In: Biochemical Engineering
Editors: F. E. Dumont and J. A. Sacco, pp.
ISBN 978-1-60741-257-1

Chapter 11

INTERACTION OF CR (VI) WITH GREEN MICROALGAE GROWTH: A COMPARATIVE STUDY

M. Alzira P. Dinis*[1]*, Vítor J. P. Vilar*[2]*,
***Álvaro A. M. G. Monteiro*[1,2]*, Rui A. R. Boaventura*[2,*]**
[1]University Fernando Pessoa, Praça 9 de Abril, 349, 4249-004 Porto, Portugal
[2]Laboratory of Separation and Reaction Engineering, Department of Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

ABSTRACT

Microalgae *Chlorella fusca* ACOI 621, *Chlorella vulgaris* ACOI 879, *Scenedesmus acutus* ACOI 538 and *Scenedesmus obliquus* ACOI 550, all native from Portugal, 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 $n = 1\big/\left(\left(1/n_0 - 1/K\right)e^{-\mu t} + 1/K\right)$ adequately describes the cellular growth. Experiments at pH = 6.5 and temperature around 24.5 ºC, in the absence of Cr(VI), led to specific growth rates (μ) 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 21 ºC, also in the absence of Cr(VI), gave μ 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 μ 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* seems to be the less resistant microalgae to changes in the environmental conditions. Looking simultaneously

---

[*] Corresponding author: Tel.: +351-22-5081683; fax: +351-22-5081674; E-mail address: madinis@ufp.edu.pt (M. Alzira P. Dinis); bventura@fe.up.pt (Rui A.R. Boaventura); vilar@fe.up.pt (Vítor J.P. Vilar); aamont@ufp.edu.pt (Álvaro A.M.G. Monteiro)

at μ 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}, μ 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 is small compared with biomass production, for all Cr(VI) concentrations. The organic carbon content of algae is 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: Microalgae; *Chlorella fusca*; *Chlorella vulgaris*; *Scenedesmus acutus*; *Scenedesmus obliquus*; growth kinetics; chromium (VI)

1. INTRODUCTION

As population, population density, and development level increase, the issue of 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 [1]. These elements make part of a list of dangerous substances with toxicity characteristics and a high potential of persistence and accumulation.

There has been considerable interest in using aquatic organisms for wastewater treatment. Portugal presents, mainly in Centre and South areas, climatic conditions ecologically favourable to green microalgae development, so the subject has been faced with increasing attention. Because of the fact that, in those areas, the limiting factor to agriculture is water, a judicious management of the hydric resources available is necessary, and so water reuse constitutes an adequate contribution for solving ecological and water shortage problems simultaneously.

Microalgae are often used for wastewater treatment due to their high growth rate and simple growth requirements. Several works refer to nitrogen and phosphorous removal by microalgae [2-6]. Lau et al. [7] show that carrageenan-immobilized *Chlorella vulgaris* cells efficiently removed N and P (95 and 100%, respectively) from wastewater. Meiring et al. [4] reported nitrogen removals of about 85%. Robinson [5] has obtained P removal efficiencies of 80% to 95% using *Chlorella emersonii* in suspension. Continuous-flow culture studies were also carried out using small-scale packed-bed reactors containing *Chlorella* immobilized in alginate beads. Reactors were maintained at 25ºC and illumination was provided at 60 μE m^{-2} s^{-1}. Gonzalez et al. [2] run 216 h-experimental cycles of batch cultures of *Chlorella* and *Scenedesmus* and concluded that *Chlorella vulgaris* was less efficient than *Scenedesmus dimorphus* in removing ammonia. Twist et al. [6] used *Scenedesmus subspicatus* cultures incubated at 20ºC with an incident photon flux density of 40 μE m^{-2} s^{-1}. The interaction of air flow rate with nutrient supply was tested under controlled temperature (19±1ºC) and light (85-95 μE m^{-2} s^{-1}).

The pH of the culture medium determines CO_2 and minerals solubility and influences directly or indirectly the algal metabolism. Becker [8] states that stopping agitation and/or

CO_2 supply can cause an increase in pH, leading to the formation of algal flocs, which will settle down. Experiments with *Scenedesmus* sp. to study the effect of pH showed that no flocculation could be obtained for pH values between 5.0 and 7.5, whereas at pH values above 8.5 almost 95% of the algal biomass could be removed.

Light is also of fundamental importance and, in algal cultures, light rapidly becomes limiting due to absorption by the algal biomass. Light intensity also interacts strongly with temperature. Algae growth rate increases with temperature until an optimal value is reached. Further increases in temperature usually lead to a rapid decline in growth rate. At temperatures close to the optimum, algae are also better able to tolerate much higher light intensity before photoinhibition sets in [9].

In a general way, microalgae growth is complex, revealing non linear behaviours in response to the alteration of several environmental parameters such as pH, temperature, light intensity and nutrients, and the interactions between these factors are not well established [8, 10].

Heavy metals removal by the green algae has been widely recognized [11-15]. According to Avery et al. [16], the accumulation of heavy metals comprises a rapid adsorption phase, reversible and metabolism independent and a slower one, metabolism dependent, often irreversible. Batch cultures of *Chlorella fusca* were used for testing the toxicity of Pb(II), Cr(III), Cr(VI) and Cd(II) [17].

Tam et al. [18] investigated the influence of the concentration of C*hlorella vulgaris* dried cells on copper removal from aqueous solution. The evolution of nitrogen and phosphorus uptake in presence and absence of Pb (10^{-6} M), at pH 7 and 4, was studied by Capelo et al. [19] using the microalgae *Selenastrum Capricornutum*. Batch cultures of *Chlorella fusca* were used for testing the toxicity of Cu(II) and Zn(II) [20] and also Pb(II), Cr(III), Cr(VI) and Cd(II) [17]. Lam et al. [13] evaluated the effect of Cd and Cu on *Chlorella vulgaris* growth. The direct toxic effect due to copper addition and the indirect impact arising from a decrease in pH following an increase in the metal ions concentration were investigated. Light intensity was in the range 85-90 $\mu E\ s^{-1}\ m^{-2}$. Lupi et al. [21] studied the inhibition of *Chlorella vulgaris* by Cu, at 27 ºC and 100 or 150 $W\ m^{-2}$ light intensity. The effect of the contact time on the binding capacity of five toxic metals (Cd, Cu, Ni, Pb and Zn) in solution by *Chlorella vulgaris* at pH 8.5 and constant temperature was also evaluated [14].

