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Water Research 37 (2003) 2097–2105

WATER RESEARCH

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Modeling of chlorine effect on floc forming and filamentous micro-organisms of activated sludges

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Received 1 September 2001; received in revised form 1 November 2002

Abstract

Chlorination is the most economical, non-specific method to control the excessive growth of filamentous micro-organisms causing bulking in activated sludge systems in the treatment of food industrial wastewaters; it was one of the first methods used to control filamentous bulking and is still widely employed. Considering that chlorination affects both floc-forming and filamentous micro-organisms and leaves undesirable disinfection by-products, it is necessary to define the adequate doses to control bulking, minimizing the effect on floc-forming bacteria.

In the present work the effect of biomass concentration and type of micro-organism on chlorine decay kinetics was evaluated; the inactivation of either a filamentous (*Sphaerotilus natans*) or a floc-forming (*Acinetobacter anitratus*) micro-organism due to chlorination was also analyzed.

For chlorine decay assays, the samples were treated in a batch system with sodium hypochlorite ranging between 9.8 and 56.6 mg Cl_2 $(gVSS)^{-1}$. Respirometric assays were used to evaluate the effect of chlorine on micro-organisms respiratory activity; in these cases, sodium hypochlorite doses ranged between 2.5 and 18 mgCl_2 $(gVSS)^{-1}$.

A model that allowed to predict simultaneously chlorine consumption and respiratory activity decay for both micro-organisms as a function of time was proposed. The model includes three coupled differential equations corresponding to respiratory inhibition, readily organic matter oxidation by chlorine and chlorine decay. The rate of chlorine decay depended on both, type and concentration of the micro-organisms in the system. Chlorine consumption rate due to *S. natans* was 2–4 times faster than *A. anitratus*. Using the proposed model initial critical chlorine doses (the lowest initial dose that leads to a total inhibition of the respiratory activity) were calculated for both micro-organisms and values of 11.9 mgCl_2 $(gVSS)^{-1}$ for *S. natans* and 4.5 mgCl_2 $(gVSS)^{-1}$ for *A. anitratus* were obtained. These critical doses indicated that in non flocculated pure cultures, floc-former bacteria *A. anitratus* was more susceptible to chlorine action than *S. natans*.

Keywords: Filamentous bulking; *Sphaerotilus natans*; *Acinetobacter anitratus*; Chlorination model

1. Introduction

Activated sludge process is the most widely used technique for wastewater treatment. However, these systems usually suffer failings of the sedimentation and thickening process due to the overgrowth of filamentous with respect to floc-forming micro-organisms, a phenomenon known as filamentous bulking.

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0043-1354/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0043-1354(02)00601-2

Nomenclature

C	chlorine concentration ($mgCl_2\,l^{-1}$)
C_0	initial chlorine concentration ($mgCl_2\,l^{-1}$)
D_C	initial critical chlorine dose ($mgCl_2$ $(gVSS)^{-1}$)
D_0	initial chlorine dose ($mgCl_2$ $(gVSS)^{-1}$)
f_z	fraction of the total biomass corresponding to the readily oxidized organic matter
F_R	bacterial respiring fraction (dimensionless)
$F_{R\infty}$	asymptotic bacterial respiring fraction (dimensionless)
K	chlorine decay kinetics coefficient ($l^{n+1}\,mgCl_2^{-1}\,mgVSS^{-n}\,min^{-1}$)
k_0	apparent second-order decay coefficient ($(mgCl_2\,l^{-1})^{-1}\,min^{-1}$)
k_z	Z component decay coefficient ($(mgCl_2\,l^{-1})^{-1}\,min^{-1}$)
k_R	respiratory activity decay coefficient ($(mgCl_2\,l^{-1})^{-1}\,min^{-1}$)
n	empirical coefficient (dimensionless)
OUR	oxygen uptake rate ($mgO_2\,l^{-1}\,h^{-1}$)
OUR_0	initial oxygen uptake rate ($mgO_2\,l^{-1}\,h^{-1}$)
t	time (min)
X_R	concentration of the respiring biomass ($mgVSS\,l^{-1}$)
X_{R0}	initial respiring biomass concentration ($mgVSS\,l^{-1}$)
X_0	total initial biomass concentration ($mgVSS\,l^{-1}$)
Z	readily oxidized organic matter concentration
Z_0	initial readily oxidized organic matter concentration
α_z	stoichiometric coefficient ($mgCl_2$ (Z unit mass)$^{-1}$)

