Role of Nano-Elements in Green Microalgae Growth in Fish Aquarium

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ABSTRACT

Fish industry suffers millions of dollars by contaminated water and its effect on fish food. It is necessary to investigate the role of nanoelements in the microflora in water. Microalgae Chlorella fusca ACOI 621, Chlorella vulgaris ACOI 879, Scenedesmus acutus ACOI 538 and Scenedesmus obliquus ACOI 550, all native in fish ponds, were characterized in terms of specific growth rate. The effect of pH and the presence of Cr(VI) in concentrations up to 25 mg l-1 (50 mg l-1 for Chlorella fusca) has been evaluated. The logistic equation of population growth adequately describes the cellular growth. Experiments at pH = 6.5 and temperature around 24.5oC, in the absence of Cr(VI), led to specific growth rates ( ) of 0.0370, 0.0284, 0.0359 and 0.0162 h-1 and maximum biomass concentrations (K) of 403.3, 369.2, 542.9 and 604.1 mg l-1 for C. fusca, C. vulgaris, S. acutus and S. obliquus, respectively. Experiments carried out with the same algae at approximately 21ºC, also in the absence of Cr(VI), gave values of 0.0241, 0.0357, 0.0272 and 0.0289 h-1 and K values of 292.6, 169.9, 263.1 and 327.8 mg l-1 for initial pH = 6.5 and values of 0.0115, 0.0177, 0.0137 and 0.0158 h-1 and K values of 35.9, 3.0, 32.8 and 54.7 mg l-1 for initial pH = 7.9. Higher pH results in a significantly lower growth rate and C. vulgaris were less resistant microalgae to changes in the environmental conditions. Looking simultaneously at and K values, the best performance in terms of growth kinetics was obtained for S. acutus and C. fusca. Growth inhibition is visible for Cr(VI) 5 mg l-1 but concentrations up to 1 mg l-1 seem not to seriously affect algal growth, even increasing the C. fusca specific growth rate. For Cr(VI) < 1 mg l-1, varies between 0.08 and 0.17 h-1, depending on the algal species. The growth of C. vulgaris is severely inhibited by Cr(VI) = 5 mg l-1. The production of metabolites was small compared with biomass production, for all Cr(VI) concentrations. The organic carbon content of algae was about 40%-50% (dry basis), except for S. obliquus (around 30%). The biomass of C. fusca and S. acutus presents the greatest sedimentation rates. The presence of high Cr(VI) concentrations negatively affects the sedimentation.

Keywords: *nanoelements, algae growth, fish aquarium, fisheries*

1 INTRODUCTION

The wastewater-related nutrient loading has grown as one of the major concerns in environmental management. On the other side, wastewaters resulting from human activities often contain heavy metals with toxicity characteristics and caught attention of governments using aquatic organisms for wastewater treatment. Microalgae are often used for wastewater treatment due to their high growth rate and simple growth requirements with ability of nitrogen and phosphorous removal by microalgae Important algae are carrageenan-immobilized *Chlorella vulgaris*, *Chlorella emersonii*, *Scenedesmus dimorphus*, *Scenedesmus subspicatus* suspensions in reactors maintained at 20-25ºC and illumination at 40-60 μE m^{-2} s^{-1} at 20ºC with an incident photon flux density of 40 μE m^{-2} s^{-1} under controlled temperature (19±1ºC) and light (85-95 μE m^{-2} s^{-1}). The pH and CO_2 of the culture medium and minerals solubility and influence directly or indirectly the algal metabolism and formation of algal flocs to settle down. Optimal light and temperature is important in algal biomass and algae growth rate.

