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ADVANCED WASTEWATER TREATMENT USING MICROALGAE IN NILE TILAPIA (*OREOCHROMIS NILOTICUS*) AQUACULTURE.

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Abstract

Removal of dissolved inorganic nutrients and heavy metals from wastewater effluent using microalgae is a subject of great interest due to its polytonal stress in aquatic ecosystems. Advanced treatment (tertiary treatment) of wastewater includes the removal of dissolved inorganic nutrients and heavy metals from secondary treated. So, *Chlorella vulgaris* and *Scenedesmus bijuga* microalgae in this study were incubated for six days in secondary treated domestic sewage in an outdoor cultivation tanks.

The results revealed that a complete depletion of ammonium and orthophosphate nutrients occurred with both algae, whereas nitrate uptake rate was 84% in *C. vulgaris* and 57.7% in *S. bijuga*. Mineral elements (Magnesium, Potassium and Sodium) nearly had the same uptake rate for both algae. Uptake rates of heavy metals (Fe, Zn, Cu, Pb & Ni) in *Chlorella* experimental tank was higher than that of *Scenedesmus* tank. On contrary, Mn heavy metal uptake rate by *Chlorella* was lower (80%) than its uptake rate (100%) by *Scenedesmus*.

Further study on using of sewage grown *Chlorella* and *Scenedesmus* with high protein content 46.5% & 52.7%, respectively in fish feeding was conducted. There were no great differences in weight gain and specific growth rate of the experimented tilapia fishes at different nutritional regime. The highest protein content (67.5%) occurred in fishes fed with *Scenedesmus* biomass grown in sewage and supplemented with 2% artificial fish food regime.

The results indicated that, cultivation of *Chlorella vulgaris* and *Scenedesmus bijuga* in secondary treated sewage effluent can be used as an advanced sewage treatment to remove inorganic N, reduce pollution problems, and to produce algal protein useful in fish feeding.

INTRODUCTION

The practical use of wastewater for various types of aquaculture is a subject of increasing interest. Wastewater aquaculture has the multiple benefits of producing potentially valuable materials, while, re-using resources and treating wastes, result in overall greater protection of the environment at lower cost (Wilde and Benemann 1993). Public health and safety concerns have traditionally been the main reasons for resisting wastewater reuse for fish farming. Potential adverse health effects in such applications could be avoided if the waste is sufficiently treated before reuse (Easa *et al.*, 1995).

There has been well-documented evidence that inorganic N and P are key nutrients causing eutrophication in aquatic environments (Oswald *et al.*, 1957; Ryther *et al.*, 1975; Gordon *et al.*, 1982). The levels of inorganic N and P are still relatively high in secondary treated sewage effluent (Environment Protection Department, 1987). In order to reduce this type of pollution from the secondary treated sewage effluent, algae have been used in the tertiary wastewater treatment of domestic sewage to remove the two elements N and P owing to their abilities to utilize them (Chan *et al.*, 1982).

Tam *et al.* (1994) suggested that immobilized *Chlorella vulgaris* cells could be used as secondary treatment process for domestic sewage. Also, *Chlorella salina* was successfully cultivated in secondarily treated domestic sewage effluent of high salinity (14 ppt) in an outdoor cultivation tank. Removal efficiencies of NH_4^+-N, NO_3^--N. PO_4^{3}-P by this alga from secondary treated sewage effluent were 89-100%. 35-66% and 100%, respectively. The high removal efficiencies of inorganic N and P means that this process can be used as a tertiary sewage treatment (Wong and Chan, 1990). In addition, sewage-grown algae have been considered as a potential protein source, which can be used as animal or fish food (Gordon *et al.*, 1982).

Chevalier and de la Noue (1985) established a wastewater treatment system with the help of *Scenedesmus* immobilized in kappa-carrageenan gel, in form of 3-mm beads. Immobilization appears to be one of the best techniques to separate physically micro-algal cells from their culture medium for the purpose of algal tertiary wastewater treatment.