For an efficient biomass separation, good flocculation must occur [4, 22]. Algae autoflocculation may result from a pH rise (>8), promoting an initial nucleation of calcium phosphate crystals. In the presence of Ca^{2+}, the calcium phosphate, positively charged, precipitates, adsorbing the negatively charged cells, agglomerating them and promoting their flocculation [8]. Under these conditions, polyvalent cations of Cr, Cu, Fe, Mn, Pb, Sr and Zn, together with Ca and Mg, tend to become incorporated into the flocs, which settle down and promote water clarification [23].

This work aims to describe the growth kinetics of pure cultures of four microalgae, *Chlorella fusca* ACOI 621, *Chlorella vulgaris* ACOI 879, *Scenedesmus acutus* ACOI 538 and *Scenedesmus obliquus* ACOI 550. Results are compared with those obtained in the presence of Cr(VI). The effect of temperature and initial pH upon algal growth was also investigated.

2. MODELLING

Growth of unicellular organisms like microalgae is often considered as a first-order autocatalytic reaction [24-26]. So, the increase in biomass concentration, n (mg l^{-1}), as a function of time, t (h), can be expressed as:

$$\frac{dn}{dt} = \mu \times n \tag{1}$$

where μ is the specific growth rate of biomass (h^{-1}), characteristic of each organism and culture medium, and is primarily governed by the growth capacity of the organism and the environmental conditions [8, 25]. This model assumes unlimited exponential growth. However, in batch systems every population is limited either by lack of nutrients or accumulation of toxic metabolites and then some allowance must be made to restrict the growth [25]. Verhulst derived the logistic equation of population growth, sometimes called Verhulst model, in 1845 [24], which is frequently presented as the initial form of a Riccati equation:

$$\frac{dn}{dt} = \mu \times n \times \left(1 - \frac{1}{K} \times n\right) \tag{2}$$

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

$$n = \frac{1}{\left(\frac{1}{n_0} - \frac{1}{K}\right) e^{-\mu t} + \frac{1}{K}} \tag{3}$$

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 $t = \infty$, $n = K$.

The logistic equation perfectly describes the exponential and stationary growth phases, but it does not include the initial lag phase and the decline phase. According to Krebs [27], there are two ways of viewing the logistic curve. One is to view it as an empirical description of how populations tend to grow in number when conditions are initially favourable. This is the more general, more flexible viewpoint. The other way is to view the logistic model as an implicit strict theory of population growth, as a “law” of population growth. This last one is, perhaps, the one that interests us most.

3. MATERIALS AND METHODS

3.1. Microalgae

Samples of *Chlorella fusca* ACOI 621, *Chlorella vulgaris* ACOI 879, *Scenedesmus acutus* ACOI 538 and *Scededesmus obliquus* ACOI 550 strains, all chlorophyceae, were obtained from stock cultures maintained by the Algal Culture Collection ACOI, Department of Botany, University of Coimbra, Portugal. The genera *Chlorella* and *Scenedesmus* belong to the order *Chlorellales* [22]. All species are unicellular green algae, without motion capacity. Species from the *Chlorella* gender are spherical or ellipsoidal, exhibiting a simple life cycle and also simple nutritional needs. They are small cells, 1 to 5 μm in diameter, which reproduce asexually, each mature cell producing 6, 8, or, more rarely, 16 autospores. Species from the *Scenedesmus* gender are largely distributed in water and soil. Cells are cylindrical with rounded or sharp extremities. They can be found laterally united in 4, 8 or 16 – more rarely – cells. They reproduce by autocolonies formation [22].

Algal cultures were stored at 4ºC, in sterilized test tubes, and renewed with algae proceeding from successive experiments performed in sterile conditions.

3.2. Growth medium

Table 1 presents the composition of the culture medium. As autoclaving leads to the precipitation of certain mineral salts [8], four concentrated solutions (Table 2) were prepared separately and aseptically filtered through 0.45 μm Gellman Sciences membranes. All solutions were prepared with dechlorinated tap water, which was previously sterilized by filtration. The only source of specific inorganic carbon was $NaHCO_3$, besides the CO_2 present 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
$MgSO_4 \cdot 7H_2O$	500
$CaCl_2 \cdot 2H_2O$	55.87
Na_2EDTA	50
NaOH	22.1
$FeSO_4 \cdot 7H_2O$	4.98

Table 2. Composition of the concentrated 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
$MgSO_4 \cdot 7H_2O$	5.0	2
$CaCl_2 \cdot 2H_2O$	0.559	2
Na_2EDTA	50	3
NaOH	22.1	3
$FeSO_4 \cdot 7H_2O$	4.98	4

One of the factors affecting microorganism growth is pH. Experiments aiming at determining kinetic parameters must be performed at constant or approximately constant pH. According to Mitchell and Slaughter [25], control of pH is also very important since in many cases it changes during the development of a microbial population. Phosphate buffers are especially useful to control pH, since phosphorus is required as nutrient and they buffer the solution at around neutral pH, where microorganisms like microalgae grow optimally. In this work, the culture medium was buffered with hydrogen / di-hydrogen phosphates.

3.3. Cr(VI) Solutions

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.

3.4. Analytical Techniques

Light intensity was measured using a luxmeter (Luxmeter HD 8366 Delta OHM).

The cell concentration was determined by optical density measurements at 680 nm, as used by Schelenz [28]. Optical densities were measured with a UV-VIS Jenway 6405 spectrophotometer, using the sterile medium culture as blank. In the range of optical densities measured, cell concentration is proportional to optical density. A calibration curve was prepared by plotting dry biomass weight against optical density. In order to eliminate the interference of cell sedimentation in optical density values, samples were previously stirred in a Vortex Mixtub, Raypa. The pH was daily measured with a 540 GLP/ WTW pH meter. Cells were counted in an improved Neubauer hemacyclometer.

Total organic carbon (TOC) and dissolved organic carbon (DOC) concentrations were measured using a Rosemount Analytical Dohrman DC-190 analyser. Prior to DOC measurements, samples were centrifuged at 5470 rpm, in an Alresa Mod Digicen centrifuge, for 15 minutes and then filtered through 0.45 μm porosity membranes (Gellman Sciences).