2 pH, TEMPERATURE, LIGHT INTENSITY AND NUTRIENTS:

Heavy metals removal by the green algae is an emerging art and the accumulation of heavy metals comprises a rapid adsorption phase, reversible and metabolism independent and a slower one, metabolism dependent, often irreversible. Major issues came across: 1. Batch culture conditions of light intensity(100 or 150 W m^{-2} or 85-90 μE s^{-1} m^{-2}), temperature 20-27 ºC, pH 4-7; 2. Presence of nanoelements (less than 10^{-6} M) of Pb(II), Cr(III), Cr(VI) and Cd(II), Cu(II) and Zn(II) and toxicity of (Cd, Cu, Ni, Pb and Zn); 3.Species specificity of evolution of nitrogen and phosphorus uptake*(Chlorella fusca*, C*hlorella vulgaris*, *Selenastrum Capricornutum); 4*. Good flocculation at pH rise (>8), promoting an initial nucleation of calcium phosphate crystals to precipitate, agglomerate and flocculate by cations of Cr, Cu, Fe, Mn, Pb, Sr, Zn, Ca and Mg, into the flocs to settle down and promote water clarification.

2.1 Example of Microalgae: For illustration, Chlorella fusca ACOI 621, Chlorella vulgaris ACOI 879, Scenedesmus acutus ACOI 538 and Scenedesmus obliquus ACOI 550 are compared with those obtained in the presence of Cr(VI) to get effect of temperature and initial pH upon algal growth. Microalgae Modelling Growth of unicellular organisms biomass concentration, n (mg l^{-1}), as a function of time, t (h), can be expressed as:

$$\frac{dn}{dt} = \mu \times n$$

where μ is the specific growth rate of biomass (h^{-1}). Riccati equation further is icon concept:

$$\frac{dn}{dt} = \mu \times n \times \left(1 - \frac{1}{K} \times n\right)$$

This equation can be easily integrated to obtain the logistic curve:

$$n = \frac{1}{\left(\frac{1}{n_0} - \frac{1}{K}\right) e^{-\mu t} + \frac{1}{K}}$$

where n_0 is the initial biomass concentration (mg l^{-1}) and K the maximum biomass concentration (mg l^{-1}). The logistic curve is sigmoidal and leads to a stationary population of size K, i.e., for $t = \infty$, $n = K$. Lacunae: initial lag phase and the decline phase missing in this implicit strict theory of population microalgae growth as a "law of population growth".

2.2 Microalgae population growth characteristics:

Chlorella fusca ACOI 621, *Chlorella vulgaris* ACOI 879, *Scenedesmus acutus* ACOI 538 and *Scededesmus obliquus* ACOI 550 strains, all chlorophyceae unicellular green algae 1 to 5 μm in diameter found in water and soil. Their algal cultures at 4ºC, in sterilized test tubes are ideal in labwork.
Growth medium composition is following (aseptically filtered through 0.45 μm Gellman Sciences with dechlorinated tap water $NaHCO_3$, and CO_2 supply in the sterile air bubbled into the medium).

Table 1. Composition of the synthetic medium

Constituent	Concentration (mg l^{-1})
$NaNO_3$	525
$NaHCO_3$	50
KH_2PO_4	626
K_2HPO_4	266
$MgSO_4.7H_2O$	500
$CaCl_2.2H_2O$	55.87
Na_2EDTA	50
NaOH	22.1
$FeSO_4.7H_2O$	4.98

Table 2. Composition of the inorganic solutions

Constituent	Concentration (g l^{-1})	Solution
$NaNO_3$	5.25	1
$NaHCO_3$	0.5	1
KH_2PO_4	6.26	1
K_2HPO_4	2.66	1
$MgSO_4.7H_2O$	5.0	2
$CaCl_2.2H_2O$	0.559	2
Na_2EDTA	50	3
NaOH	22.1	3
$FeSO_4.7H_2O$	4.98	4

Microorganism growth is pH dependent. Phosphate buffers (hydrogen / di-hydrogen phosphates
) control pH, while phosphorus is also required as nutrient.

- Hexavalent chromium solution was prepared by dissolving $K_2Cr_2O_7$ in distilled and demineralised water. Test cultures were spiked with pre-defined volumes of this solution in order to get different Cr(VI) concentrations.
- Light intensity by luxmeter (Luxmeter HD 8366 Delta OHM) measures optical density at 680 nm on UV-VIS Jenway 6405 spectrophotometer.
- The pH was daily measured with a 540 GLP/ WTW pH meter. Cells were counted in an improved Neubauer hemacyclometer. (3)
- Total organic carbon (TOC) and dissolved organic carbon (DOC) concentrations were measured by Rosemount Analytical Dohrman DC-190 analyser.