Filamentous bulking can be controlled by specific and/or non-specific methods. Specific methods tend to address the main causes of filamentous micro-organism proliferation thus, these causes should be identified previously. Examples of specific approaches are selectors [1], and modifications of plant configuration or operating conditions [2]. Chlorination was one of the first non-specific methods used to control filamentous bulking [3] and is still widely employed [4,5]. When chlorination is used as a non-specific method to control filamentous bulking, the relative survival between floc-forming and filamentous micro-organisms should be evaluated. Among filamentous bacteria, *Sphaerotilus natans* is one of the main causes of bulking [6,7]. On the other hand, floc-forming micro-organisms *Acinetobacter* occur naturally in soil and water [8] and are also present in activated sludge systems [9]. Different authors reported that genus *Acinetobacter* represents 48–73% of the total microbial population in activated sludges [10–12]. Even though chlorination is very effective for the inactivation of filamentous micro-organisms it is not selective because it also affects nitrification process [13], and phosphate and COD biological removal [14]. Besides, an excess of residual chlorine should also be avoided in order to prevent the formation of undesirable compounds like trihalomethanes and other disinfection by-products [4]. In general, chlorination starts with low concentrations (below $5\,mgCl_2\,l^{-1}$) in the aeration tank, and continues with increasing doses as needed. Neethling [15] working on different activated sludge plants found that chlorine doses to control bulking ranged between 0.7 and $20\,mgCl_2\,l^{-1}$ and recommended not to exceed concentrations above $35\,mgCl_2l^{-1}$. Lakay et al. [14] reported doses of $8\,mgCl_2$ $(gVSS)^{-1}$ to control filamentous bulking, while Jenkins et al. [2] recommended doses between 1 and $15\,mgCl_2$ $(gVSS)^{-1}$.

Several models that describe micro-organism inactivation by disinfectants like chlorine or its derivatives have been developed [16–20]. However, most of them do not consider effects such as the decay of disinfectant concentration with time or biomass (particulate organic) concentration. In addition, since these models are empirical it is difficult to assign a physical meaning to the constants. Besides, there are some models that describe chlorine decay. The simplest one is a first-order decay model in which the chlorine concentration is assumed to decay exponentially [21–23]. However, other authors have suggested a kinetic model in which two separated decay equations were used, one was defined for the first few hours and the other for larger contact times [24–26]. Because the initial decay is rapid, they suggested a second-order decay equation for the first phase and a first-order decay equation for the second one. Ventresque et al. [27], Jadas-Hécart et al. [21] and Dossier-Berne et al. [28] even suggested the use of a multiple parameter decay equation for the second phase to describe the chlorine decay behavior more accurately. Fang Hua et al. [29] developed a semi-empirical model for chlorine decay based on a global reaction with a certain physical meaning. In spite of the great amount of proposed models available in the literature, none of them considered both chorine decay and its effect on micro-organisms viability simultaneously under different conditions. Although there is a wide available information, chlorine dosage is still an empirical subject mainly due to the difficulty of translating laboratory scale results to practical applications.

The objectives of the present work were: (a) to study the effect of concentration and type of micro-organisms on chlorine decay kinetics, (b) to evaluate the inactivation of either a filamentous (*S. natans*) or a floc-forming (*Acinetobacter anitratus*) micro-organisms by chlorine,

and (c) to develop a kinetic model that describe simultaneously chlorine decay and inactivation of micro-organisms.

2. Materials and methods

2.1. Bacterial strains and culture medium

A strain of *S. natans* ATCC 29329 obtained from American Type Culture Collection was used as a typical filamentous micro-organism. The floc-forming strain E932 was isolated from a lab-scale activated sludge wastewater treatment plant using a specific isolation technique for floc-former bacteria [30]; this strain was identified as *A. anitratus* using the biochemical test system Sensident-E (Merck).

