PRODUCTION OF *SCENEDESMUS DIMORPHUS* GROWN ON SOME WASTES (POULTRY MANURE) USED IN AQUACULTURE

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Abstract

As compared to Bold's basal medium (B.B.M.), high yields of *Scenedesmus dimorphus* in terms of cell number, protein, chlorophyll "a" and algal dry weight contents were achieved at low concentrations of poultry manure water extract (0.2% - 0.6%). Cell diameter and dry weight increased with increasing waste concentration. Nucleic acid contents of *Scenedesmus dimorphus* grown on poultry manure were lower than that values obtained when the alga was grown on Bold's basal medium (B.B.M.). A well balanced distribution of protein amino acids was found in *Scenedesmus dimorphus* grown on water extract of poultry manure, which was comparable with that of FAO reference protein. Also it was found that such protein had a chemical score of 61.1 while the protein of *Scenedesmus dimorphus* grown on Bold's basal medium had a chemical score, which equals 47.4.
Key words: Bold's basal medium, poultry manure, protein high nutritive. *Scenedesmus dimorphus*.

Introduction

Since the world is alarmed by the population explosion and food crisis, one may ask whether there is any sense today in looking for non-conventional sources of protein for animal and possibly, human nutrition? Indeed protein is a vitally important food material required by man (Fathy and Seyam, 1989 and De Pauw *et al*., 1998). 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 conversions efficiencies and no need for harvesting, drying and storage as the animals or food chains could use the algae as produced (Allen and Nelson, 1974; Benemann, 1992).

Out of the intensive research in many countries, one can conclude that, microalgae provide a valuable source of protein and other chemical compounds (Becker, 1994). High productivity of microalgae is only achieved in tropical or subtropical countries, where the climatic conditions favor continuous cultivation all year round. Microalgae may provide such countries with a potential direct and indirect protein source which can be locally produced. In the commercial and semi-commercial production of algae for feed and food, important advances have

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been made in South East Asia (Saleh *et al*., 1979; Zaret *et al*., 2001 and Persoone *et al*., 2002).

For biologists, algae offer a number of distinct advantages. In addition to being photosynthetic autotrophs unicellular algae posses a number of microbial characteristics including rapid growth rate, high protein content and variable metabolism, which respond rapidly to environmental changes. Some studies have been carried out to investigate the chemical composition of microalgae. Microalgae are rich in vitamins and fats, also their protein quality, in terms of the amounts of important amino acids are high. Badawy (2005) found that crude protein content of *Chlorella* spp. was 46.7 %, the crude fat content was 14.8 %, total carbohydrate content was 11.6 %, ash was 17.5 %, crude fiber was 9.30 %, nucleic acid content (RNA 2.63 % and DNA 1.72 %), and vitamins group antioxidant B_6, B_{12}, E, C, ß-carotene (µg/g dry weight) were found to be 0.05, 0.08, 2.25, 16.0 and 2384.0, respectively. Meanwhile, *Scenedesmus* spp. contained 52.3 % crude protein, 12.20 % crude fat, 10.06 % Carbohydrate, 14.92 % ash, 8.83 % crude fiber, 3.16 RNA and 1.43 DNA, 0.27 B_6, 0.78 B_{12}, 0.01 E, 21.8 C and 1890.0ß-carotene (µg\g dry weight). El-Fouly *et al*. (1984) reported that, five amino acids (aspartic acid, serine, alanine, leucine and glycine) were collectively responsible for 50% or more of the total dry matter content of *Chlorella* and *Scenedesmus* algae, However, they were deficient in sulfur-amino acids. Natural food still remains the major feed for Tilapia rearing so a timely supply of microalgae in sufficient quantity ensures the success of a tilapia hatchery. Microalgae and cyanobacteria grown on wastes can be used as a dietary supplement for animals and for any other biotechnological purposes.

Recycling of poultry manure and evaluation of *Scenedesmus dimorphus* grown on it is the aim of the present study. The present study was carried out in the Central Laboratory for Aquaculture Research (CLAR) and International Central Laboratory for Aquaculture Research Management. The World Fish Center, Regional Center for Africa and West Asia, Abbassa, Abu-Hammad, Sharkia, Egypt.