3.5. Experimental Procedures

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}, which has given the best results in preliminary experiments. A flask containing only culture medium was also used as blank for optical density measurements, sterilization control and pH variation. Experiments were carried out at temperature approximately constant. Mixing was accomplished and some CO_2 was provided by bubbling sterile air into the culture medium. As source light, special Osram L36W/72 Biolux lamps, giving a spectrum similar to sunlight, were used. Experiments were performed under a 24 h light photoperiod. All glass material was sterilized at 180ºC for 1 h 30 min.

In the first set of experiments, room temperature was controlled at 24.6 ± 1.0ºC (mean ± standard deviation). Daily pH measurements were carried out but no pH corrections were performed.

Mixing was maintained approximately uniform in all flasks by adjusting the diffused air flowrate. Besides, flasks were also manually stirred several times a day, to minimize the formation of a biofilm onto the flask wall.

Light intensity values ranged from 2000 to 3000 lux (32-48 μE m^{-2} s^{-1}). Twist et al. [6, 29], Minowa and Sawayama [30] used similar values, 40, 30 and 40-60 μE m^{-2} s^{-1}, respectively.

A second set of experiments was carried out to evaluate the effect of pH on microalgae growth rate. Experiments performed with buffered culture medium at pH ≈ 8 were compared with experiments where initial pH = 6.5. All the operating conditions were as before, except temperature, which was around 20.8 ± 1.8ºC.

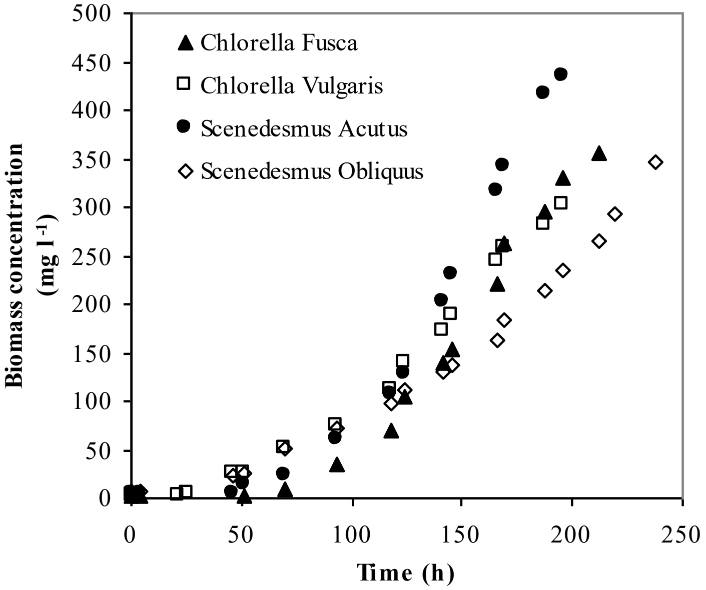

Figure 1. Microalgal growth as a function of time. (T = 24.6 ºC; pH = 6.5).

To study the effect of the addition of Cr(VI), pre-defined volumes of metal stock solution were added simultaneously to 2000 ml-glass flasks containing 1500 ml of each algal culture once a density of 100 000 cells ml^{-1} was reached. Experiments with Cr(VI) concentrations of 0, 1, 5 and 25 mg l^{-1} (50 mg l^{-1} for *Chlorella fusca* culture) were carried out at room temperature approximately constant: 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, respectively. No pH corrections were performed. An Erlenmeyer flask containing only sterile culture medium was submitted to the same operating conditions, for each set of experiments, and used as blank to correct optical density measurements over time. Daily samples were collected both in test and control flasks for pH, biomass (optical density) and total, dissolved and suspended organic carbon.

Flocculation properties of algal cultures were evaluated through settling tests. Initial optical densities were compared with those of the supernatants after a 6 h-settling period.

4. RESULTS AND DISCUSSION

4.1. Experiments under the Same Initial pH Value (T = 24.6 ºC, pH = 6.5)

For each algae, the parameters μ, K and the initial biomass concentration, n_0, were determined by fitting the logistic equation to the experimental results, using the non-linear least squares technique. Adjusting n_0 allows overcoming high experimental errors associated with the measurement of low biomass concentrations. The growth curve obtained for all algae species involved in this study are shown in Figure 1. All curves present a similar growth pattern, although *Scenedesmus acutus* growth seems to increase after 150 h operation and *Chlorella fusca* shows a slower growth rate in the beginning.

Growth curves obtained for each algae by fitting the logistic equation to the experimental data are also presented 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 increased from 6.4-6.5 to 7.2-7.7, after about 190 h operation, as observed in Figure 3. As it could be expected, algae species growing faster are those presenting the highest pH variation, which is clearly visible for C*hlorella fusca*, by comparing Figure 3 with Figure 2.

4.2. Experiments with Different Initial pH Values (T = 20.8 ºC, pH = 6.5 and pH = 7.9)

4.2.1. Effect of pH

The influence of the initial pH on algal growth was investigated, although maintaining the temperature at a lower value than in the previous experiments. The initial pH was kept around 6.5 in some experiments, whereas in others it was increased up to 7.9. Figure 4 shows the experimental and predicted growth curves obtained and the parameters μ, K and n_0, determined by fitting the logistic equation to the experimental results.