The following culture medium was used: monohydrate citric acid 3480 mg l^{-1}, $(NH_4)_2SO_4$ 1000 mg l^{-1}, $MgSO_4 \cdot 7H_2O$ 400 mg l^{-1}, $CaCl_2 \cdot 2H_2O$ 50 mg l^{-1}, KH_2PO_4 250 mg l^{-1}, $Na_2HPO_4 \cdot 12H_2O$ 1000 mg l^{-1}, vitamin B12 100 μg l^{-1}, $FeSO_4 \cdot 7H_2O$ 15 mg l^{-1}, $ZnSO_4 \cdot 7H_2O$ 5 mg l^{-1}, $MnSO_4 \cdot H_2O$ 3 mg l^{-1}, $CuSO_4 \cdot 5H_2O$ 0.75 mg l^{-1}, $CoCl_2 \cdot 6H_2O$ 0.15 mg l^{-1}, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ 0.5 mg l^{-1}, BO_3H_3 0.1 mg l^{-1}, IK 0.1 mg l^{-1}. The medium was autoclaved at 121°C for 45–60 min. Vitamin B12 was sterilized by membrane filtration (0.45 μm Millipore HA) and was added to the previously sterilized medium.

All pure culture samples were obtained from a chemostat apparatus. Dilution rates ranged between 0.07 and 0.36 h^{-1}. The system was considered to run under steady-state conditions after operating for a period of at least five residence times [31]. The operation temperature was 30°C, pH = 7.0 and the dissolved oxygen concentration was above 2 mgO_2 l^{-1}. Biomass concentration was determined by absorbance measurements at 620 nm and transformed into volatile suspended solids (VSS) using a calibration curve previously determined [32]. Optical density measurements were carried out with a spectrophotometer DU 650 Beckman. In order to check the presence of flocs or clumps, microscopy observations were made using a Leitz microscope model Ortholux II (Germany).

2.2. Chlorine decay assays

In these assays the effect of micro-organism type (*S. natans* or *A. anitratus*) and its concentration on the chlorine decay was studied. Chlorine decay assays were performed in a flask with magnetic stirring and controlled temperature (30°C). Aliquots of 300 ml of a pure culture obtained from the biorreactor operating under steady state were poured into the flask with different volumes of fresh NaClO stock solution. The initial chlorine concentrations ranged between 8.6 and 11.0 mgCl_2 l^{-1} obtaining initial doses (D_0) between 9.8 and 56.6 mgCl_2 (gVSS)$^{-1}$. At different times, a sample was taken and total and free chorine concentrations were determined with specific kits (Hach, methods 8021 and 8167, respectively). All determinations were done in duplicates and results were expressed as the average value. Chlorine decay assays lasted from 50 to 300 min depending on the initial doses. All the experimental design (micro-organism type and D_0) was repeated twice.

2.3. Inactivation assays (respirometric technique)

Chlorine effect on micro-organisms was evaluated by respirometry. Oxygen uptake rate (OUR) is a widely used indicator of metabolic activity for aerobic micro-organisms [33,34]. Respirometry, has been used to determine microbial kinetic parameters [35–41], biochemical oxygen demand [33,42] and to study the toxicity of different agents [33,34,43,44].

Respirometric technique allows to determined the effect of chlorine on the respiratory activity of the biomass by the following equation:

$$F_R = \frac{OUR}{OUR_0} = \frac{X_R}{X_{R0}}, \quad (1)$$

where F_R is the bacterial respiring fraction; OUR_0 is the initial oxygen uptake rate, X_{R0} is the initial respiring biomass concentration (before chlorination) and OUR and X_R correspond to the values after chlorination.

The respirometer consisted of an hermetically sealed flask with an oxygen electrode (YSI Model 58), an aerator, magnetic stirring and a temperature control system (30°C). Data from the oxygen probe were recorded with a micro-processor at 1 Hz frequency. Microbial OUR was measured by placing in the respirometer aliquots of 20 ml of pure cultures (with a known biomass concentration) obtained from the biorreactor operating under steady state; 1 ml of citric acid solution (3.5%, pH = 7) was added as oxidizable substrate and the mixture was aerated. When the aeration stopped, a linear decrease of dissolved oxygen concentration was found; the slope of this line corresponded to OUR of the control sample (OUR_0).

For the chlorine treatments, 300 ml of pure culture obtained from the chemostat were placed in a flask under magnetic stirring and different NaClO doses were added (2.5–18 mgCl_2 (gVSS)$^{-1}$). At different contact times, 20 ml were drawn and placed in the respirometer; the remaining chlorine in these samples was neutralized with sodium thiosulfate. After 1 min, 1 ml of the citric acid solution was added and the culture was aerated again to determine the OUR after chlorine treatment. These assays lasted between 25 and 40 min depending on chlorine dose. All OUR determinations were done in duplicates and results were expressed as the average

value. For each tested condition (micro-organism type and D_0) two runs were performed and results were expressed as the average value.