Materials and Methods

Algal strain and Nutrient solution:

A pure strain of *Scenedesmus dimorphus were* obtained from the Department of Botany, National Research Center, Dokki, Cairo, Egypt. The inoculum was prepared in laboratory, 250 ml Erlenmeyer flasks each containing 100 ml of sterilized media was inoculated with *Scenedesmus dimorphus* in a concentration of 1.5 x 10^6 cells/ml. The control medium (nutrient standard solutions) Bold's basal medium (B.B.M.) according to Bischoff and Bold (1963). B.B.M. composition is illustrated in Table (1). Algae cultures were incubated in the growth room at 25 ^{O}C, adjusted by air condition, continuous illumination provided by 6 fluorescent lamps which gave a light intensity of 5000 Lux, under photoperiods 16h light/ 8h dark cycle. The pH was adjusted daily to 8.0; fluorescent lamps as well as photoperiods were adjusted by electricity timer.

Table (1): Chemical composition of B.B.M. medium

Chemicals	Final concentration
A. Macronutrients: Consist of six stock solutions:	
1-Sodium nitrate ($NaNO_3$)	25 g/L
2- Potassium dihydrogen phosphate ($KH_2 PO_4$)	17.5 g/L
3-Dipotassium hydrogen phosphate ($K_2H\ _2PO_4$)	7.5 g/L
4-Magnesium sulfate ($MgSO_4$)	7.5 g/L
5-Calcium chloride ($CaCl\ _2$)	2.5 g/L
6-Sodium chloride ($NaCl\ _2$)	2.5 g/L
B. Micronutrients: Consist of four stock solution:	
1-Na_2-EDTA + KOH	50 g plus 31 g/L
2-$FeSO_4.7H\ _2O$	4.98 g/L
3-$H_3\ BO_3$ Boric acid	11.42 g/L
4-The following four salts: All were dissolved in one liter of distilled water.	
- $ZnSO_4.7H_2O$	8.82 g/L
- $MnCl\ _2$	1.44 g/L
- $CuSO_4$	1.57 g/L
- $Co(NO_3)_2.6H_2O$	0.49 g/L

To prepare one liter of Bold's basal medium added 10 ml of each six stock solution (macroelements) and 1 ml of each four stock solution (microelements) and add 936 ml distilled water. Macronutrient consists of six stock solutions as well as micronutrient consists of four stock solutions, each amount of salts is dissolved separately in 100 ml warm water and diluted into one liter distilled water.

Extraction of waste:

Poultry manure samples were randomly collected in polyethelene bags. The samples were air-dried in the laboratory for 5 weeks. Three equal samples of the air dried poultry manure were ground (by a mortar and pestle) for homogencity and to facilitate extraction. They were extracted with equal volumes of distilled water (each 200 ml) for 20 min. at different temperatures (22°C, 100°C and 120°C). The supernatant extracts were obtained by centrifugation at 7000 r.p.m.

for 20 min. From each extract six different concentrations (0.2%, 0.4%, 0.6% & 1.2% concerning the solid waste) were prepared by dilution using distilled water. The pH of each trial was adjusted at 8.0 using diluted HCL or NaOH solution. Results of the chemical analysis of poultry manure are shown in Table (2). To study utilization of waste constituents by *Scenedesmus dimorphus* two sets of culture flasks (each contained 100 ml 0.4% sterilized poultry manure pH 8.0) were prepared. The first set of flasks was inoculated by *Scenedesmus dimorphus* in a concentration of 1.5 x 10^6 cells/ml under aseptic conditions. The second set of flasks (control) was left without inoculation. Both sets were incubated for 4 days, at the end of which the final pH was determined. The growth (number of cells/ml) was measured and the contents of flasks of both sets were centrifuged (7000 rpm for 15 min). The supernatants were analyzed for soluble carbohydrates, nitrate, free ammonia and amino acids.

