BIOMASS PRODUCTION AND NUTRIENT REMOVAL IN SEMICONTINUOUS CULTURES OF SCENEDESMUS SP. (CHLOROPHYCEAE) IN ARTIFICIAL WASTEWATER, UNDER A SIMULATED DAY-NIGHT CYCLE

D Voltolina, H Gómez-Villa, G Correa

VIE MILIEU, 2004, 54 (1) : 21-25

BIOMASS PRODUCTION AND NUTRIENT REMOVAL IN SEMICONTINUOUS CULTURES OF *SCENEDESMUS* SP. (CHLOROPHYCEAE) IN ARTIFICIAL WASTEWATER, UNDER A SIMULATED DAY-NIGHT CYCLE

D. VOLTOLINA[1], *H. GÓMEZ-VILLA*[1], *G. CORREA*[2]

[1]*Centro de Investigaciones Biológicas del Noroeste, Laboratorio UAS-CIBNOR, P.O. Box 1132, Mazatlán, Sin., Mexico*

[2]*Centro de Investigación Científica y de Educación Superior de Ensenada, Departamento de Acuicultura, P.O. Box 2732, Ensenada, B.C., Mexico*

microalgas@mzt.megared.net.mx; voltolin@cibnor.mx

SCENEDESMUS
WASTEWATER TREATMENT
NUTRIENT REMOVAL
NUTRIENT RECYCLING
BIOMASS PRODUCTION

ABSTRACT. – The possibility of short-term nutrient removal from wastewater with microalgae was evaluated with semicontinuous cultures of *Scenedesmus* sp. in artificial wastewater, with 30, 40 and 50% renewal of the medium every 24 h and a simulated day-night cycle. Organic biomass production ranged from 33.8 to 39.5 $mg \cdot l^{-1}$, with protein contents of 52-54%. Nitrate removal was high (69 to 79%), but the initial concentration of ammonia decreased only by 8 to 13%. The total inorganic nitrogen removed in 24 h varied from 17 to 22%, and 42 to 44% of the amount removed was recycled into new organic biomass. The concentration of phosphorus decreased by between 29 and 43%, with an estimated efficiency of recycling of 35 to 45%. As with nitrogen, removal was related to the availability of light, which favours active uptake and causes photosynthesis – related changes in water chemistry due to the high pH of the medium, with fast ammonia stripping and phosphate precipitation. Given that in outdoor cultures light is discontinuous, short-term nutrient removal does not seem feasible with the solar technology available for tertiary biological treatment.

SCENEDESMUS
TRAITEMENT DES EAUX USÉES
ÉLIMINATION DES SELS NUTRITIFS
RECYCLAGE DES SELS NUTRITIFS
PRODUCTION DE LA BIOMASSE

RÉSUMÉ. – La possibilité d'élimination rapide des sels nutritifs des eaux usées par les micro-algues a été évaluée en effectuant des cultures de *Scenedesmus* sp. semi-continues dans des eaux d'épuration artificielles, en renouvelant le milieu à 30, 40 et 50 % toutes les 24 h et en maintenant un cycle jour-nuit simulé. La production de la biomasse organique a été de 33,8 à 39,5 $mg \cdot l^{-1}$, avec un taux protéinique de 52 à 54 %. L'élimination des nitrates est élevée (69 à 79 %) mais la concentration initiale en ammonium a baissé de 8 à 13 % seulement. L'azote total inorganique est éliminé en 24 h, variant de 17 à 22 %, tandis que 42 à 44 % de cette quantité éliminée sont recyclés en nouvelle biomasse organique. La concentration en phosphore a baissé de 29 à 43 % avec une efficacité estimée du recyclage de 35 à 45 %. Comme pour l'azote, l'élimination est liée à l'éclairement, celui-ci favorisant une assimilation active et provoquant les changements liés à la photosynthèse dans l'eau, en raison d'un pH élevé du milieu, avec une élimination rapide de l'ammonium et la précipitation des phosphates. Du fait que la lumière est discontinue dans les cultures à l'extérieur, l'élimination rapide des sels nutritifs ne semble pas réalisable avec une technique d'insolation dans le traitement tertiaire biologique.

INTRODUCTION

Microalgae mass cultures have been suggested as a convenient strategy for tertiary wastewater treatment, because they are effective in nutrient removal and yield potentially valuable biomass (Oswald & Gotaas 1957, Talbot & De la Noüe 1993). Their efficiency is usually measured in experiments with batch cultures or recirculating bioreactors run for several days (Chevalier & De la Noüe 1985, González *et al*. 1997), which is consistent with the long retention times of wastewater in integrated ponding systems (Abeliovich 1986, Oswald 1988a).