The growth in a microalgal culture is normally described by biomass increase expressed as cell number, dry weight, chlorophyll or protein content. The latter two parameters to express the biomass content are more reliable and a lot more precise because chlorophyll is one of the major pigments responsible for the algal coloration and it is present at all growth (Fresnedo & Serra, 1992).

The starvation approach was devised to deplete more quickly the dissolved nutrients in wastewater, especially ammonium and orthophosphate ions, which are the major causes of microalgal and aquatic plant blooms. With mass algal cultivation in open ponds, NH_4-N or PO_4-P removal usually take three to six days to obtain over 90% of efficiency depending on the concentration and size of the inoculum. In a laboratory study, the green algae *Chlorella vulgaris* and *Scenedesmus bijuga* were selected, because of their high growth rate, for cultivation in secondary treated sewage effluent (Niklas, 1994). Uptake of inorganic N and P from sewage effluent by these algae is extremely efficient under controlled environmental conditions (Talbot & de la Noue, 1993).

The present communication reports the detailed results of the outdoor cultivation of *C. vulgaris* and *S. bijuga* in secondary treated domestic sewage effluent, at Central Lab. for Aquaculture Research (CLAR) at Abbassa, Abou-Hammad, Sharkia, Egypt. The removal efficiencies of inorganic N and P by these algae from secondarily treated sewage effluent as a tertiary sewage treatment and the use of these sewage-grown algae biomass as a live food for the monosex Nile tilapia (*Oreochromis nilotica*), which is an economically important fish cultivated in Egypt were evaluated in this study.

MATERIALS AND METHODS

The two algae *Chlorella vulgaris* and *Scenedesmus bijuga* were isolated and cultivated on sterile nutrient solution using of Bold's basal medium (BBM) as described by Bischoff and Bold (1963). Sterilized medium (121 °C, 15 min) was inoculated at 25 °C 2 and 14/10 light-dark cycle (5000 lux by fluorescent lamps) for culturing of algal organisms. The sewage effluent was obtained from the sewage at Central Lab. for Aquaculture Research (CLAR) at Abbassa, Abou-Hammad, Sharkia, Egypt. Before using sewage effluent for cultivation experiments, it was filtered by a series of two Aqua-pure dirt/rust filters (APCT AP117) to remove most of the suspended particles. The inorganic nitrogen (NH^{4+}-N and NH^{3+}-N) and pH were measured by Hach comparison apparatus (American Public Health Association, APHA, 1985). The conductivity meter YSI model 33 S.C.T. Meter was used for measuring salinity. The standard methods (APHA, 1985) was used for measuring available phosphorus and nitrate-N.

The removal efficiency of inorganic N and P were determined by (Chan *et al.*, 1979). The dry weight of algal cells and Chlorophyll "a" content were measured as shown as in Boyd (1984). The cell numbering was done by means of a haemacytometer cell.

Crude protein, crude fat and carbohydrate of algae biomass and fish tissues were determined according to APHA (1985). Ash content was measured according to Wong and Chan (1980), after drying at 110 °C.

Minerals and heavy metals of the secondary sewage water cultures were determined by atomic absorption spectrophotometer (Wong, 1979). The glass aquaria (80 x 40 x 50 cm) were filled with fresh water, approximately 100 litre for feeding experiment. The air stones was used for providing the aeration from pumps. The water temperature was kept between 22-28 °C. The monosex Nile tilapia (*Oreochromis nilotica*) fish fry with an average body weight of about (0.4 g) were provided from a fish hatchery. Each aquarium contained 30 fishes, and each treatment and control were in triplicates. Each group of fish was fed on one of the following 5 diets; 1-AF (by using 5% Artificial fish food, a commercial feed which contained 25% protein as control), 2-CF (by using living *C. vulgaris* cells cultured in secondary treated sewage effluent water at concentration of 1×10^5 cells ml^{-1}), 3-SF (by using living *S. bijuga* cells cultured in secondary treated sewage effluent water at concentration 1×10^5 cells ml^{-1}) 4-CAF (by using living *C. vulgaris* cells cultured in secondary treated sewage effluent water 1×10^5 cells ml^{-1} with 2% artificial food (AF)); 5-SAF (by using living *S. bijuga* cells cultured in secondarily treated sewage effluent water 1×10^5 cells ml^{-1} with 2% AF). Growth of the experimental fishes was monitored by measuring the total body weight of each fish after a period of 90 days.