Table (2): Chemical analyses of poultry manure (% D.M basis)

Item	Poultry manure
Crude protein (CP)	**26.5**
Crude fiber (CF)	**15.9**
Nitrogen free extract (NFE)	**25.4**
Ether extract (EE)	**2.20**
Ash	**30.0**
Dry matter (DM)	**84.7**
Moisture	**13.3**

Chemical analysis:

The following analysis were carried out: total nitrogen (APHA, 1985), (the crude protein content was calculated as total nitrogen content x 6.25), ether extract, crude fiber, ash and moisture (A.O.A.C. 1995), cell of *Scenedesmus dimorphus* were harvested by centrifugation at 7000 r.p.m., chlorophyll "a" content was measured spectrophotometricaly according to (Vollenweider 1969 & APHA, 1985). Nucleic acids were determined according to Morse and Carter (1969). To determine the algal dry weight, samples were oven dried at 60-65°C for 72 hr. up to constant dry weight.

Results and Discussion

The ranges obtained in this investigation for microalgae protein production were similar to the values reported by other authors (Flaak and Epifanio, 1978; El-Fouly *et al.*, 1979; Becker, 1994). Water extracts of poultry manure supported excellent algal growth when compared with the control medium (B.B.M.) especially with low concentrations (0.2%, 0.4% and 0.6%) whatever the extraction temperature. However, the algal growth which was obtained at 0.4% (optimum) chicken manure extracted at 22 ^{O}C was found to be higher than that at 100 ^{O}C and 120 ^{O}C (Figures 1, 2 and 3). These results are in agreement with those of Becker (1987); Allen and Garret (1977); El-Fouly *et al.*, (1998).

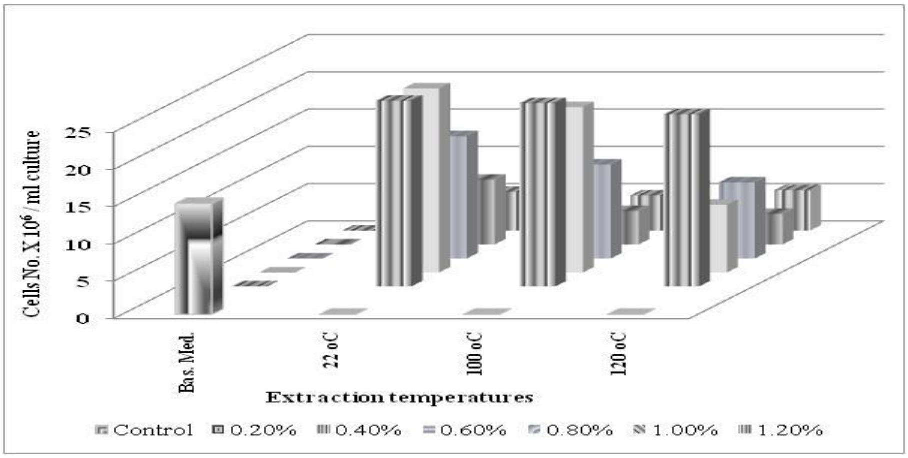

Figure (1): The growth of *Scenedesmus dimorphus* grown on Bold's basal medium and different concentration of poultry manure extracted at different temperatures

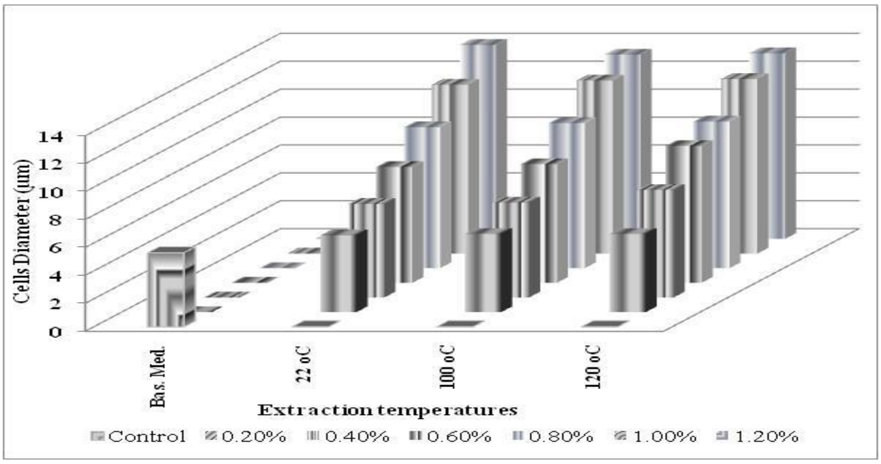

Figure (2): The cell diameter of *Scenedesmus dimorphus* grown on Bold's basal medium and different concentration of poultry manure extracted at different temperatures