However, because of the large volumes involved, urban wastewater is generally treated to the secondary level in high rate biodigesters with typical turnover times of less than 12 hours (Fahey *et al*. 1975). For operational reasons, these are located close to the city, where tertiary treatment of their effluents for several days would not be feasible, because the real state values of suburban areas are high and space is at premium.

On the other hand, semicontinuous cultures of *Scenedesmus* spp. maintained with 30 to 70% daily renovations of the artificial wastewater used as growth medium, removed between 50 and 70% of its dissolved inorganic nitrogen content in only 24 h (Voltolina *et al*. 1998, Nuñez *et al*. 2001).

This suggested the possibility of achieving nutrient concentrations adequate for direct disposal to the environment with short-term biological treatment, though those results were obtained under continuous light, which caused important ammonia losses due to the sustained high pH of the medium. In this paper we assess the feasibility of short-term treatment with microalgae, using semicontinuous cultures of *Scenedesmus* sp. maintained with different daily dilutions and under a simulated day-night cycle.

MATERIALS AND METHODS

The artificial wastewater (AWW) was prepared with 1-µm filtered tap water, enriched as in previous studies with 11.83, 39.83 and 4.46 mg·l^{-1} of N-NO_3^-, N-NH_4^+ and P-PO_4^{3-}, which were the mean concentrations of the effluent of the secondary treatment plant of the city of Ensenada, Baja California, Mexico, in 1992-1993 (Voltolina *et al*. 1998).

The microalga used was an unidentified species of the chlorophyte *Scenedesmus*, isolated from a hypereutrophic environment close to the same city and maintained as strain SC-X-2 in the culture collection of the Centro de Investigación Científica y de Educación Superior de Ensenada (Trujillo Valle 1993).

The experiments were run in a ventilated climatic chamber. Culture conditions were 14:10 h LD photoperiod and temperatures of 25.5 and 17.0 ºC in daylight and during the night, which are the mean summer values recorded in Ensenada (Correa Reyes 1996). The photon fluence rate, measured during the light period at the center of the empty culture vessels with a QSL-100 quantum scalar irradiance meter (Biospherical Instruments Inc., San Diego, CA, U.S.A.) was 365.5 µmol·m^{-2}·s^{-1}, for a daily total of 18.4 mol·m^{-2}.

The cultures were maintained with continuous air bubbling in three 3.8-l round bottom polycarbonate flasks with 3 l of AWW, harvesting at the end of each dark period 30, 40 or 50% of the culture volume, which was replaced with new medium. When five or six daily optical density readings at 550 nm, backed by occasional direct cell counts with a haemacytometer, showed that the cultures were in steady state, triplicate samples were obtained for five consecutive days from each culture, immediately after dilution and at the end of the light and of the dark period. These were used for dissolved nutrient analysis and to determine the concentration of the organic biomass and its protein content.

Dissolved nitrogen and phosphorus were measured in Whatman GF/C-filtered water samples, using the following reagents and methods (Hach 1997): N-NO_3^-: NitraVer 5, #8171; N-NO_2^-: NitriVer 3, #8507; N-NH_4^+: Nessler, #8038; P-PO_4^{3-}: PhosVer 3, #8048.

Total (organic and acid-hydrolizable) dissolved phosphorus was measured after acid persulfate digestion (method #8190) only for the 30% dilution, because the results were similar to those of reactive phosphates.

The ash-free dry weight of the biomass was obtained after Sorokin (1973), by difference between the total and the inorganic dry weights of samples of known volumes concentrated on precalibrated Whatman GF/C glass fiber filters, dried to constant weight in an oven at 60 ºC and ashed at 450 ºC in a muffle furnace.

Similar samples were used to measure the protein content of the algae biomass with the modified biuret method by Dorsey *et al*. (1997). This is particularly suitable for *Scenedesmus* and other single-cell microalgae resistant to normal procedures of protein extraction, because it recovers, on average, 90% of the Kjeldahl organic nitrogen (Dorsey *et al*. 1978).

For this reason, the efficiency of nitrogen recycling of this microalga was estimated dividing each protein concentration by the usual 6.25 factor and adding 10% to the resulting values. In the case of phosphorus, the cell quota was assumed to be equivalent to 1.3% of the weight of the organic biomass, calculated from the traditional molar ratios $C_{106} H_{181} O_{45} N_{16} P$ (Oswald 1988a).

RESULTS

The mean amount of organic biomass produced during the light period with the 30% dilution was lower than those determined with the other regimes of daily renewal of the growth medium.

