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The use of vermicompost in cultivation and production of *Spirulina platensis*

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Abstracts

The cyanobacterium *Spirulina platensis* is quite spread all over the world due to its high nutritional value. It has been used as a protein source and vitamin supplement in healthy diet, in aquaculture and fisheries. Cyanobacterium *Spirulina* is capable to grow in various kinds of culture media.

Furthermore, decomposed organic and inorganic nutrients media have been proven to be a good source of culture medium for *Spirulina* by many researchers. In this work, *S. platensis* was cultivated using dry vermicompost from fish sludge (VCFS) or cow manure (VCCM). 100 g of dry vermicompost from each source (VCFS / VCCM) collected and compared with Aiba and Ogawa liquid medium which was used as a culture medium. The results obtained showed that the highest protein and "chlorophyll a" ratio of *S. platensis* were observed in the biomass harvested from T2 (VCCM) and T3 (Aiba and Ogawa liquid medium). The highest dried *S. platensis* biomass was harvested from T2 (VCCM) while the lowest came from T1 (VCFS). The chemical composition (as % of dry weight) revealed that (VCCM) was higher in total phosphorus, total nitrogen and organic matter than (VCFS).

Keywords: *Spirulina platensis*, vermicompost.

INTRODUCTION

The quality of aquaculture waste materials are highly variable source of metabolic products and food wastes (**Beveridge *et al.* 1992**). Solid wastes represent a significant proportion of the applied feed**. Schuenhoff *et al.*, (2003)** showed that more than 80 % of the feed nitrogen content and more than 75 % of the phosphorus content are excreted in the water in both dissolved and particulate forms. Compost made from fish manure (sludge) could provide an good source of nutrient-rich organic matter. Instead of creating a disposal problem, vermicomposting these organic wastes can create a useful and potentially marketable product. Vermicomposting has been suggested and demonstrated as practical treatment method for fish processing wastes and mortalities (**Frederick, 1991; Liao *et al.*, 1993**). Earthworm is suggested as an effective mean to convert the fish solid wastes into vermicompost. They can also consume sludge from the cardboard industry; recycling plant (**Vermitech, 2004**) Fish; blood wastes; animal mortalities and Pre-composted food wastes (**Frederickson *et al.*, 1997**).

Worm castings/ compost contain up to 5 times the plant available nutrients found in average potting soil mixes. Chemical analysis of the castings was conducted (**Ruz-Jerez *et al.*, 1992; Parkin and Berry 1994**) and showed that it contains 5 times the available nitrogen, 7 times the available potash and 1.5 times calcium more than that found in 15 cm of good top soil.

Spirulina is a planktonic photosynthetic filamentous cyanobacterium that forms massive populations in tropical and subtropical bodies of water which have high levels of carbonate, bicarbonate and alkaline pH values of up to 11.

Spirulina from Chad Lake in Africa and Texcoco Lake in Mexico have been harvested as a source of food for fish **(Vonshak, 1997)**. *Spirulina* has been studied for single cell protein (SPC) **(Anupama, 2000),** vitamins, minerals, proteins and polyunsaturated fatty acids (gammalinolenic acid) **(Miranda *et al.*, 1998),** therapeutic properties **(Belay *et al.*, 1993),** and antioxidant activity **(Estrada *et al.*, 2001).** Several cultivation methods like; open ponds **(Lee YK, 1997),** tubular photobioreactors **(Torzillo *et al.*, 1986)**, inclined glass panels **(Hu Q, *et al.*, 1996)** have been tried.

Cost and composition of cultivation media along with growth rate of the algae were challenging factors for commercially viable production. Different media have been tried for cultivation of spirulina such as Zarrouk's media **(Zarrouk, C. 1966**), **Aiba and Ogawa, 1977**, Bangladesh medium (**Khatum *et al.*, 1994),** CFTIR media **(Venkataraman *et al.*, 1995**), Rao's media **(Singh, S. 2006**), OFERR media **(Singh, S. 2006**) and Revised media (6) **Raoof *et al.*, 2006**.