Where high concentrations of wastes extracted at high temperatures decreased the algal growth. These results indicate that a temperature of 22°C may account for the release of essential nutrients and growth promoting compounds.

Higher temperature may produce growth delimiting compounds. The cell volume and the algal dry weight (Figs. 2 and 4) were clearly varied.

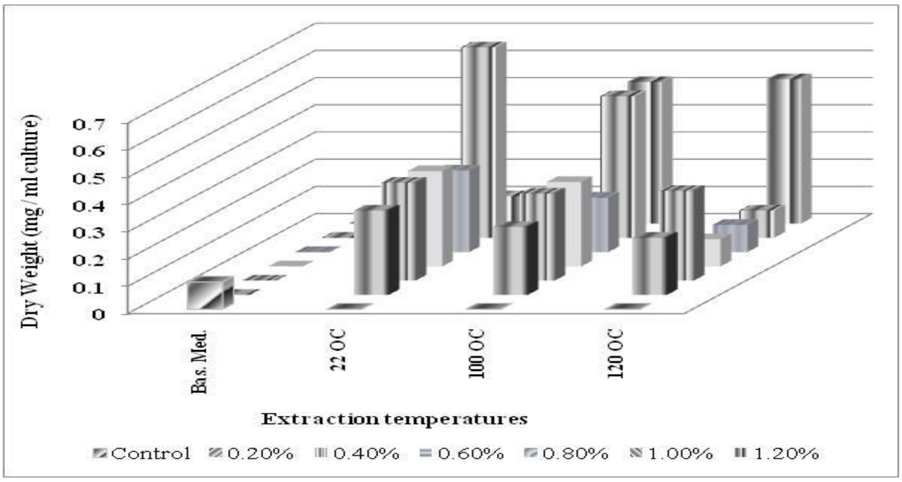

Figure (3): Dry weight of *Scenedesmus dimorphus* grown on Bold's basal medium and different concentration of poultry manure extracted at different temperatures

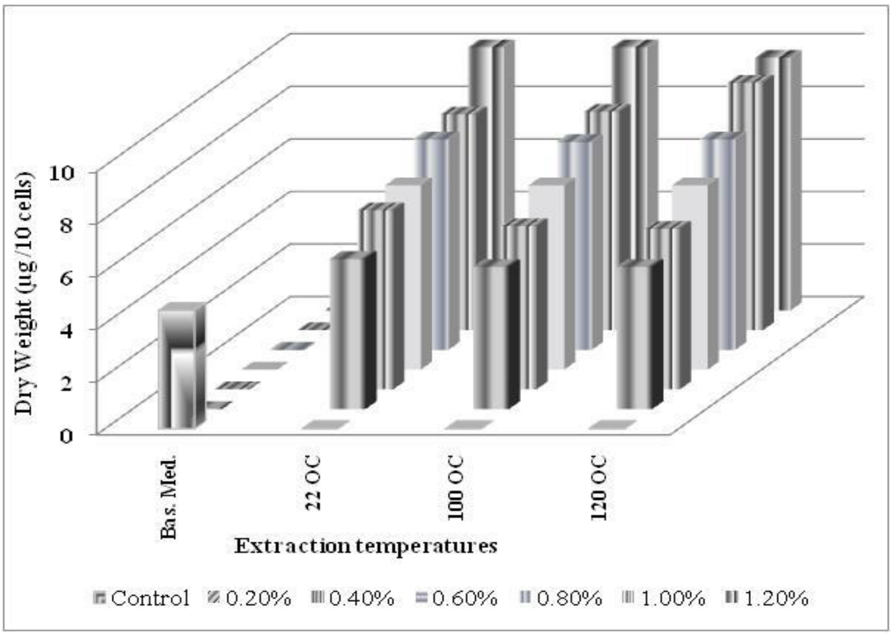

Figure (4): Dry weight of *Scenedesmus dimorphus* grown on Bold's basal medium and different concentration of poultry manure extracted at different temperatures