However, at 30% dilution biomass synthesis continued at a slow rate during the night, whereas dark respiration caused net losses of 2.4 and 8.2 mg·l^{-1} with the 40 and 50% dilutions.

Proteins showed similar trends with the highest and lowest dilutions, and remained unchanged at the end of the night for the daily harvests of 40%. After 24 hours, biomass yields ranged between 33.8 and 39.4 mg·l^{-1}·d^{-1} and the protein percentages increased in parallel with the amount of AWW renewed, from 47 to 55% of the ash-free dry weight of the biomass (Table I).

The amounts of inorganic nitrogen removed were low, from 7.1 to 8.8 mg·l^{-1}. As a consequence of the increasing volumes of AWW exchanged daily, mean concentrations after 24 hours were progressively higher, from 28.8 to 32.0 and 37.2 mg·l^{-1} for the 30, 40 and 50% renewals of the medium.

Ammonia removal ranged between 13 and 8% of the initial concentrations, whereas the average percentages of nitrates removed after 24 h varied from 69 to 79%. For both, removal was light-related; with the 30% dilution it continued at a far lower rate during the night, and occurred only in daytime with the other regimes of dilution (Table II).

Table I. – Mean values (± standard deviation) of organic biomass and protein concentrations, in mg·l^{-1}, in semicontinuous cultures of *Scenedesmus* sp., measured after 30, 40 and 50% dilutions (initial) and after 14 and 24 hours of incubation with a 14:10 hours light: dark cycle. Δ: difference between the initial and the 24 h values. n:15 in all cases.

	30%	40%	50%
		Biomass	
Initial	90.38 ± 3.94	59.44 ± 1.94	39.26 ± 9.11
14h (L)	122.77 ± 2.89	101.21 ± 1.65	81.28 ± 6.09
24h (L+D)	127.76 ± 4.81	98.79 ± 2.76	73.11 ± 4.26
Δ 24h	37.38 ± 8.22	39.35 ± 4.16	33.83 ± 10.2
		Proteins	
Initial	45.51 ± 3.11	31.21 ± 1.27	21.17±3.22
14h (L)	59.55 ± 2.62	52.41 ± 2.53	42.73 ± 5.44
24h (L+D)	62.80 ± 3.81	52.27 ± 2.40	39.66 ± 2.79
Δ 24h	17.69 ± 2.64	21.06 ± 2.66	18.55 ± 5.87

Table II. – Top, mean concentrations (± standard deviation) in mg·l^{-1} of N-NH_4^+, N-NO_3^-, and of total inorganic and organic N (ΣNi and N org.) in semicontinuous cultures of *Scenedesmus* sp. after 30, 40 and 50% dilutions and after 14 and 24 hours of incubation under a 14:10 hours light: dark cycle. Δ: difference from the initial value. n:15 in all cases. Below, mean values (± standard deviation) of dissolved P-PO_4^{3-}, in mg·l^{-1}, in semicontinuous cultures of *Scenedesmus* sp., measured after 30, 40 and 50% dilutions and after 14 and 24 hours of incubation under a 14:10 hours light: dark cycle. Δ: difference from the initial value. P org. = estimated phosphorus content of the biomass. n:15 in all cases.

Dilutions (%)		Initial	14h (L)	24h (L+D)	Δ (L+D)
30	N-NH_4^+	31.44 ± 0.54	28.10 ± 0.48	27.45 ± 0.83	-3.99
	N-NO_3^-	4.48 ± 0.09	1.53 ± 0.24	1.38 ± 0.24	-3.10
	ΣNi	35.92	29.63	28.83	-7.09
	N org.	7.94 ± 0.43	10.48 ± 0.42	11.05 ± 0.61	3.11
40	N-NH_4^+	35.25 ± 1.49	30.35 ± 0.30	30.83 ± 0.68	-4.42
	N-NO_3^-	5.57 ± 0.03	1.15 ± 0.12	1.19 ± 0.05	-4.38
	ΣNi	40.82	31.50	32.02	-8.80
	N org.	5.49 +0.20	9.22 ± 0.41	9.19 ± 0.38	3.71
50	N-NH_4^+	38.82 ± 3.99	35.52 ± 3.70	35.55 ± 3.16	-3.27
	N-NO_3^-	5.82 ± 0.11	1.71 ± 0.19	1.66 ± 0.18	-4.16
	ΣNi	44.64	37.23	37.21	-7.43
	N org.	3.73 ± 0.51	7.52 ± 0.87	6.98 ± 0.45	3.26