Spirulina platensis alga is rich in protein and vitamins, and can be used to improve the immunity capacity of both human and the animals which consume it. When *Spirulina* alga is used as feed for young prawns and fingerlings, the fish exhibit good coloring, as well as reducing mortality rate and increasing growth rate **(Sermwattanakul & Bamrungtham, 2000).** Therefore, one of the possible acceptable way to utilize vermicompost is in the production of microalgae.

The conventional nitrogen source for *S. platensis* is nitrate. However, recent researches have evaluated the potential of using animal wastes as a low-cost nitrogen source **(Gantar *et al.*, 1991 & Olguin *et al.*, 2001 and Thepparath *et al.*, 2009).** The present study aims to investigate the potentiality of two different sources for vermicompost production, which are fish sludge (VCFS) and cow manure (VCCM) in the production of *S. platensis*, in comparison with traditional media (modified Aiba and Ogawa, 1977 liquid medium).

Materials and Methods

Vermicompost production:

The Fish bio-solid wastes were collected from the tilapia brood-stock cement tanks, at the Nile Tilapia hatchery of the Central Laboratory for Aquaculture Research (CLAR). It was spread in a thin layer and left to dry for 5days and stored until being used in earthworm feeding and vemicompost production. Cow manure was provided by a farmer nearby the CLAR and introduced fresh to the earthworms. Three different species of composting earthworm, *Eisenia fetida*, *Perionyx excavatus*, and *Lumbricus rubellus*, were used in mixed population to produce vermicopmost in stereo-foam boxes with diminsions of 60 X 40 X 30 cm at a density of 150 g worms / box.

The fish sludge was re-moistened before introducing to the earthworm. Each of organic wastes, fish sludge and cow manure was introduced separately to the production boxes at a rate of half worm biomass weight daily for 6 day a week.

After getting the bottom of the box covered with vermicompost and organic wastes, after about 2 months, the vermicompost was harvested and collected, each kind of vermicompost separately, vermicompost fish sludge (VCFS); vermicompost cow manure (VCCM), in other boxes and left for one month to get cocoons hatched and young worms collected, and for more maturation of vermicompost.

CULTIVATION AND PRODUCTION OF SPIRULINA:

Spirulina platensis was obtained from phytoplankton lab belonged to limnology department - CLAR (Central Lab. for Aquaculture Research). The culture was routinely maintained in modified **Aiba and Ogawa, 1977** liquid medium.

Culture medium:

In-door cultivation and production of *Spirulina* using vermicompost (from fish sludge or cow manure) and Aiba and Ogawa liquid medium:

The culture medium used was 100 g of dry vermicompost from each source (VC). The vermicompost was suspended in 10 l of aerated tap water for 2 days before being used as a vermicopmost. Sodium metabisulfite (50 mg / 10 l media) was added to prevent microbial contamination. After 24 h, sodium bicarbonate 850 g / 10 l media was added before the beginning of the experiment (**Thepparath *et al*., 2009**).

The experiment started by inoculation (10 ml / l) from the *Spirulina platensis* that cultivated before in Aiba and Ogawa liquid.

Experimental design:

Three treatments each of 3 replicates in glass aquaria (30 x 30 x 30 cm) as follow:

1- T1: VCFS vermicompost from the fish sludge of ponds in the rate of 100 g VCFS / 10 l tap water.

2- T2: VCCM vermicompost from manure in the rate of 100 g VCCM from cow manure / 10 l tap water.

3- T3: Inoculated alga in 10 l of modified **Aiba and Ogawa, 1977** liquid medium (standard media SM).

Table 1. Aiba and Ogawa, 1977 medium (standard medium, SM).