The algal cells grown on the basal medium attained the smallest volume compared to those grown on the waste extracts. As shown from figure 5, the maximum growth rate of *Scenedesmus dimorphus* was achieved after 4 days on 0.4% waste concentration. Therefore, the incubation period for all culture experiments subjected to biochemical analysis were conducted for 4 days using 0.4% waste.

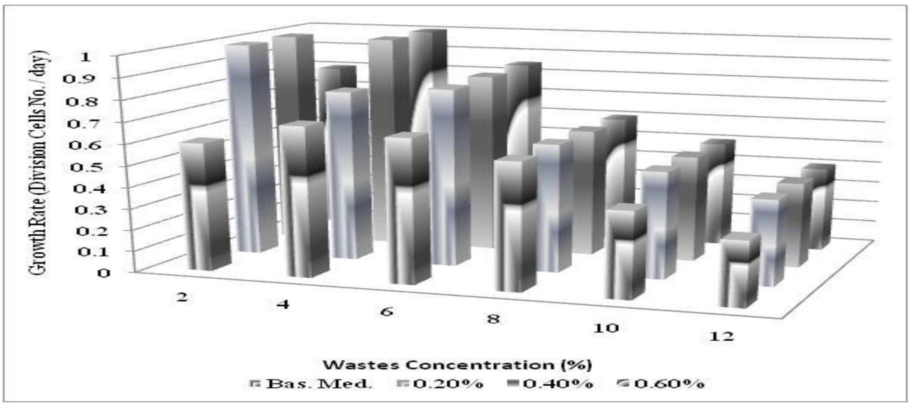

Figure (5): Growth rate of *Scenedesmus dimorphus* grown on Bold's basal medium and different concentration of poultry manure under different incubation periods

The results obtained by El-Fouly *et al*., (1984) showed that the maximum growth rate of *Scenedesmus dimorphus* of optimum concentration of sludge was achieved after 9 days. The contents of chlorophyll a were extracted at different temperature, from cultures supplied with different wasted (Fig.6).

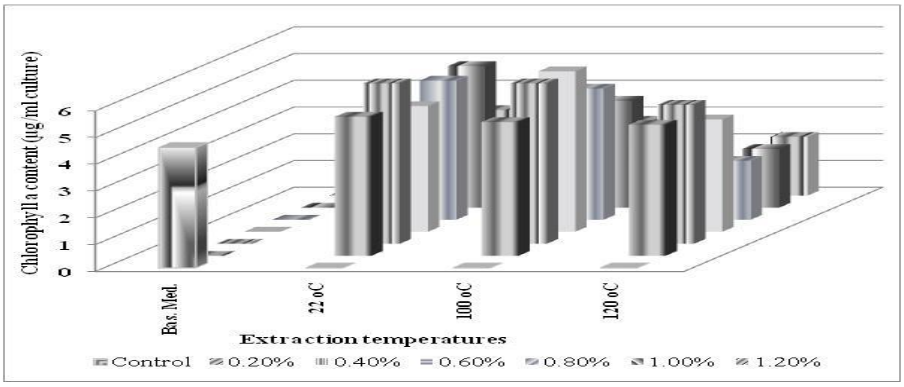

Figure (6): Chlorophyll (a) content of *Scenedesmus dimorphus* grown on Bold's basal medium and different concentration of poultry manure extracted at different temperatures

The results varied according to the concentration of poultry manure and to the extraction temperature. Concerning evaluation of *Scenedesmus dimorphus* protein obtained in the present work, the comparative analysis of *Chlorella* (Payer *et al.*, 2001). The FAO reference protein (FAO, 1983) is presented in Table (3) and Fig. (7). The results showed that the chemical score and the concentration of all amino acids in the protein of *Scenedesmus dimorphus* grown on poultry manure extract were higher than that of the algae grown on the medium. Also, the content of lysine the most important amino acid in *Scenedesmus dimorphus* grown on poultry manure extract was the highest when compared to that reported in *Chlorella*, *Spirullina* and even in FAO reference.