Dilutions (%)		Initial	14h (L)	24h (L+D)	Δ (L+D)
30	P-PO_4^{3-}	3.74 ± 1.05	3.06 ± 0.63	2.67 ± 0.54	- 1.07 ± 0.88
	P org.	1.18	1.60	1.66	0.49
40	P-PO_4^{3-}	3.27 ± 0.76	2.15 ± 0.40	1.89 ± 0.15	- 1.39 ± 0.63
	P org.	0.77	1.32	1.28	0.51
50	P-PO_4^{3-}	3.82 ± 1.17	3.03 ± 0.32	2.55 ± 0.28	- 1.27 ± 1.39
	P org.	0.51	1.06	0.95	0.44

The percentages of the nitrogen removed that were recycled into new biomass varied from 42 to 44%, and were presumably due only to active nitrate uptake. For the 30% dilution, the increase in particulate organic nitrogen was equal to the amount of $N\text{-}NO_3^-$ that disappeared from the medium, but the increases were lower than the nitrates removed in the other two cases.

This was probably due to algal settling and denitrification in the resulting deposits, which would explain the presence of nitrites in the medium. These were not used for AWW preparation, but were consistently found in concentrations varying from 0.01 to 0.04 $mg\cdot l^{-1}$ (not reported in table II, because the method used for nitrate analysis measures the concentration of both species).

Initial phosphate values varied widely, as a consequence of the variable quantities removed in the previous 24 h. In all cases dissolved phosphorus concentrations decreased in daytime and during the night, although with the 40 and 50% dilutions the removal was higher after the light period.

The estimated phosphorus incorporation was also related to the light-dark cycle, with important increases during the first 14 h of incubation. In parallel with biomass changes, cell phosphorus contents remained practically unchanged after the 10 dark hours with the lowest dilution, but they decreased with the others.

The total estimated uptakes varied from 0.44 to 0.51 $mg\cdot l^{-1}\cdot d^{-1}$, with efficiencies of recycling decreasing progressively with increasing volumes of AWW renewed daily, from 45 to 35% for the lowest and the highest dilutions (Table II).

DISCUSSION

The organic biomass yields obtained in previous experiments with continuous light ranged from 100 to 154 $mg\cdot l^{-1}\cdot d^{-1}$, and the protein content was low, from 33.5 to 34.6% of the ash-free dry weight (Voltolina *et al*. 1998, Nuñez *et al*. 2001).

With the light-dark cycle, the organic production was only 34 to 39 $mg\cdot l^{-1}\cdot d^{-1}$, but the protein percentages were substantially higher, possibly because of the photoperiod-related changes in the proximate composition of *Scenedesmus* spp. mentioned by Becker (1994), who suggested morning harvests of this microalga to increase protein yields.

If these levels could be maintained outdoors, the daily production of a 0.3 m-deep pond would vary from 10 to 12 $g\cdot m^{-2}$ of organic biomass, which is within the range reported by several sources for outdoor mass cultures of single-cell chlorophytes, run for biomass production or for wastewater treatment (Becker 1994).

During summer, the mean photon flux calculated from the solar radiation values mentioned by Oswald (1988b) for the latitude of Ensenada (close to 30ºN), range from 90 to 108 $mol\cdot m^{-2}\cdot d^{-1}$ for the months of September and July respectively, which are from 5 to 6 times higher than the total irradiance supplied to our cultures.

This might increase biomass yields of outdoor cultures in summer because, with similar accumulated degrees·hour^{-1} (552 ºC·d^{-1}, compared to the 527 ºC·d^{-1} of our experiment) and the same 50% dilutions, the biomass obtained with total irradiances of 27.0 and 36.3 $mol\cdot m^{-2}\cdot d^{-1}$ was 120 and 154 $mg\cdot l^{-1}\cdot d^{-1}$ (Voltolina *et al*. 1998, Nuñez *et al*. 2001), which are higher than the 34 mg obtained in this work with 18.4 $mol\cdot m^{-2}\cdot d^{-1}$.

On the other hand, the high light availability may cause photoinhibition in outdoor cultures (Molina Grima *et al*. 1999, Acién Fernández *et al*. 1998) and, though solar radiation is lower in winter (from 52 to 55 $mol\cdot m^{-2}\cdot d^{-1}$ in January and December), the accumulated temperatures are only 360-380 ºC·d^{-1}, which would limit the productivity of these cultures and make them less effective for biomass production.

In addition, the algal biomass is only a desirable byproduct of tertiary treatment, whose targeted end product is nutrient-poor water. With the traditional technology, this may be obtained only if there is sufficient land available at little or no cost, which is rarely the case of urban communities.

