.E per kilogram. To avoid such high costs as algal slurry was dried using drum drier apparatus or steam generator, using of algae on freeze form and following press especially for biological purposes. The obtained biomass was calculated and the consumed costs are listed in Table (4).

Table (4): Individual costs during out-door cultivation (*batch)

Element	Amount	Total market price L.E
N (urea)	**15000L x 0.5g = 7.5kg**	**13.5**
P (H_3PO_4)	**15000L x 0.1ml = 1.5 L**	**13.125**
K (K_2SO_4)	**15000L x 0.01g = 0.15kg**	**1.2**
Night illumination	**4 x 12h x 19d x 40w =72.96kw**	**9.12**
Aeration (Paddle wheel)	**0.37kw x 24 x 19 = 168.72**	**42.18**
Water	**15m^3**	**6.0**
Micronutrients	**1.5L**	**60.0**
Total		**155.925**

*Mean of three ponds

As mentioned before, Costs of the first batch included the costs of indoor cultivation and the two open plate units. Thus, the actual cost could be account as (155.925+ 150.714= 306.639 L.E). However, the interior costs must be discarded out of calculation if continuous mass production will achieved.

Second batch which present the actual production costs after 20 days represented a slight difference in yield (82 kg), but in a short period in concern to dilution rate which in turn reduced the production cost. Costs of the second batch could be calculated as the same of first batch, however more yield was obtained. Accordingly, one kilogram of fresh algal bulk required 1.90 L.E. Continuous cultivation reduced the batches of harvesting to about 10 day. By such time, the consumed production costs could be downed as listed in Table (5) to 95.225 L.E.

Table (5): Individual costs during out door cultivation (*continuous batch).

Element	Amount	Market price L.E
N (urea)	**15000L x 0.5g = 7.5kg**	**13.5**
P (H_3PO_4)	**15000L x 0.1ml = 1.5 L**	**13.125**
K (K_2SO_4)	**15000L x 0.01g = 0.15kg**	**1.2**
Night illumination	**4x12h x 10d x 40w = 19.2kw**	**4.8**
Aeration (Paddle wheel)	**0.37kw x 24 x 10= 88.8**	**22.2**
Water	**$1m^3$**	**0.4**
Micronutrients	**1.0L**	**40.0**
Total		**95.225**

*Mean of three ponds

As shown in Fig. 4, most of the consumed costs were found during indoor cultivation and increasing of growth volume led to the minimizing of such cost. The rise of production cost not only attributed to the long time of growth and high consumed power for aeration and illumination, but return to the long adaptation for alga to the ambient conditions and transferring from solid to liquid phase.

It may be also concluded that; in all cases of cultivation; most of production costs goes back to the electric power. During indoor cultivation, illumination represents the maximum cost and aeration took place during the first outdoor step (open plate). Lowest costs were found in the water used for cultivation and recycling of water during open pond cultivation makes it costless. In addition, the rise of production cost in the case of first open pond goes back to the long adaptation of alga to out door condition especially agitation and low culture width which prevent the phenomenon of self shading that protect alga against light chock (Fig.5).

The obtained data concluded that the amount of nutrients addition not depend on the duration of cultivation, but only depended upon the growth rate and recycling of the used water minimized the production costs. In addition, during the first treatment period (143 days) growth was progress proportionally and

sigmoid growth curve was observed due to dilution rate. Top of growth curve represented the net growth and bottom of growth curve represented the dilution. Rate of dilution (24-48 hours) described the high growth rate of the cultivated strain and sufficient of nutrition as well. The differences between batches were found to be due to different treatments.

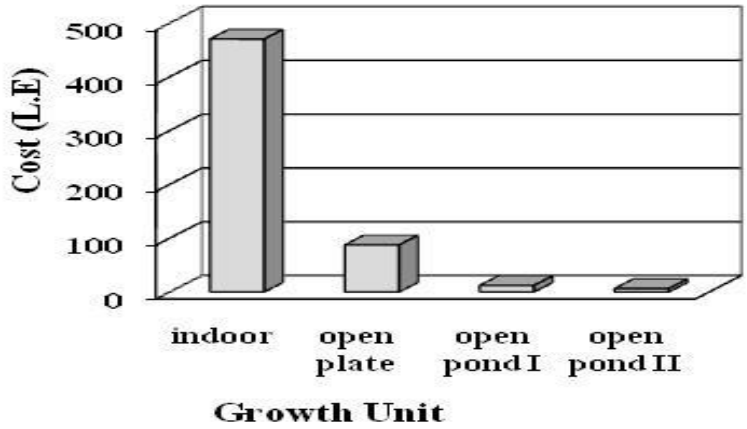

Figure (4): Different consumed costs for the mass production of the green alga *Scenedesums* sp.