Table (3): Amino acids composition and chemical score of the protein of *Scenedesmus dimorphus* on Bold's basal medium (B.B.M.) and 0.4% water extract of poultry manure

Amino acids	Concentration (g/100 g protein)				
	1*	2*	3*	4*	FAO
Aspartic acid	1.09	2.40	8.20	10.10	--
Threonine	--	--	3.90	5.52	4.0
Serine	--	--	2.7	4.35	---
Glutamic acid	9.13	9.52	9.9	10.1	---
Proline	0.95	1.35	2.8	6.74	---
Glycine	1.46	2.7	4.4	5.7	----
Alanine	2.19	7.7	6.03	7.15	----
Cysteine	0.44	0.45	0.38	0.59	----
Valine	0.7	1.61	3.9	2.47	5.0
Methionine	0.59	1.66	1.28	1.55	3.5
Isoleucine	1.13	2.21	3.74	5.61	4.0
Leucine	0.64	2.15	6.75	7.12	7.0
Tyrosine	1.15	2.58	5.8	6.65	---
Phenylalanine	0.5	1.38	2.05	4.62	6.0
Histidine	1.45	1.2	1.4	2.0	---
Lysine	1.85	1.91	4.17	5.03	5.5
Arginine	4.24	4.14	4.32	5.20	---
Chemical score	---	---	47.4	61.1	100

1*= Total free amino acids of *Scenedesmus dimorphus* grown on Bold's basal medium.
2*= Total free amino acids of *Scenedesmus dimorphus* grown on poultry manure extract.
3*= Bounded amino acids of *Scenedesmus dimorphus* grown on Bold's basal medium.
4*= Bounded amino acids of *Scenedesmus dimorphus* grown on poultry manure extract.

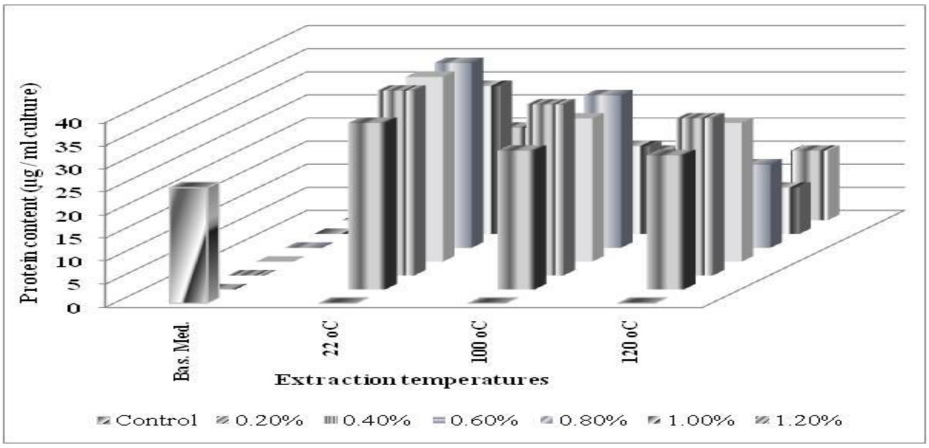

Figure (7): Protein content of *Scenedesmus dimorphus* grown on Bold's basal medium and different concentration of poultry manure extracted at different temperatures

Protein-tryptophan was not detected because it was destroyed during acid hydrolysis of algal protein (Dam *et al.*, 2002). Regarding, the results of nucleic acids determination (Fig. 8), *Scenedesmus dimorphus* cells grown on Bold's basal medium contained higher concentrations of nucleic acids than that of cells grown on 0.4% poultry manure at both 22°C and 120°C. From the previously given results, the nucleic acids per cent in relation to the algal dry weight (Fig. 9) was found to be of minimum value in the algal cells grown on 0.4% poultry manure (regardless higher concentration which produce lower algal growth) water extract prepared at 22°C.