As an example, and using conservative figures, a daily per capita water consumption of 300 l and four days of retention in a 0.3 m-deep high rate pond, a city like Ensenada would need 120 ha of prime industrial or agricultural land to treat to the tertiary level, the water used by the approximately 300,000 inhabitants it had in 1992-1993 (Soto Sainz 1993), which is close to the 130 ha calculated from the area of high rate ponds of the integrated ponding system serving the Hollister (California, U.S.A.) 14,000 persons community (Oswald 1988a).

Our data show that these figures may not be improved with the available solar technology, because a short-term adequate level of nutrient removal is largely dependent on the photosynthesis-induced changes in water chemistry caused by the sustained high pH of the medium, which are the main causes of nutrient depletion.

REFERENCES

Abeliovich A 1986. Algae in wastewater oxidation ponds. *In* Richmond A ed, Handbook of Microalgae Mass Cultures. CRC Press, Boca Raton: 331-338.

Acién Fernández FG, García Camacho F, Sánchez Pérez J A, Fernández Sevilla J M, Molina Grima E 1998. Modeling of biomass productivity in tubular photobioreactors for microalgal cultures: effects of dilution rate, tube diameter and solar irradiance. *Biotechnol Bioeng* 58: 605-616.

Becker E W 1994. Microalgae: Biotechnology and Microbiology. Cambridge University Press, Cambridge, 293 p.

Chevalier P, De la Noüe J 1985. Efficiency of immobilized hyperconcentrated algae for ammonium and orthophosphate removal from wastewaters. *Biotech Lett* 7: 395-400.

Correa Reyes J G 1996. Remoción de nutrientes con microalgas en agua residual sintética. M. Sc. Thesis. Centr Investi Cien Educ Sup Ensenada. Ensenada, B.C., México, 60 p.

Dorsey T E, McDonald P W, Roels O A 1977. A heated biuret-Folin protein assay which gives equal absorbance with different proteins. *Anal Biochem* 78: 156-164.

Dorsey T E, McDonald P W, Roels O A 1978. Measurement of phytoplankton-protein content with the heated biuret-Folin assay. *J Phycol* 14: 167-171.

Fahey J, Perrine R L, Dracup J A eds 1975. Waste Nutrient Recycling Using Hydroponic and Aquacultural Methods. Environmental Science & Engineering and Institute of Evolutionary & Environmental Biology. Univ California, Los Angeles, 170 p.

González L E, Cañizares R O, Baena S 1997. Efficiency of ammonia and phosphorus removal from a Colombian agroindustrial wastewater by the microalgae *Chlorella vulgaris* and *Scenedesmus dimorphus*. *Biores Technol* 60: 259-262.

Hach 1997. Water Analysis Handbook, 3rd Ed. Hach Company, Colorado, Loveland, 1309 p.

Molina Grima E, Acién Fernández F G, García Camacho F, Chisti Y 1999. Photobioreactors: light regime, mass transfer and scaleup. *J Biotechnol* 70: 231-247.

Nuñez V J, Voltolina D, Nieves M, Piña P, Medina A. Guerrero M 2001. Nitrogen budget in *Scenedesmus obliquus* cultures with artificial wastewater. *Biores Technol* 78: 161-164.

Oswald W J 1988a. Microalgae and wastewater treatment. *In* Borowitzka MA & Borowitzka L J eds, Micro-algal Biotechnology. Cambridge Univ Press, Cambridge: 305-328.

Oswald W J 1988b. Large-scale algal culture systems (engineering aspects). *In* Borowitzka M A & Borowitzka L J eds, Micro-algal Biotechnology. Cambridge Univ Press, Cambridge: 357-394.

Sorokin C 1973. Dry weight, packed volume and optical density. *In* Stein JR ed, Handbook of Phycological Methods. Culture Methods and Growth Measurement. Cambridge Univ Press, Cambridge: 321-343.

Soto Sainz L P 1993. Remoción de nutrientes de agua residual sintética, utilizando la microalga *Scenedesmus* sp. B. Sc. Thesis. Esc Cien Mar, Univ Autón Sinaloa, Mazatlán, Sinaloa, México, 41 p.

Talbot P, De la Noüe J 1993. Tertiary treatment of wastewater with *Phormidium bohneri* (Schmidle) under various light and temperature conditions. *Wat Res* 27: 153-159.

Trujillo Valle M L 1993. La Colección de Microalgas del CICESE. Centr Invest Cie Educ Sup Ensenada, Ensenada, B.C., México, Com. Acad. CIACT 9301, 103 p.

Voltolina D, Cordero B, Nieves M, Soto L P 1998. Growth of *Scenedesmus* sp. in artificial wastewater. *Biores Technol* 68: 265-268.

Reçu le 17 juillet 2003; received July 17, 2003
Accepté le 19 novembre 2003; accepted November 19, 2003