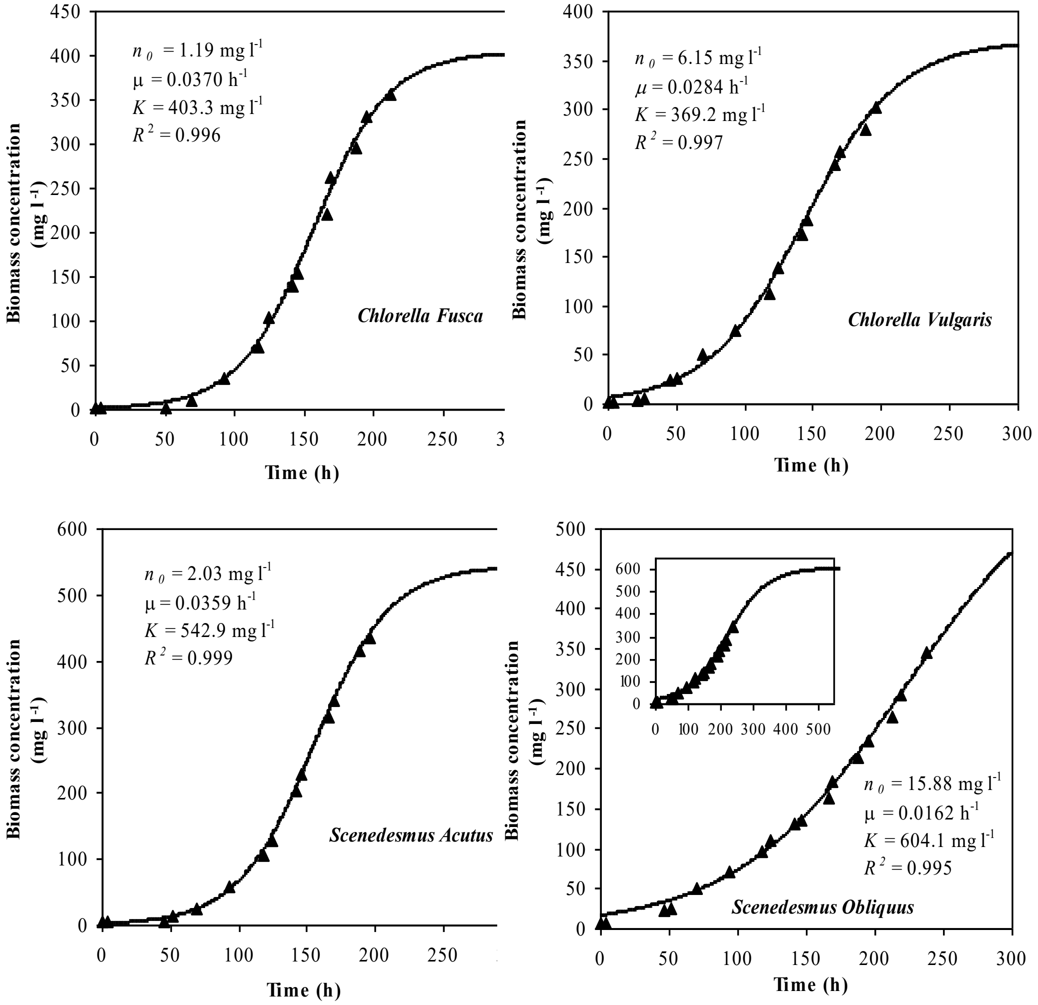

Figure 2. Microalgal growth (▲) as a function of time. (—) Logistic model fittings (T = 24.6 ºC; pH = 6.5).

As indicated by K values, there is no significant growth of biomass when the initial pH was adjusted to around 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.

In the experiments performed at a temperature of 20.8 ºC and initial pH = 6.5, it was obtained an increase in pH of around 0.5 after about 230 h incubation. Starting with a pH = 7.9, no visible alterations were visible during the same operating time. At higher pH values, the amount of CO_2 available is smaller, thus affecting algal growth. Results confirm that a high initial pH acts as an inhibitory factor preventing algae from developing normally [8].

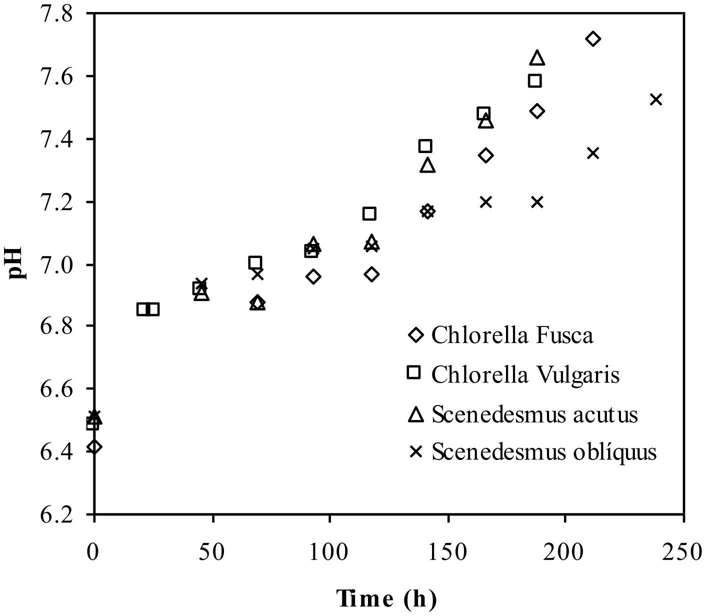

Figure 3. pH evolution over time for each alga (T = 24.6 ºC; pH = 6.5).

4.2.2. Temperature Influence

Comparing the values of the model parameters for the experiments performed at pH = 6.5 and two different temperatures (24.6 and 20.8 ºC), it can be concluded (Figure 2 and Figure 4) that although the variation of the specific growth rate of biomass,μ, is casual, the maximum biomass concentration, K, increases with temperature.

At 20.8 ºC, 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.

The variation of kinetic parameters due to temperature variation can be also associated to environmental stresses, as, according to Becker [8], living organisms like algae are extremely susceptible to changes occurring in the environment.

4.3. Effect of Cr(VI) on Growth Kinetics

Figure 5 shows the experimental results of biomass growth for each algal species. Growth in the presence of different Cr(VI) concentrations is compared with that obtained using Cr(VI)-free culture medium. Cr(VI) concentrations of 1 mg l^{-1} do not affect or slightly improve algal growth. However, higher concentrations cause an inhibitory influence on growth kinetics. The more marked effect is visible in *C. vulgaris* culture, since growth is almost interrupted for 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). As it could be expected, pH rises more slowly or even remains constant when inhibition occurs.