Constituents	Composition (g / l)
$NaHCO_3$	13.61
$NaNO_3$	2.50
$K2HPO_4$	0.5
K_2SO_4	1.0
NaCl	1.0
$CaCl_2.2H_2O$	0.04
Na_2EDTA	0.08
$MgSO_4.7H_2O$	0.2
$FeSO_4.7H_2O$	0.01
Trace metal sol.*	5 ml / l

*Trace metal solution consists of: EDTA (Disodium salt) 0.8 g / l, $FeSO_4.7H_2O$ 0.7 g / l , $ZnSO_4.7H_2O$ 1 mg / l, $MnSO_4.7H_2O$ 2 mg / l, H_3BO_3 10 mg / l , Co (NO_3) $2.6H_2O$ 1 mg / l, $Na_2MoO_4.2H_2O$ 1 mg / l and $CuSO_4.5H_2O$ 0.005 mg / l.
NP: evaporated tab water was compensated every 24 h.

The experiment was carried out in phytoplankton lab (photo-period 12 / 12, (3000 – 5000 lux), in appropriate temperature (30 ± 2 °C) and pH (8.5 – 10.5). Each culture was agitated by aeration.

In stationary phase (after 15 days from the *Spirulina platensis* inoculation) the algal culture reached maximum growth, in this phase the circulation provided by the pumping system was stopped and the algal cells harvested by filtrated the glass aquaria using a piece of cloth of nylon (its mesh 16µ). The algal cells (Cyanophyta) were dried in oven at 60 ºC and ground in an electric coffee mill to form algal powder.

Analytical methods:

Spirulina platensis powder obtained from the different three sources were analyzed for the following aspects:

1- Chlorophyll a was determined by measuring absorbance at 665 and 750 nm. (**APHA, 1989**).
2- The biomass dry weight was determined by the filtration of 50 ml sample through Whatman filter paper No.4 after washing with distilled water. The biomass obtained was then washed twice by distilled water (d.w.) and dried at 80ºC for 4 h according to (**Olguin *et al*., 2001**).
3- Dry matter (DM), organic matter (OM), crude protein (CP), crude fiber (CF), and ether extract (EE) were determined according to **A.O.A.C. (1995)**.

Statistical analysis

Statistical analysis was applied according to **Steel and Torrie (1980)**, data were analyzed using the GLM procedure with one way analysis of variance **(SAS, 2002),** Differences between means were tested for significance according to Duncan's multiple rang test **(Duncan, 1955)**.

Results and Discussion

Table (2) revealing that cow manure vermicompost was higher in total phosphorus, total nitrogen and organic matter than fish sludge vermicompost. Phosphorus is an element required for microalgae growth, especially for generating and transforming metabolic energy (**Sun and Wang 2009**) Phosphorus is an essential nutrient that constitutes cells, nucleotides and nucleic acids **(Correll, 1999).**

Table (2). The chemical composition (% dry weight) of vermicompost from fish sludge (VCFS) and from cow manure (VCCM):

	VCFS	VCCM
OM %	27.52	52.96

TP %	0.227	0.625
TN %	1.485	2.755

Nitrogen is required for the synthesis of amino acids which make up proteins and other cellular components.

Table (3). The chlorophyll a (µg/l) concentration in *Spirulina platensis* that cultivated by using different media: (T1) VC FS , (T2) VCCM and (T3) SM standard media

Treatments	3 day	6 day	9 day	12 day	15 day
T1(VCFS)	$3.89^{ab} \pm 0.3$	$12^{a} \pm 0.4$	$30.6^{a} \pm 0.4$	$30.74^{b} \pm 0.2$	$32.9^{b} \pm 0.3$
T2(VCCM)	$4.2^{a} \pm 0.9$	$7.06^{b} \pm 0.8$	$17.9^{c} \pm 0.8$	$34.2^{ab} \pm 0.6$	$46.9^{a} \pm 0.8$
T3 (SM)	$3.94^{ab} \pm 0.8$	$6.81^{c} \pm 0.3$	$15.04^{cd} \pm 1$	$39.6^{a} \pm 0.2$	$47^{a} \pm 0.8$

Means ± se in column with different superscripts are statistically different at significant level of 0.05 when compared.