Statistical analysis

SAS (statistical Analysis System) computer software and IBM 3033 computer was used for data analysis. Specific growth rate (SGR) was estimated according to Degani and Viola (1987) as follows:

$$SGR = \frac{\text{Ln Wt} - \text{Ln Wo}}{T} \times 100$$

Where:

Wo = weight (g) at the beginning of the experimental period.

Wt = weight (g) at the end of the experimental period.

T = time (days)

Ln = Anti-log.

RESULTS AND DISCUSSION

Table 1 shows the inorganic N and P, pH and salinity of the sewage effluent. The mean value of total inorganic N (NH_4^+-N + NO_3^--N) was 4.39 PPM, while, the level of inorganic P (PO_4^{3-}-P) was 0.6 PPM.

The removal efficiencies of NH_4^+-N by the two microalgae were about 97 - 100%, while, the removal efficiencies of NO_3^--N by *C. vulgaris* and *S. bijuga* were comparatively higher (78-83% 57.7-72.7%) respectively, (Table 2). The same removal efficiencies for the PO_4^{3-}-P by the two algae were about 100%. The removal efficiencies of nitrogen from sewage effluent by marine phytoplankton alone or in combination with aquaculture have been reported to be 22- 96% (Gordon and Chapman, 1977). The removal efficiencies of phosphorus was 50-90% (Bogan *et al.*, 1960, Gordon and Chapman, 1977).

Table 3 shows the yield of the two microalgae in the outdoor cultivation tank. The cell density of *C. vulgaris* and *S. bijuga* were about 5.8 x 10^6 & 5.11x 10^6 cells ml^{-1}, respectively, while, the chlorophyll "a" contents were 4.71 & 4.85 mg l^{-1} and the dry weights were 0.7 & 0.8 g l^{-1}, respectively for the same algae. Therefore, the cultivation of *C. vulgaris* and *S. bijuga* can be used as tertiary treatment process in secondary sewage treatment. Tam *et al.* (1994) suggested that immobilized Chlorella cells can be used as secondary treatment process for domestic sewage.

The chemical composition for *C. vulgaris* and *S. bijuga* are tabulated in Table 4. The crude protein contents were 46.48 & 52.66 g/100g dry algae for *C. vulgaris* and *S. bijuga*, respectively (Young and Scrimanshaw, 1975), whereas, the crude fat contents were 15.3% for *C. vulgaris* and 13.39% for *S. bijuga*. The total carbohydrate contents of *C. vulgaris* and *S. bijuga* were 10.1 and 9.2%, respectively. A substantial fraction of the carbohydrate of the two algae was incorporated in the cell wall polysaccharides which were often poorly digestible, so that the carbohydrate digestibility of two algae may be moderate or low (Schulz & Oslage, 1976). The ash % of the *C. vulgaris* was 19.12% and for *S. bijuga* was 16.75% in this study which represented lower quantities of inorganic matter (ash as mentioned by Milamena *et al.* 1990). The crude fiber of *C. vulgaris* was 9.16%, while, for *S. bijuga* was 7.7% which were high values of fiber content. These values were mentioned by (Millamena *et al.*, 1990) who found that *C. vulgaris* had the highest fiber content which may be attributed to its cellulosic cell wall.