Microalgae Growth Set up: Four 250-ml Erlenmeyer flasks containing 250 ml of medium were inoculated with the above referred microalgae species to obtain an initial cell concentration around 100 000 cells ml^{-1} in presence of CO2, light (2000 to 3000 lux) (32- 48 μE m^{-2} s^{-1}), Osram L36W/72 Biolux lamps, at 180ºC for 1 h 30 min, room temperature 24.6 ± 1.0ºC (mean ± standard deviation), pH with stirring.

What is Outcome?
Figure 1. Effect of pH on microalgae growth rate at 20.8 ± 1.8ºC.

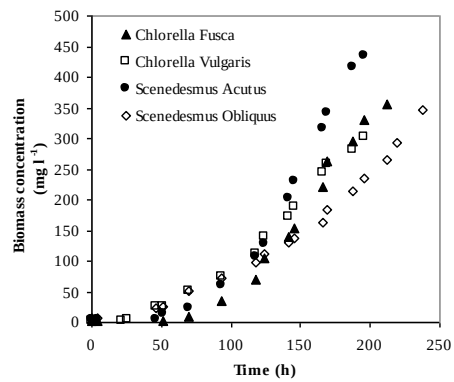

Figure 1. Microalgal growth as a function of time. (T = 24.6 ºC; pH = 6.5).

Cr(VI) added to1500 ml of four algal cultures (density of 100 000 cells ml^{-1}). Experiments with Cr(VI) concentrations of 0, 1, 5 and 25 mg l^{-1} (50 mg l^{-1} for *Chlorella fusca* culture) at room temperature: 21.6±1.1, 22.4±0.6, 21.0±0.71 and 21.0±0.3 ºC (mean ± standard deviation) for *Chlorella fusca*, *Chlorella vulgaris*, *Scenedesmus acutus* and *Scenedesmus obliquus* cultures, and *d*aily samples were collected both in test and control flasks for pH, biomass (optical density) and total, dissolved and suspended organic carbon. Same growth curves under same Initial pH Value (T = 24.6 ºC, pH = 6.5) for each algae are shown in Figure 1. In Figure 2. *Scenedesmus acutus* and *Chlorella fusca* show higher lag phases. Maximum biomass concentration, K, ranges from 369 mg l^{-1} for *Chlorella vulgaris* to 604 mg l^{-1} for *Scenedesmus obliquus*. The pH effect increased from 6.4-6.5 to 7.2-7.7, after about 190 h-operation, as shown in Figure 3. Different Initial pH Values (T = 20.8ºC, pH = 6.5 and pH = 7.9) showed influence of the initial pH on algal growth in Figure 4 as experimental and predicted growth curves and the parameters μ, K and n_0. K values show no significant growth of biomass at pH 7.9: the highest K value is ≈54 mg l^{-1} for *Scenedesmus obliquus*, whereas for *Chlorella vulgaris* is only ≈3 mg l^{-1}. The maximum biomass concentration follows the same order as for pH = 6.5:

Scenedesmus obliquus > *Chlorella fusca* > *Scenedesmus acutus* > *Chlorella vulgaris*. The specific growth rate of biomass μ is also reduced by about 50% for all species, when compared to the values obtained at initial pH = 6.5.

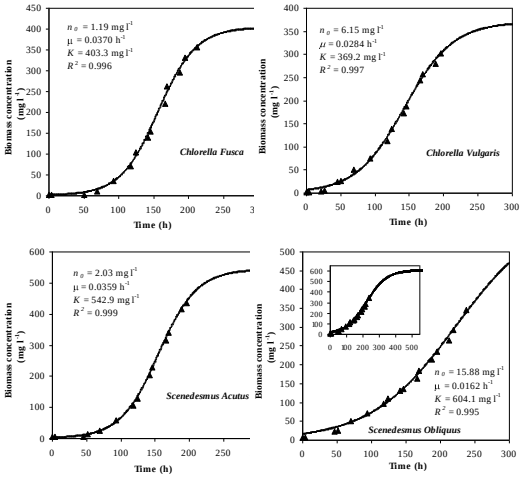

Figure 2. Microalgal growth (□) as a function of time. (—) Logistic model fittings (T = 24.6 ºC; pH = 6.5).