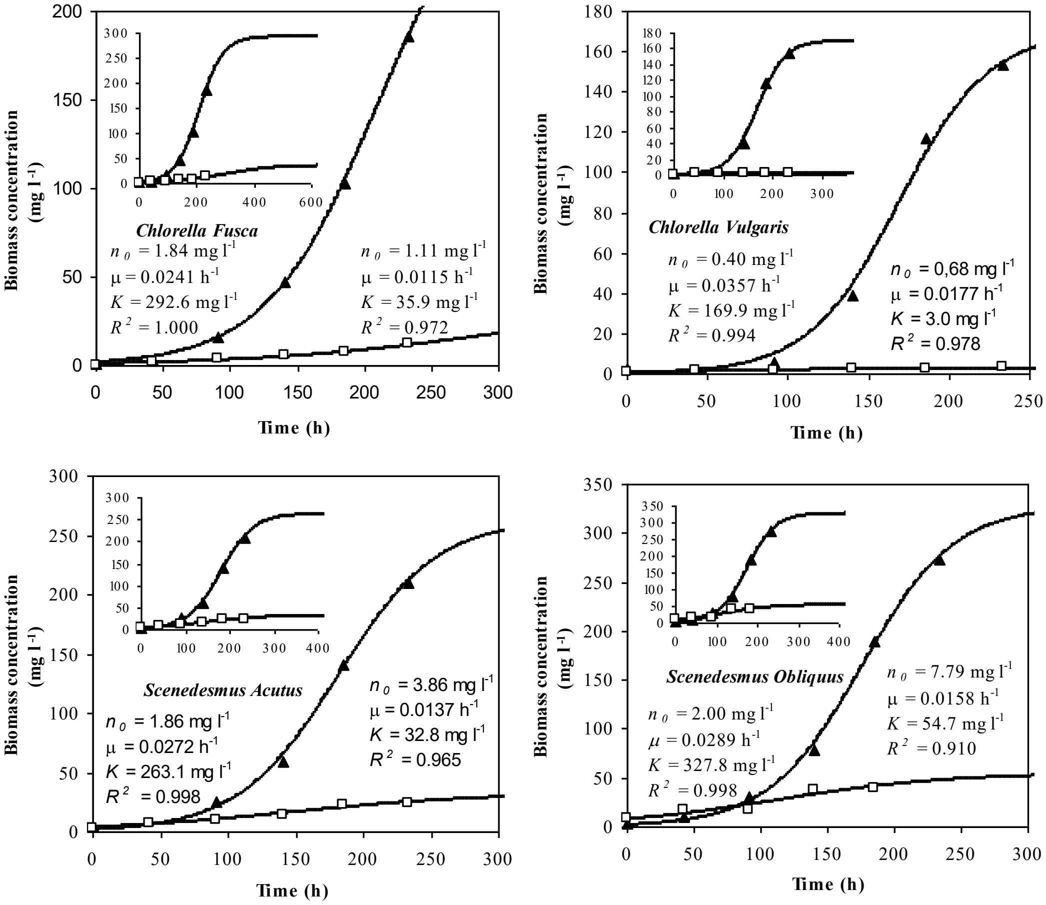

Figure 4. Microalgal growth as a function of time for different initial pH values. (▲) pH = 6.5; (☐) pH = 7.9. (—) Logistic model fittings (T = 20.8 ºC).

The logistic fittings to the experimental results are also displayed in Figure 5 and the corresponding parameter values are presented in Table 3. Hexavalent chromium concentrations up to 5 mg l^{-1} seem not to negatively affect the initial specific growth rate of biomass (μ) of *C. fusca* and the presence of 1 mg l^{-1} even increases μ. However, the maximum biomass concentration (K) continuously decreases as Cr(VI) concentration increases. According to Wong et al. [20], *C. fusca* is known for its tolerance to heavy metals, which explains this behaviour. On the other hand, *C. vulgaris* (Figure 5) is less tolerant to Cr(VI) than *C. fusca*. Although the addition of 1 mg l^{-1} does not affect the normal development of the population, growth is interrupted within a few hours for Cr(VI) = 5 mg l^{-1}. The effect of the presence of Cr(VI) on growth kinetics of *S. acutus* is similar to that observed for *C. fusca*, even though a lower maximum biomass concentration (K) has been reached for Cr(VI) = 5 mg l^{-1}. 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. However, K values are significantly higher than those obtained for the other species. For Cr(VI) = 25 mg l^{-1} the algal growth is completely inhibited.

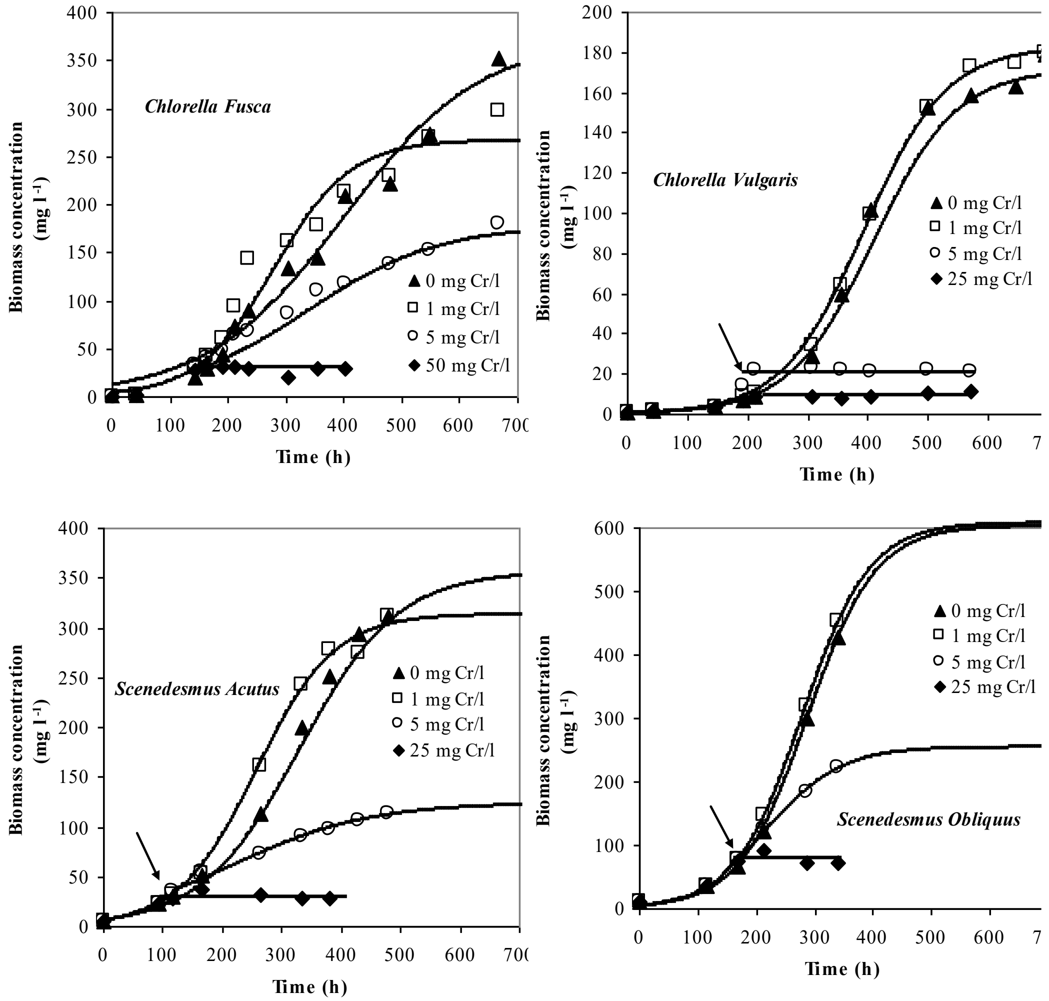

Figure 5. Effect of Cr(VI) on algal growth as a function of time (T = 21-22 ºC; initial pH = 6.6-6.7; arrow indicates the moment of Cr addition). (—) Logistic model fittings.