Table (5) provide the levels of minerals and heavy metals of sewage effluent before and after cultivation with *C. vulgaris* and *S. bijuga*. The levels of Magnesium, Potassium, Sodium and Iron were 23.9, 13.91, 228.2 & 0.20 mg l^{-1} before cultivation, which were reduced by 47.7%, 34.5%, 39.8% & 15.0% after cultivation with *C. vulgaris*, respectively, while, they were reduced to 50.2%, 32.4%, 35.1% & 50.0% after treatment with *S. bijuga*. The levels of heavy metals including Zinc, Copper, Lead, Manganese and Nickel in the secondary sewage effluent were 0.23, 0.333, 0.288, 0.05 & 0.13 mg l^{-1} before cultivation, which were reduced by 26.1%, 48.0%, 27.8%, 20.0% & 53.8% after cultivation with *C. vulgaris* respectively, while they were reduced to 47.8%, 54.1%, 31.3%, 0.0 & 61.5% after treatment with *S. bijuga*. respectively. The relatively low metal content enables the use of the algal biomass as a supplement for animal feed (Wong and Chan, 1990).

Table 6 shows that the uptake rates of iron and zing by the *C. vulgaris* were 85% and 73.9%, respectively, which are higher than the uptake rates, by the *S. bijuga* giving 50 & 52.1%, respectively for the same elements.

The mineral fraction in microalgal cells constitutes a major proportion of the dry weight, ranging from 6 to 39% (Brown *et al.*, 1989). Metals concentrations in cells vary considerably with species and environment (Eisler, 1981).

Results of the feeding experiment are shown in Table 7. The better growth (gain and net daily gain) and better specific growth rate were found in feeding the monosex Nile tilapia with live *C. vulgaris* alga cultivated in secondary sewage effluent water with 2% AF (CAF). Such treatment recorded highest values than those of fish fed the control (artificial fish food, AF) and other diets. Meanwhile, our data are on contrary to those reported by Parazo (1990) and Fowler (1991).This may be due to experimental periods, water temperature, feeding rate and system of fish culture indoors or outdoors.

The biochemical compostion of experimental fish is shown in Table 8. The crude protein % (CP) in fish bodies received live *S. bijuga* alga with 2% AF (67.5%) was higher than that of fish fed other diets but the crude fat % was (19.1%) which is lower than those of the treatments. Dress-out percentage increased slightly as increasing protein level in diets (Lovell *et al.*, 1973). Body fat decreased as dietary protein increased. They added that the probable reason for the lower dressing percentage of the fish on the lower protein diets was the higher amount of fat in abdominal cavity, so, body protein percentage was inversely related to body fat content. These results support the idea that sewage-grown algae can be used as fish food.

Table 1. Inorganic N and P, pH and salinity of the sewage effluent.

Composition	Concentration[1]
NH_4^+-N (PPM)	3.7 1.7
NO_3^--N (PPM)	0.7 0.2
Total inorganic nitrogen[2]	4.4
PO_4^{3-}-P (PPM)	0.6 0.1
pH	7.7 0.3
Salinity (ë)	0.2 0.1

[1] Mean value (standard deviation of 8 measurements.

[2] Total inorganic nitrogen = NH_4^+-N plus NO_3^--N (ppm).

Table 2. Removal efficiencies of inorganic N and P from the sewage effluent by *C. vulgaris* and *S. bijuga* in outdoor cultivation tank.

Inorganic N or P	Level before cultivation (ppm)	Level after cultivation (ppm)		Removal efficiency (%)	
		C.v 1	*S.b* 1	*C.v*	*S.b*
Experiment No. 1					
NH_4^+-N	3.75	ND1	ND	100	100
NO_3^--N	0.317	0.053	0.134	83.3	57.7
Total inorganic N[3]	4.067	0.053	0.134	98.7	96.7
PO_4^{3-}-P	0.515	ND	ND	100	100
Experiment No. 2					
NH_4^+-N	3.69	ND	0.09	100	97.6
NO_3^--N	295	0.065	0.124	78	58
Total inorganic N	3.985	0.065	0.214	98.3	94.6
PO_4^{3-}-P	0.54	ND	ND	100	100
Experiment No. 3					
NH_4^+-N	3.62	0.104	0.114	97.1	96.9
NO_3^--N	0.33	0.07	0.09	78.8	72.7
Total inorganic N	3.95	0.104	0.114	97.4	97.1
PO_4^{3-}-P	0.46	ND	ND	100	100

[1] C.v = C. vulgaris and S.b = S. bijuga

[2] ND = non-detectable

[3] Total inorganic nitrogen = NH_4^+-N plus NO_3^--N (ppm)

Table 3. Final density, chlorophyll "a" content and dry weight of *C. vulgaris* and *S. bijuga* cultivation after 6 day from incubation on sewage effluent.