Table (3) showing that, T1 had the highest chlorophyll "a" concentration after 6 and 9 days, while after 12 days till the end of the experiment; T3 and T2 had higher chlorophyll "a" concentration than T1. Results agree with **(Piorreck *et al.*, 1984)** who reported that lower concentrations of chlorophyll "a" were obtained from cultivations that obtained from limited nitrogen sources.

Lower chlorophyll a content in VCFS can also be attributed, partially, to the lower phosphorus content, this element is necessary for many processes in plants and algae, such as photosynthesis and metabolisms. **Navarro *et al.*, (2008) stated that** phosphorus takes part in many metabolic processes, such as signal transduction, energy conversion and photosynthesis**.**

Table (4). Chemical composition (% dry weight) of *S. platensis* grown on different media (T1,T2 and T3)

Treatments	Protein%	Fiber %	Lipid %
T1 (VCFS)	$31.25^{c} \pm 0.004$	$8.78^{c} \pm 0.02$	$19.62^{a} \pm 0.07$
T2 (VCCM)	$66.31^{a} \pm 0.005$	$14.96^{a} \pm 0.06$	$5.21^{c} \pm 0.01$
T3 (SM)	$61.04^{b} \pm 0.003$	$13.77^{b} \pm 0.04$	$17.66^{b} \pm 0.02$

Means ± se in column with different superscripts are statistically different at significant level of 0.05 when compared.

As shown in Table (4); the significantly highest protein % content was recorded in T2 (66.31 %) while the lowest was obtained in T1 (31.25 %). The increased protein content was apparently due to the increased nitrogen level in the medium where the total nitrogen in vermicompost of cow manure was 2.755 % while that of fish sludge vermicompost was 1.485 %. The present results are matching with the founds of **Piorreck *et al.*, 1984** who reported that increasing the nitrogen level in the nutrient medium leads to a corresponding increase in the biomass and protein content of *Spirulina*. On the other hand,**Theodorou *et al.* (1991)** reported that soluble protein content in the Green Alga *Selenastrum minutum* was decreased under phosphorus limitation, which explains the lower protein content in S. *platensis* cultivated in VCFS. They suggested that this decrease may be due to the hypothesis that the synthesis of nonessential proteins could be repressed during phosphorus limitation because the enzymes that are responsible for protein synthesis affected by phosphorus concentration **(Theodorou *et al.*,1991)**. **Mutlu *et al.* (2011)** noted that protein level in *C. vulgaris* decreased from 50.8 % to 38.16 % during phosphate starvation. As shown in Table (4) the highest lipids percentage was in T1 (19.62 %) while the lowest was in T2 (5.21 %), which could be attributed to the decrease in TN concentration in T1 which resulted in

a significant change in cell composition, favoring the accumulation of lipid components and decrease protein content in *S. platensis* during the growth. our results agree with **(Leyla *et al.*,2011)** who found that *Spirulina* biomass harvested at the stationary phase, 67.4, 53.5, 5.6 % protein and 5.78, ١٣,٦٦ % and ١٧,٠٥ lipid were recorded for the groups of control (SM), 50 % N deficiencies and 100 % N deficiencies, respectively. The highest lipid content were recorded from the culture to which treated 100 % N deficiencies. **Tedesco and Duerr (1989)** also indicated that, lack of N in *S. platensis* culture medium increased total lipid ratio.

As illustrated in Fig (1), the highest biomass was in T2 and the lowest was in T1. In mass cultures of microalgae, nutrition condition is one of the key factors that control their growth and productivity (**Vonshak and Richmond, 1988; Faintuch *et al.*, 1991)**. Among the nutrients required for the growth of algae, phosphorus is considered as an important element which plays an essential role in maintaining high production rates of microalgae **(Mostert and Grobbelaar, 1987)**, which agrees with the present study results. **Fried *et al*. (2003**) concluded that both nitrogen and phosphates enhanced algal biomass. They affect on algal growth autonomous of each other and there is no interaction between them.