Comparing the results obtained without Cr(VI) addition (Figure 4) with those presented in Figure 5, one can conclude that growth rates are lower in the first experiments. The difference may be due to the higher medium volume used in the second study (1500 ml instead of 250 ml). As light intensity over the flasks was the same, the available light for algal growth was lower. As light is one of the most important environmental factors influencing the growth and development of photosynthetic organisms like chlorophyceae [21], the maximum concentration of growing algae in a continuously mixed culture is determined by the degree of penetration of light into the culture.

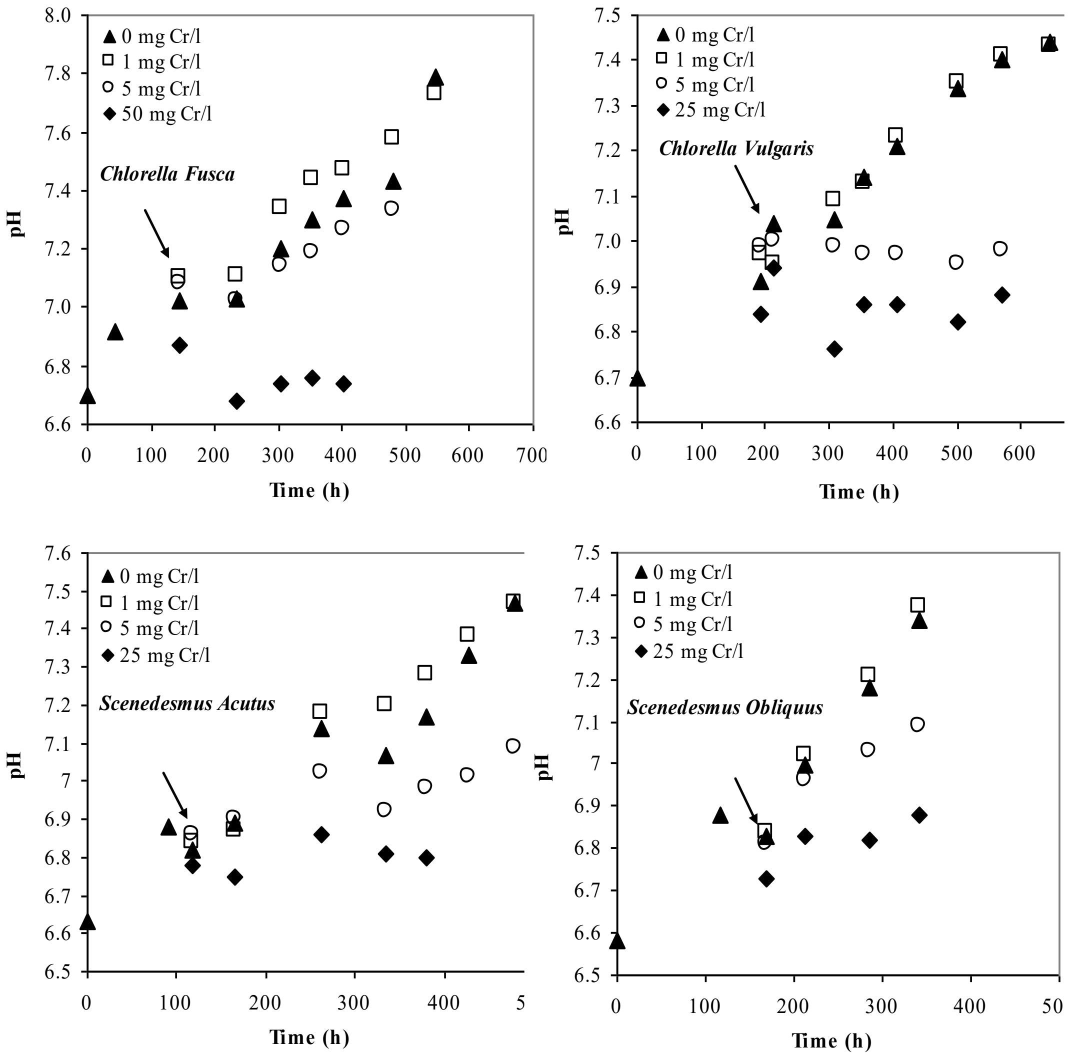

Figure 6. Evolution of pH over time (T = 21-22 ºC; initial pH = 6.6-6.7; arrow indicates the moment of Cr addition).

4.4. Effect of Cr(VI) on Metabolites Production

Total, dissolved and suspended organic carbon was measured over time in each algal culture. Table 4 presents the mean production of metabolites (in terms of dissolved organic carbon) for different Cr(VI) concentrations. Dissolved organic carbon concentration is small when compared with total organic carbon concentration. There are no indications that cellular growth by non photosynthetic routes may have occurred. Results also indicate that an outstanding dissolved organic carbon production due to the metal presence seems not to occur. It is also possible to observe that *Chlorella fusca* yields the lowest dissolved organic carbon concentration.

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

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

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 ± standard deviation) (mg l^{-1})			
Chlorella fusca (CP)	11.0±4.9	8.0±4.1	8.9±3.9	16.2±6.7
Chlorella vulgaris	29.1±9.7	26.8±5.3	24.6±1.0	24.2±2.4
Scenedesmus acutus	29.2±4.3	40.9±29.6	38.4±34.6	28.9±5.2
Scenedesmus obliquus	26.2±3.7	28.3±8.6	35.3±21.8	26.2±2.6

Suspended organic carbon was plotted against biomass for each Cr(VI) concentration and no significant dependence on Cr(VI) was observed. A global linear correlation between biomass concentration and suspended organic carbon for each algal culture was found, as shown in Figure 7. The percentage of organic carbon in the algal biomass varies from 29% (*S. obliquus*) to 53% (*C. vulgaris*). These results are in agreement with those reported by several authors that present values of the same order of magnitude by using empirical formulas [23, 30-33].