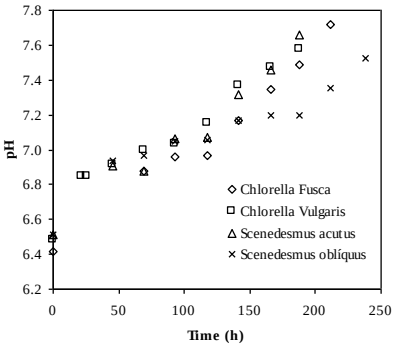

Figure 3. pH evolution over time (□) for each alga (T = 24.6 oC; pH = 6.5).

Temperature influence at pH = 6.5 at two different temperatures (24.6 and 20.8 ^{o}C) shown in Figure 2 and Figure 4 variation of the specific growth rate of biomass, μ, is casual, the maximum biomass concentration, *K*, increases with temperature. At 20.8ºC, it was possible to observe that the pH increased from 6.5-6.6 to 7.0-7.1, after about 190 h operation, not reaching values so high as at 24.6 ºC (Figure 3). The lower production of biomass at 20.8 ºC originates a lower impact on the initial pH. Effect of Cr(VI) on Growth Kinetics in Figure 5 shows biomass growth for four algal species in the presence of different Cr(VI) concentrations. of 1 mg l^{-1} do not affect or slightly improve algal growth. Higher concentrations cause an inhibitory influence on growth kinetics. *C. vulgaris* culture showed interruption by Cr(VI) concentration as low as 5 mg l^{-1}. In the absence of Cr(VI) or for low Cr(VI) concentrations (1 mg l^{-1}), pH increases over time from the initial value around 6.5 to about 7.4 - 7.8 (Figure 6). Figure 5 and Table 3 suggest Cr(VI) concentrations up to 5 mg l^{-1} do not affect the initial specific growth rate of biomass (μ) of *C. fusca* and the presence of 1 mg l^{-1} even increases μ. *C. fusca* has tolerance to heavy metal *C. vulgaris* < *C. fusca* or lower maximum biomass concentration (*K*) reached for Cr(VI) = 5 mg l^{-1} .

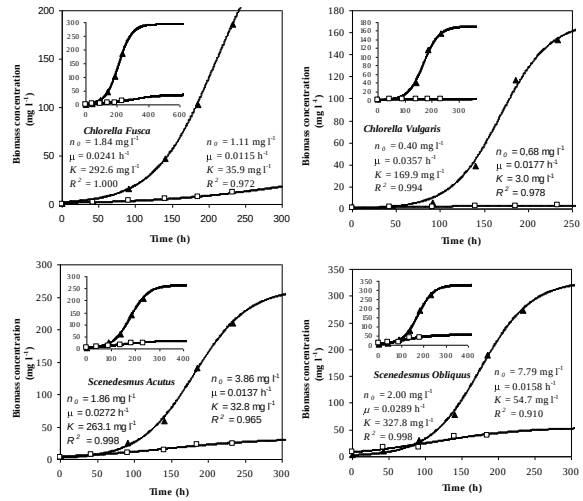

Figure 4. Microalgal growth as a function of time for different initial pH values. (□) pH = 6.5; (□) pH = 7.9. (—) Logistic model fittings (T = 20.8ºC).

The presence of Cr(VI) up to 5 mg l^{-1} practically does not affect the biomass specific growth rate of *S. obliquus*, but the maximum biomass concentration (*K*) markedly decreases. For Cr(VI) = 25 mg l^{-1} the algal growth is completely inhibited.