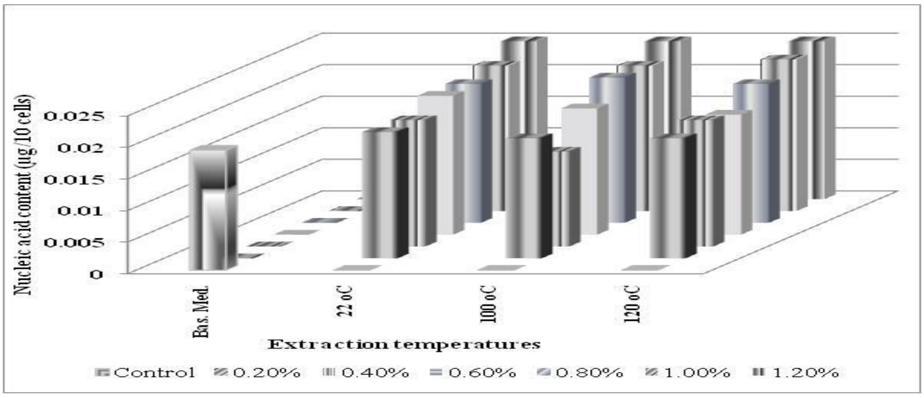

Figure (8): Nucleic acids content of *Scenedesmus dimorphus* grown on Bold's basal medium and different concentration of poultry manure extracted at different temperatures

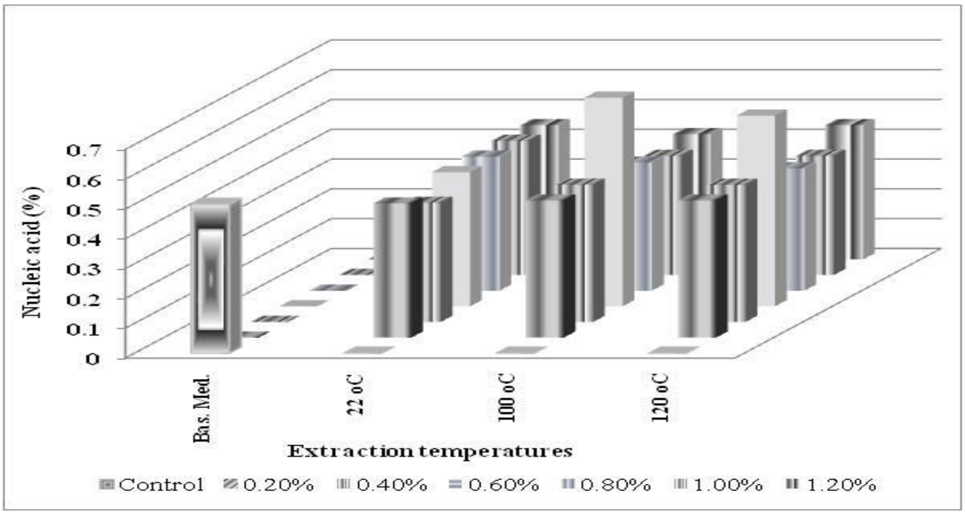

Figure (9): Nucleic acids percent of *Scenedesmus dimorphus* grown on Bold's basal medium and different concentration of poultry manure extracted at different temperatures

A constant worry in the utilization of microbial cells as food or feed may be refered to their high nucleic acids content. However, algae which were used as unicellular protein (such as *Chlorella*, *Spirullina* and *Scenedesmus*) contain definitely lower total nucleic acids contents than bacteria and yeasts (Wang *et al*., 2005). In accordance with this, Data reveled that *Scenedesmus dimorphus* cells grown on poultry manure extracts contained 0.22% - 34% nucleic acids while that grown on B.B.M. contained 0.42% on dry weight basis. Considering that *Scenedesmus* has autotrophic, mixotrophic and heterotrophic modes of nutrition (Gonzalez *et al*., 2001), the results (Table 4) indicated that *Scenedesmus dimorphus* absorbed about 82% of the free amino acids and about 15% of water soluble carbohydrates.