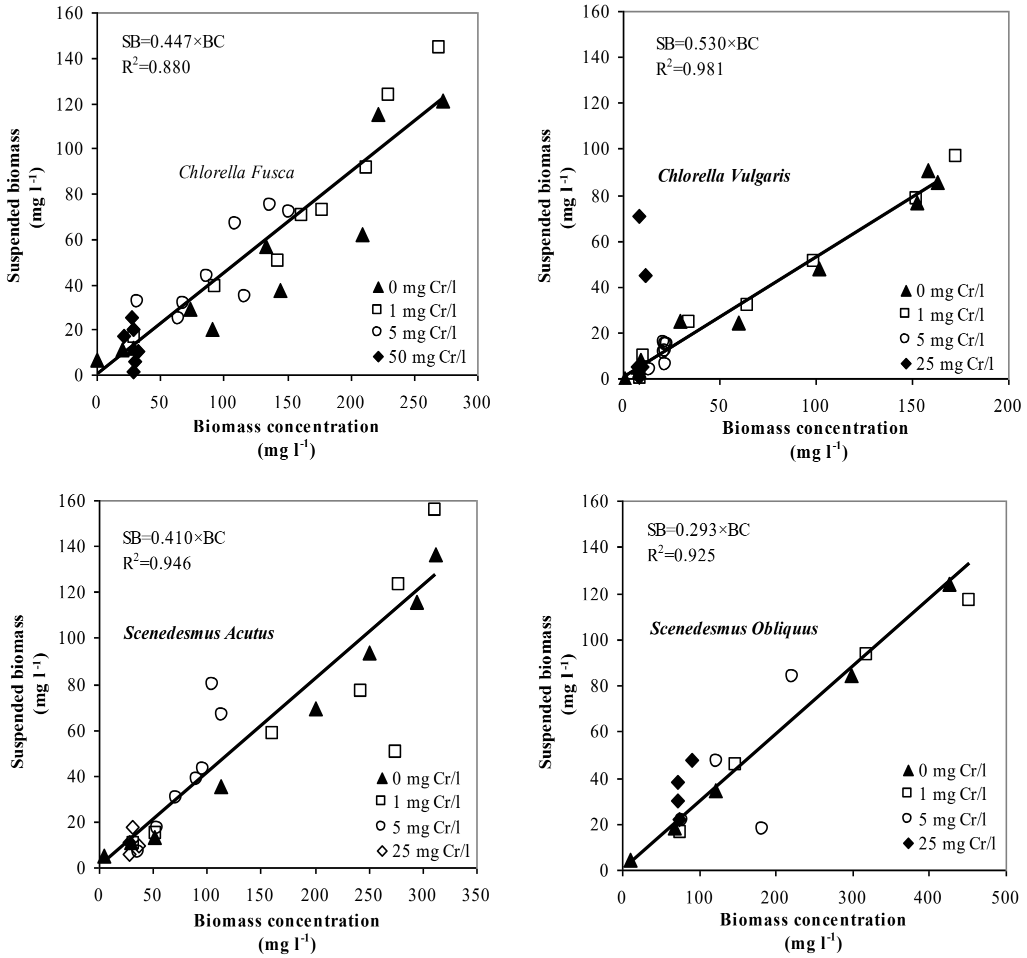

Figure 7. Suspended biomass as a function of dry biomass concentration. (—) Linear regression. SB = Suspended Biomass; BC = Biomass Concentration.

4.5. Effect of Cr(VI) on Biomass Settling

The ability of algal biomass to settle down has been expressed in terms of the decrease of the suspended biomass concentration after a 6 h-settling period. Results of the biomass-settling rate for each algae species and 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*. As Cr(VI) concentration increases, the settling rate decreases for all algal cultures but the effect is particularly evident for *C. vulgaris* and *S. acutus*. Results suggest that Cr(VI) $\geq$ 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

nd – not determined.

5. CONCLUSIONS

Microalgae growth kinetics is adequately described by the logistic equation. Experiments carried out at 24.6 ºC and initial pH = 6.5 led to values of the specific 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*, respectively. The corresponding maximum biomass concentration, K, was 604.1, 369.2, 542.9 and 403.3 mg l^{-1} for the same algal species. In spite of the low growth rate, *Scenedesmus obliquus* culture reaches the highest biomass concentration. A decrease in temperature operation to around 21 ºC originated 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 to about 7.9 led to drastic decreases in the specific growth rate of biomass (below 0.0177 h^{-1}) and the maximum biomass concentration (below 54.7 mg l^{-1}). Results show that pH plays a central role in microalgae growth. *Chlorella vulgaris* seems to be the less resistant microalgae to changes in pH and temperature. Looking simultaneously at μ and K values, the best performance was obtained with *Scenedesmus acutus* and *Chlorella fusca.*

Growth inhibition of *Chlorella fusca*, *Chlorella vulgaris*, *Scenedesmus acutus* and *Scenedesmus obliquus* by Cr(VI) occurs for concentrations ≥ 5 mg l^{-1}, but concentrations up to 1 mg l^{-1} do not seem to seriously affect growth or even may increase it slightly. The more marked effect of Cr(VI) on algal growth is observed in *C. vulgaris* culture. Metabolites production has proved to be in small quantity either in presence or absence of chromium, thus indicating that there was no relevant growth based on a non photosynthetic process. Microalgal biomass produced in all experiments is composed of approximately 40 - 50% organic carbon. *C. fusca* and *S. obliquus* are the algal species that present a higher degree of autoflocullation, then being more easily separated from the liquid phase. However, 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.

ACKNOWLEDGEMENTS

Financial and logistic support for this work was in part provided by Fernando Pessoa Foundation Teaching and Culture and by LSRE financing by FEDER/POCI/2010, for which the authors are thankful.

REFERENCES

[1] Patterson JW. *Industrial Wastewater Treatment Technology.* Boston, Butterworth, 1985.