Species	*C. vulgaris*	*S. bijuga*
Density (x 10^6 cell ml $^{-1}$)	5.8	5.11
Chlorophyll "a" (mg l $^{-1}$)	4.71	4.85
Dry weight (g l $^{-1}$)	0.7	0.8

Initial (0 day): Density x 10^6 cell ml $^{-1}$ = 0.2, chlorophyll "a" (mg l $^{-1}$) = 1.11 and dry weight g l $^{-1}$ = 0.2 for *C. vulgaris* and density x 10^6 cell ml $^{-1}$ = 0.24, chlorophyll "a" (mg l $^{-1}$) = 1.6 and dry weight g l $^{-1}$ = 0.26 for *S. bijuga*.

Table 4. Chemical composition of *C. vulgaris* and *S. Bijuga* cultivated in sewage effluent.

Composition	g per 100 g dry algae	
	C. vulgaris	*S. bijuga*
Moisture %	5.5 ± 0.2	4 .00 ± 1.0
Crude protein (N x 6.25)	46.48 ± 1.1	52.66 ± 1.6
Crude fat	15.3 ± 1.2	13.39 ± 1.3
Carbohydrate	10.1 ± 0.8	9.20 ± 0.6
Ash	19.12 ± 1.9	16.75 ± 2
Crude fiber	9.16 ± 1.0	7.70 ± 0.9

[1] Mean value ± standard deviation.

Table 5. Minerals and heavy metals of sewage effluent before and after cultivation with *C. vulgaris* and *S. bijuga* after 6 day incubation periods.

	Before cultivation	After cultivation			
		C. vulgaris	% of change	*S. bijuga*	% of change
Minerals					
Magnesium	23.900 ± 0.20	11.41 ± 0.4		12.00 ± 0.30	50.2
Potassium	13.910 ± 0.20	4.80 ± 0.10	34.5	4.50 ± 0.10	32.4
Sodium	228.200 ± 45	90.80 ± 20.1	39.8	82.3 ± 0.18	36.1
Iron	0.200 ± 0.04	0.03 ± 0.01	15.0	0.10 ±0.02	50.0
Heavy metals					
Zinc	0.230 ± 0.06	0.06 ± 0.01		0.11 ± 0.02	
Copper	0.333 ± 0.02	0.16 ± 0.01	48.0	0.18 ± 0.03	54.1
Lead	0.288 ± 0.02	0.08 ± 0.02	27.8	0.09 ± 0.01	31.3
Manganese	0.050 ± 0.01	0.01 ± 0.003	20.0	0.0	0.0
Nickel	0.130 ± 0.02	0.07 ± 0.010	53.8	0.08 ± 0.01	61.5

Table 6. Uptake rate of heavy metals from sewage effluent using two microalgae.

	Before incubation	After incubation		Uptake rate %	
		C. vulgaris	*S. bijuga*	*C. vulgaris*	*S. bijuga*
Minerals					
Magnesium	24	11.4	12	52.5	50
Potassium	14	4.8	4.5	65.7	68
Sodium	228	90.8	82.3	60.2	63.9
Iron	0.2	0.03	0.1	85	50
Heavy metals					
Zinc	0.23	0.06	0.11	73.9	52.1
Copper	0.33	0.16	0.18	51.5	45.5
Lead	0.29	0.08	0.09	72.4	69
Manganese	0.05	0.01	ND[1]	80	100
Nickel	0.13	0.07	0.08	46.2	38.5

[1] ND = non-detectable

Table 7. growth performance of experimental fish.