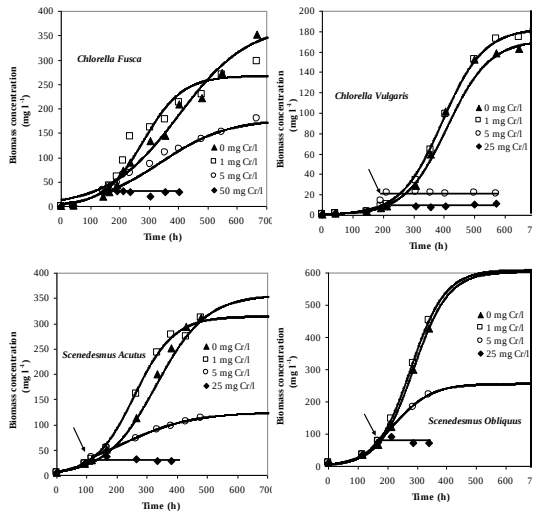

Figure 5. Effect of Cr(VI) on algal growth as a function of time (T = 21-22 C; initial pH = 6.6-6.7; arrow indicates the moment of Cr addition). (—) Logistic model fittings.

Light is one of the most important environmental factors influencing the growth and maximum concentration of growing algae in a continuously mixed culture is determined by the degree of penetration of light into the culture.

Effect of Cr(VI) on Metabolites Production is shown in Table 4 at different Cr(VI) concentrations. Suspended organic carbon vs biomass for each Cr(VI) concentration showed linear correlation as shown in Figure 7.

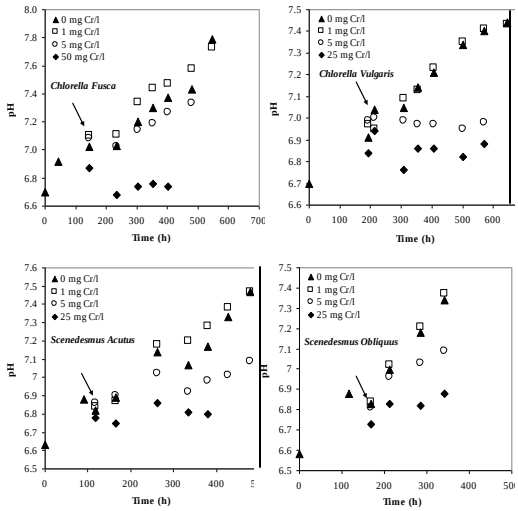

Figure 6. Evolution of pH over time (T = 21-22 C; initial pH = 6.6-6.7; arrow indicates the moment of Cr addition).

Table 3. Specific growth rates of biomass (□) and maximum biomass concentrations (*K*) obtained by fitting the experimental data to the logistic equation

Concn of Cr(VI)(mg l^{-1})	**Microalgae**	μ (h^{-1})	*K* (mg l^{-1})	R^2
0	***C fusca***	**0.008**	**372.4**	**0.97756**
	C vulgaris	0.014	171.4	0.98586
	S acutus	0.013	356.1	0.99839
	S obliquus	0.017	603.0	0.99914
1	***C fusca***	**0.015**	**267.9**	**0.93015**
	C vulgaris	0.015	182.7	0.99860
	S acutus	0.015	313.6	0.99351
	S obliquus	0.017	607.7	0.99973
5	***C fusca***	**0.009**	**178.1**	**0.97919**
	C vulgaris	0	-	-
	S acutus	0.009	124.0	0.98879
	Sobliquus	0.016	254.6	0.99525
25 (50 for *CF*)	***Chlorella fusca (CF)***	**0**	-	-
	Chlorella vulgaris	0	-	-
	Scenedesmus acutus	0	-	-
	Scenedesmus obliquus	0	-	-

Effect of Cr(VI) on Biomass Settling down is expressed in as "decrease of the suspended biomass concentration after a 6 h-settling period" at different Cr(VI) concentrations are presented in Table 5. In the absence of Cr(VI) and for Cr(VI) = 1 mg l^{-1}, the settling rate is similar for *C. fusca*, *S. acutus* and *S. obliquus* but much lower for *C. vulgaris*.