[2] González LE, Cañizares RO, Baena S. Efficiency of ammonia and phosphorus removal from a colombian agroindustrial wastewater by the microalgae Chlorella vulgaris and Scenedesmus dimorphus. *Bioresource Technology* 60(3): 259-262, 1997.

[3] Lau PS, Tam NFY, Wong YS. Operational optimization of batchwise nutrient removal from wastewater by carrageenan immobilized Chlorella vulgaris. *Water Science and Technology* 38(1): 185-192, 1998.

[4] Meiring PGJ, Rose PD, Shipin OV. Algal aid puts a sparkle on effluent. *WQI* 2(30-32, 1994.

[5] Robinson PK. Immobilized algal technology for wastewater treatment purposes. In: Wong Y-S, Tam NFY, eds. *Wastewater Treatment with Algae,*vol USA, Springer-Verlag, 1-16; 1998.

[6] Twist H, Edwards AC, Codd GA. A novel in-situ biomonitor using alginate immobilised algae (Scenedesmus subspicatus) for the assessment of eutrophication in flowing surface waters. *Water Research* 31(8): 2066-2072, 1997.

[7] Lau PS, Tam NFY, Wong Y-S. Carrageean as a matrix for immobilizing microalgal cells for wastewater nutrients removal. In: Wong Y-S, Tam NFY, eds. *Wastewater Treatment with Algae,v*ol USA, Springer-Verlag, 1998.

[8] Becker EW. *Biotechnology and Microbiology.* Great Britain, Cambridge University Press, 1994.

[9] Borowitzka M. Limits to growth. In: Wong Y-S, Tam NFY, eds. *Wastewater Treatment with Algae,*vol USA, Springer-Verlag, 203-226; 1998.

[10] Sterner RW, Grover JP. Algal growth in warm temperate reservoirs: kinetic examination of nitrogen, temperature, light, and other nutrients. *Water Research* 32(12): 3539-3548, 1998.

[11] Cervantes C, Campos-Garcia J, Devars S, Gutierrez-Corona F, Loza-Tavera H, Torres-Guzman JC, Moreno-Sanchez R. Interactions of chromium with microorganisms and plants. *FEMS Microbiology Reviews* 25(3): 335-347, 2001.

[12] Corradi MG, Gorbi G, Morsi A-E-MH, Torelli A, Bassi M. Exudates from The Wild Type and a Cr Tolerant Strain of Scenedesmus acutus Influence Differently Cr(VI) Toxicity to Algae. *Chemosphere 3*7(14-15): 3019-3025, 1998.

[13] Lam PKS, Wut PF, Chan ACW, Wu RSS. Individual and combined effects of cadmium and copper on the growth response of Chlorella vulgaris. *Environmental Toxicology* 14(347-353, 1999.

[14] Soto EG, Rodríguez MVG, Suárez EL, Romero JMC, Iglesias JP, Solís JMF. Binding of metals in solution by Chlorella vulgaris. *Annali di Chimica* 89(323-326): 1999.

[15] Travieso L, Pellón A, Benitéz F, Sánchez E, Borja R, O´Farril N, Weiland P. BIOALGA reactor: preliminary studies for heavy metals removal. *Biochemical Engineering Journal* 12(87.91, 2002.

[16] Avery SV, Codd GA, Gadd GM. Microalgal removal of organic and inorganic metal species from aqueous solution. In: Wong Y-S, Tam NFY, eds. *Wastewater Treatment with Algae*,vol USA, Springer-Verlag, 55-72; 1988.

[17] Bundy K, Mowat F. Speciation studies and toxicity assessment of complex heavy metal mixtures. In *HSRC/WERC Joint Conference on the Environment,v*ol 1996.

[18] Tam NFY, Wong YS, Simpson CG. Removal of copper and immobilized microalgae, Chlorella vulgaris. In: Wong Y-S, Tam NFY, eds. *Wastewater Treatment with Algae,*vol Springer-Verlag, 17-36; 1998.

[19] Capelo S, Vilhena MF, Gonçalves MLSS, Sampayo MA. Effect of lead on the uptake of nutrients by unicellular algae. *Water Research* 27(1563-1568, 1993.

[20] Wong SL, Wainwright JF, Pimenta J. Quantification of total and metal toxicity in wastewater using algal biomass. *Aquatic Toxicology* 31(57-75): 1995.

[21] Lupi FM, Fernandes HML, Sá-Correia I. Increase of copper toxicity to growth of Chlorella vulgaris with increase of light intensity. *Microbial Ecology* 35(193-198, 1998.

[22] Richmond A. Large scale microalgal culture and applications. In: Chapman DG, Round FE, eds. *Progress in Phycological Research,*vol 7. USA, Biopress Ltd., 1-62; 1990.

[23] Oswald WJ. Micro-algae and wastewater treatment. In: Borowitzka MA, Borowitzka LJ, eds. *MicroAlgal Biotechnology*,vol Great Britain: Cambridge, University Press, 305-328; 1988.

[24] Bailey JE, Ollis DF. *Biochemical Engineering Fundamentals.* USA, McGraw-Hill Book Company, 1977.

[25] Mitchell MJ, Slaughter JC. *Biology and Biochemistry for Chemists and Chemical Engineers.* USA, Ellis Horwood Limited, 1989.

[26] Wilson EO, Bossert WH. *A Primer of Population Biology.* USA, Sinauer Associates Inc, 1971.

[27] Krebs CJ. *Ecology: The Experimental Analysis of Distribution and Abundance.* Menlo Park, California, Benjamin Cummings, 2001.

[28] Schelenz T. Relationships between algistatic activity and physicochemical properties of 5-amino.1-aryl-1H-tetrazoles. . *Journal fur Praktishe Chemie Chemiker Zeitung* 342(2): 205-210, 2000.

[29] Twist H, Edwards AC, Codd GA. Algal growth responses to waters of contrasting tributaries of the River Dee, North-East Scotland. *Water Research* 32(8): 2471-2479, 1998.

[30] Minowa T, Sawayama S. A novel microalgal system for energy production with nitrogen cycling. *Fuel and Energy Abstracts* 78(1213-1215, 1999.

[31] Fogg GE. *The Metabolism of Algae.* USA, John Wiley & Sons Inc., 1953.

[32] Gloyna EF. *Bassins de Stabilization des Eaux Usées.* OMS 1972.

[33] Stumm W, Morgan JJ. *Aquatic Chemistry.* New York, John Wiley & Sons, 1996.