	Individual mean wt (g)					Net daily	Specific
Diet	Initial	SD	Final	SD	Gain[2]	gain (g)[3]	growth rate
AF	0.433	0.10	3.617	0.30	3.184	0.035	2.123
CF	0.413	0.07	4.000	0.20	3.587	0.400	2.520
SF	0.432	0.06	4.230	0.14	3.798	0.042	2.535
CAF	0.424	0.09	4.730	0.19	4.306	0.048	2.680
SAF	0.419	0.08	4.512	0.20	4.093	0.045	2.640

[1] Mean value ± standard deviation of 8 measurements.

[2] Gain = final weight gain - initial weight gain.

[3] Net daily gain = gain / time of experiment.

Table 8. Proximate analysis of whole fish bodies.

Diet	% Ash	% Fat	% protein
AF	11.3 ± 2.0	26.3 ± 2.0	62.26 ± 1.1
CF	12.7 ± 1.0	20.9 ± 1.1	66.10 ± 0.9
SF	13.0 ± 0.8	20.3 ± 1.6	66.40 ± 1.3
CAF	12.9 ± 1.4	19.9 ± 1.2	66.90 ± 1.0
SAF	13.3 ± 1.1	19.1 ± 1.3	67.50 ± 1.2

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إستخدام الطحالب الدقيقة فى المعالجة المتقدمة لمياه المخلفات فى الإستزراع السمكى للبلطى النيلى

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٢ *كلية العلوم جامعة بنها*

إن عملية إزالة المغذيات الغير عضوية الذائبة والمعادن الثقيلة من المخلفات السائلة باستخدام الطحالب الدقيقة هى موضوع ذو أهمية كبيرة نظراً لما تحدثه من تلوث فى الأنظمة البيئية المائية. تتضمن مرحلة المعالجة المتقدمة (المعالجة الثلاثية) للمخلفات السائلة إزالة المغذيات الغير عضوية الذائبة والمعادن الثقيلة من المخلفات السائلة المعالجة ثانوياً. لهذا أجريت دراسة زراعة طحلبى كلوريلا فولجاس والسندسمس بيوجا من الطحالب الدقيقة لمدة ستة أيام فى مخلف مجارى معالج ثانوياً فى آنية زراعية خارج المعمل.

أظهرت النتائج نضوب كامل لكلٍ من أمونيوم والفوسفات مع كل الطحلبين بينما كان معدل استهلاك النترات ٨٤٪ مع كلوريلا و٥٧,٥٪ مع سيندسمس. كما اتضح أن معدل استهلاك العناصر المغضوذية (ماغنسيوم ، بوتاسيوم ، صوديوم) مع كل من الطحلبين كان متقارباً. كان معدل استهلاك المعادن الثقيلة مع كلوريلا اعلى من نظيره مع سيندسمس عنه مع كلوريلا.

ولقد أجريت دراسة لايضاح مدى امكانية الطحالب ذات المحتوى البروتينى المرتفع مثل (كلوريد ٤٦,٥٪ وسيدسمس ٥٢,٧٪) والنامية على مياه المجارى فى تغذية الاسماك. دلت النتائج على عدم وجود فروق فى الوزن ومعدل النمو النوعى لاسماك البلطى النيلى فى النظم الغذائية المختلفة. بينما ارتفع المحتوى البروتينى (٦٧,٥٪) لاسماك البلطى النيلى فى النظام الغذائى المحتوى على طحلب سيندسمس نمى على مياه مجارى مضاف اليه ٢٪ علف صناعى.

دلت النتائج السابقة على أن زراعة كلوريلا وسيندسمس على مياه المجارى المعالج ثانوياً يمكن أن تستخدم كمعالجة متقدمة *للمخلف* وذلك لازالة عناصر التغذية النيتروجينية والفوسفاتية الغير عضوية والتى قد تؤدى زيادتها إلى مشاكل تلوث، كما يمكن استخدامها كمصدر بروتينى فى التغذية السمكية بعد ذلك.