Table (4): Utilization of some constituents of poultry manure by *Scenedesmus dimorphus*

Constitutes	Control medium	Inoculated medium	Consumption%
Final pH	**7.96**	**8.95**	**--**
Number of cells x 10^6/ml	**--**	**12.02**	**--**
Total soluble carbohydrates (p.p.m)	**40.2**	**34**	**15.4**
Nitrate (p.p.m)	**5.7**	**4.9**	**14.0**
Free ammonia (p.p.m)	**4.8**	**3.1**	**35.4**
Free amino acids (p.p.m)	**10.5**	**1.9**	**81.9**

Also the results indicated that *Scenedesmus dimorphus* absorbed about 35% of free ammonia and about 14% of nitrate. This result coincided with that reported by Molina *et al.,* (2007) where they observed that there is general nutritional performance among fresh-water microalgae for ammonia followed by nitrate.

$$\textbf{Chemical score} = \frac{\textbf{\% Sulphur amino acids in sample protein}}{\textbf{\% Sulphur amino acids in FAO reference protein}} \times 100$$

In conclusion, the present study showed that poultry manure can be used as a potential growth medium for mass production of highly nutritive *Scenedesmus dimorphus* as a good source of protein.

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انـتـاج طحلب ا لسندزمــس على بعض ا لمخلــفات ا لمستخدمة فى المزارع السمكية

ترتيل السيد محمد بدوى

المعمل المركزى لبحوث الثروة السمكية بالعباسة – قسم بحوث البيئة والبيولوجى – مركز البحوث الزراعية - وزارة الزراعة – الدقى – مصر.

يتميز طحلب السندزمس بقيمة غذائية لما يحتويه من نسبة عالية من البروتين وكذ لك نسبة من الدهون والكربوهيدرات وبعض العناصر المعد نية وتعتبر أحد أهم فوائده استخدامه كغذاء متكامل للمراحل الاولى للاسماك ويتم استخدام العلائق المكونة من الطحالب الدقيقة فى الاستزراع السمكى فى الوقت الحالى بصورة أساسية فى انتاج اليرقات والاصبعيات كما تستخدم أيضا فى تنمية الاحتياجات المطلوبة من الهائمات الحيوانية اللازمة لتغذية اليرقات. وتهدف هذه الدراسة الى امكانية تنمية طحلب السندزمس على بعض المخلفات التى تستخدم فى تسميد أحواض المزارع السمكية (زرق الدواجن) كبيئة غير تقليدية وغير مكلفة لتحقيق أعلى معدل انتاج لخلايا الطحلب بأقل التكاليف بحيث تحتوى الخلايا الناتجة على أعلى نسبة من البروتين وايضا تقليل المشاكل التى تنتج من تسميد أحواض المزارع السمكية بزرق الدواجن0 قد تم فى هذا البحث أستخدام البيئة الصناعية بولدز بازل (B.B.M.) بالاضافة الى أستخدام مستخلص من هذا المخلف ووجد ان الطريقة المثلى لاستخلاصه هى نقعه فى الماء لمدة عشرون دقيقة عند درجة حرارة 22°م ، وعند تحليل مخلف الدواجن وجد انه يحتوى على مواد وعناصر غذائية بتركيزات كافية لنمو خلايا الطحلب عليها وأظهرت النتائج ان خلايا طحلب السندمس تستهلك من مستخلص هذا المخلف تقريبا 15.5% كربوهيدرات ، 14% من النترات ، 35% من الامونيا ، 82% من الاحماض الامينية وتحتوى الخلايا على نسبة من الاحماض النووية لاتتعدى النسبة الموجودة فى ا لخلايا النامية على البيئة الصناعية ، وقد اتضح ايضا من هذه الدراسة ان أعلى معدل لنمو الخلايا الطحلبية على مخلف زرق الدواجن تحقق فى اليوم الرابع وان مرحلة الكمون للطحلب المستزرع على المخلف تمتد لفترة أطول منها للطحلب المستزرع على البيئة الصناعية.