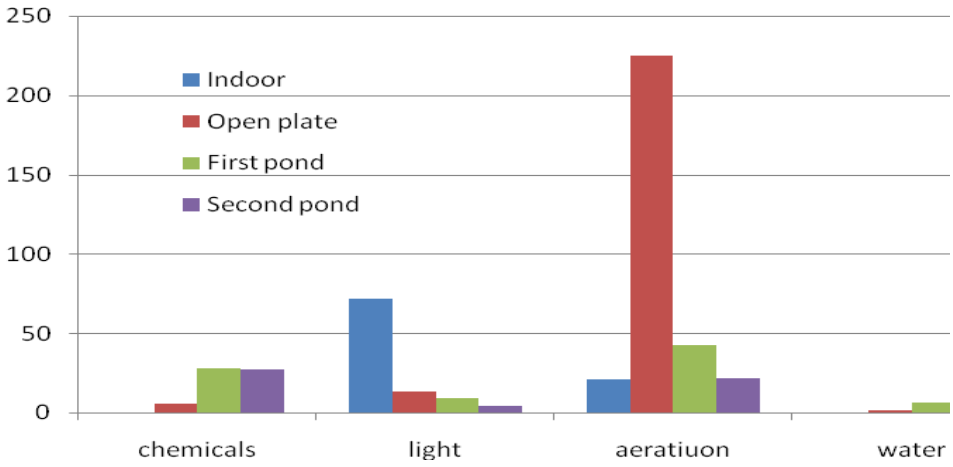

Figure (5): Different input costs for the mass production of the green alga *Scenedesums* sp.

Concerning the consumed power for algal collection and separation, about 18 L.E are required for pumping system and centrifuge apparatus (2kw x 3h x

0.25 = 1.5 + 22kw x 3h x 0.25=16.5) = 18L.E. More minimizing could be performed as growth yield as increased to be more than 1.0 $g.L^{-1}$.

Conclusion

Different categories were found to be interoperating in algae mass production. Of these are mineral nutrition, electric power, and water used. Electric power including light, aeration, agitation, pumping and harvesting represented the main category of algal cost production. Intensive algae cultivation as well as water re-cycling could be serving as the main effort for minimizing the algal production cost.

Acknowledgment

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التقييم الإقتصادى لإنتاج طحلب السندزموس المكثف خارج المعمل

أبوالخير بدوى السيد

قسم تكنولوجيا التسميد – المركز القومى للبحوث – الدقى – القاهرة.

يتم زراعة الطحلب الأخضر من جنس سندزموس بصورة مكثفة بالمركز القومى للبحوث للإستخدام العلمى والتجارى فى أحواض مفتوحة سعة 15 م^3 للحوض الواحد. يستخدم فى عملية الزراعة محلولا مغذيا يحتوى على النيتروجين والفوسفور والبوتاسيوم بالتركيز الموصى به من مصادر تجارية. التخفيف وإضافة المحلول المغذى تتم تبعا لدرجة النمو. وعندما يصل النمو للحد الإقتصادى (اجم/لتر تقريبا) يتم جمع الطحالب بإستخدام جهاز الطرد المركزى المستمر (5000لتر/ساعة).

فى هذه الدراسة تم زراعة الطحلب داخل المعمل مستخدما وحدات الزراعة المختلفة (فلاسكات 5لتر– بولى إيثيلين 200لتر) وخارج المعمل مستخدما الوحدة المفتوحة 1000لتر والمعدلة 2000لتر والأحواض الأرضية المفتوحة15م^3 للحوض الواحد بنفس التركيزات المستخدمة من العناصر المغذية الكبرى.

عند تتبع تكاليف الزراعة والإنتاج خلال هذه المراحل من مياه – محلول مغذى – إضاءة – تقليب بالهواء المضغوط والطلمبات والبدالات – طرد مركزى, وجد أن الإستهلاك والتكلفة العالية تحت ظروف الزراعة الناجحة ترجع إلى الطاقة الكهربائية المستهلكة. وجد ايضا أن الزراعة خارج المعمل أقل تكلفة من الزراعة داخل المعمل وأن الطاقة الكهربائية المستهلكة تتناقص بزيادة حجم المزرعة. ومن الوجهة الغذائية وجد أن زيادة تركيز اليوريا مع العناصر الصغرى تزيد من كمية الناتج مما يقلل تكلفة الطاقة الكهربائية المستهلكة. ويمكن خفض التكلفة أيضا بتواى عملية الزراعة بإعادة أستخدام مياه التشغيلة السابقة.