Table 4. Effect of Cr(VI) addition on the production of metabolites (expressed as dissolved organic carbon, DOC) by the algal cells

Microalgae	Concentration of Cr(VI) (mg l^{-1})			
	0	1	5	25 (50 for *CF*)
	Metabolites (DOC average ± sd) (mg l^{-1})			
C fusca	11.0±4.9	8.0±4.1	8.9±3.9	16.2±6.7
C vulgaris	29.1±9.7	26.8±5.3	24.6±1.0	24.2±2.4
S acutus	29.2±4.3	40.9±29.6	38.4±34.6	28.9±5.2
S obliquus	26.2±3.7	28.3±8.6	35.3±21.8	26.2±2.6

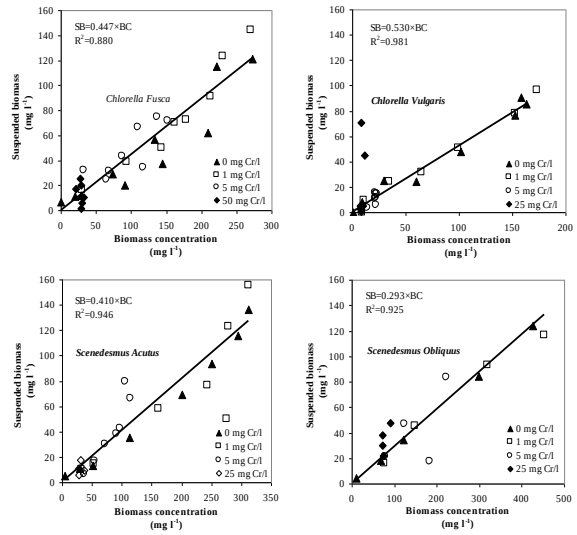

Figure 7. Suspended biomass as a function of dry biomass concentration. (—) Linear regression. SB = Suspended Biomass; BC = Biomass Concentration.

As Cr(VI) concentration increases, the settling rate decreases for all algal cultures and Cr(VI) ≥ 5 mg l^{-1} impairs algae autoflocculation.

Table 5. Biomass settling rate (mg l^{-1} h^{-1})

Concentration of Cr(VI) (mg l^{-1})	*Chlorella fusca* (*CF*)	*Chlorella vulgaris*	*Scenedesmus acutus*	*Scenedesmus obliquus*
0	55.1	13.4	41.1	42.7
1	45.8	12.3	46.4	54.6
5	26.5	1.4	16.0	33.2
25 (50 for *CF*)	nd	0.52	nd	6.5

CONCLUSION

Microalgae growth kinetics at 24.6ºC, pH = 6.5 showed growth rate of biomass, μ, of 0.0162, 0.0284, 0.0359 and 0.0370 h^{-1} for *Scenedesmus obliquus*, *Chlorella vulgaris*, *Scenedesmus acutus* and *Chlorella fusca*, biomass concentration, *K*, was 604.1, 369.2, 542.9 and 403.3 mg l^{-1}. Temperature 21 ºC lower μ (between 0.0241 and 0.0357 h^{-1}) and *K* (between 169.9 and 327.8 mg l^{-1}) values. An increase in pH 7.9 decreases specific growth rate of biomass (below 0.0177 h^{-1}) and the maximum biomass concentration (below 54.7 mg l^{-1}). *Chlorella vulgaris* is sensitive to pH and temperature. μ and *K* values showed best performance with *Scenedesmus acutus* and *Chlorella fusca*. Cr(VI) concentrations ≥ 5 mg l^{-1} seriously affect growth in *C. vulgaris* culture. Microalgal biomass produced has 40 - 50% organic carbon. *C. fusca* and *S. obliquus* present a higher degree of autofloculation. Cr(VI) ≥ 5 mg l^{-1} impairs algae autoflocculation particularly of *C. vulgaris* and *S. acutus* cultures. For Cr(VI) ≤ 1 mg l^{-1} only *C. vulgaris* flocs present a low settling rate.

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