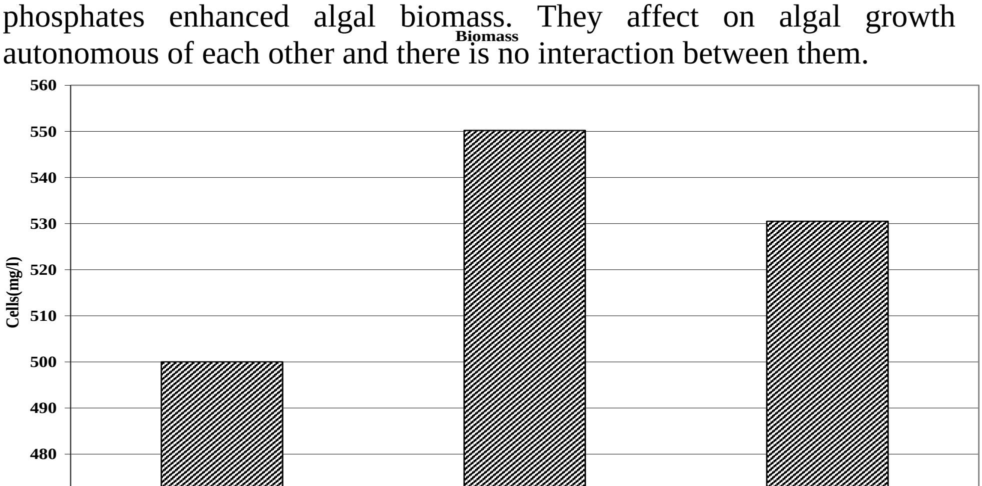

Fig. (1): biomass of *Spirulina platensis* cells (mg / l) at the end of growth in the three treatments

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إستخدام الفيرمكومبوست فى زراعة وإنتاج طحلب الإسبيرولينا بلاتينسيس

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الملخص العربي

البكتيريا الخضراء المزرقه (إسبيرولينا بلاتينسيس) شائعة في العالم بسبب محتواها الغذائي العالي. وقد استخدمت كمكملات للبروتين والفيتامينات في صناعة الغذاء، المستخدم في أنظمة تربية الأحياء المائية ومصائد الأسماك، لكونها قادرة على النمو في أنواع مختلفة من الأوساط الغذائية. علاوة على ذلك، فقد أثبتت المغذيات العضوية وغير العضوية المتحللة أنها مصدر جيد كوسط غذائي لزراعة الإسبيرولينا من قبل العديد من الباحثين. في هذه الدراسة، تمت زراعة الإسبيرولينا بلاتينسيس بإستخدام ثلاث معاملات, المعاملة الأولى تم أستخدام الفيرمكومبوست الناتج من مخلفات الأحواض السمكية وفي المعاملة الثانية تم أستخدام الفيرمكومبوست الناتج من مخلفات البقر بمعدل ١٠٠ جرام/ ١٠ لتر من ماء الصنبور لكلا المعاملتان وفى المعاملة الثالثة تم إستخدام ميديا (**Aiba and Ogawa, 1977**) التقليدية فى ١٠ لتر من ماء الصنبور لمقارنة نمو طحلب الأسبيرولينا مع نمو الطحلب بإستخدام الفيرمكومبوست وذلك تم بداخل أحواض زجاجية فى ثلاث مكررات لكل معاملة. وقد بينت النتائج المتحصل عليها في نهاية الدراسة أن أعلى نسبة من البروتين والكلوروفيل "أ" كانت من الإسبيرولينا بلاتينسيس الجافة الناتجة من المعاملة الثانية و الثالثة. تم حصاد أعلى كتلة حيوية جافة من المعاملة الثانية في حين أن أقلها تم الحصول عليه من المعاملة الأولى. يوضح التركيب الكيميائي (٪ الوزن الجاف) للفيرميكومبوست بنوعيه أن الفيرميكومبوست الناتج من مخلفات البقر كان أعلى في الفوسفور الكلي و النيتروجين الكلي والمواد العضوية من الفيرميكومبوست الناتج من مخلفات الأحواض السمكية.