2.4. Data fit and numerical simulation

Equations were fitted to experimental data by non-linear regression analysis using Sigma Plot 2.0 software. Numerical solution of the coupled first order differential equations was performed by means of a fourth-order Runge–Kutta method (Sigma Plot 2.0).

3. Results and discussion

3.1. Modeling of total chlorine decay

Previous studies [45] showed that chlorine reacts rapidly with the ammonium present in the cultures at the assayed temperature and pH. After one minute, chloramines corresponded to more than 95% of the total chlorine added. According to Palin [46], mono, di, and trichloroamines are produced by the reaction between chlorine and ammonium. The resulting distribution of chloramines concentration is governed by the relative formation rates which depend on the relative chlorine and ammonium concentrations, pH and temperature. For equimolar chlorine–ammonium concentrations, 25°C and pH between 7 and 8, the amount of dichloroamine was below 2%. Besides, the formation of nitrogen trichlorure only took place at pH below 4.4. Thus, under the present assay conditions, 98% of the combined chlorine should correspond to monochloroamine.

Micro-organisms were separated by centrifugation and the reaction between chlorine and the supernatant alone was tested. These experiments showed that chlorine concentration was almost constant with time. Thus, chlorine decay with time can only be attributed to its reaction with the biomass.

Fig. 1 shows examples of typical results of chlorine decay with time as affected by different initial biomass concentrations (X_0) of pure cultures of *S. natans* and strain E932. Fig. 1 shows that the rate of chlorine decay depended on both, type of micro-organisms and VSS concentration. In order to model chlorine decay as a function of time several authors proposed a second-order decay equation with respect to total chlorine concentration ([24–26]):

$$\frac{dC}{dt} = -k_0 C^2. \tag{2}$$

Integrating and reordering Eq. (2) the following was obtained:

$$\frac{1}{C} = \frac{1}{C_0} + k_0 t, \tag{3}$$

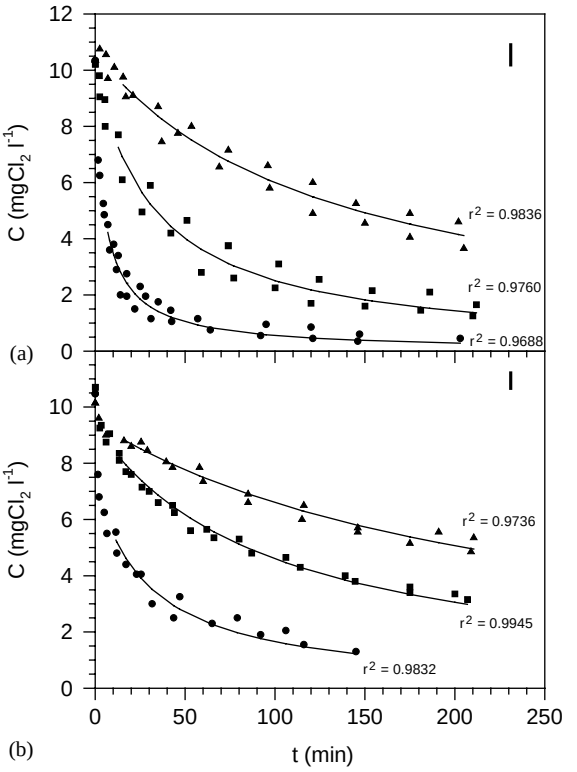

Fig. 1. Chlorine decay as a function of time for different biomass concentrations (gVSS l^{-1}). (a) *S. natans*: (●) 0.97, (■) 0.37, (▲) 0.19 (b) strain E932: (●) 1.15, (■) 0.33, (▲) 0.18; (—) Eq. (3). Bars in the upper right corner indicate the mean standard deviation.

where k_0 is the second-order decay coefficient ($(mgCl_2\,l^{-1})^{-1}\,min^{-1}$).

A typical representation of the experimental data following a second-order kinetics of Eq. (3) is shown in Fig. 2. The second-order model fitted experimental data for times above 5–10 min; however, at shorter times the experimental chlorine decay rate was higher, indicating the presence of a parallel process for chlorine uptake. Due to the observed deviation, experimental data at short contact times were omitted in the estimation of k_0.

In order to take into account the effect of X_0 on k_0 the following equation was proposed:

$$k_0 = kX_0^n, \tag{4}$$

where k and n are empirical coefficients.

For each micro-organism coefficients k and n from Eq. (4) were determined from a log–log plot of k_0 vs. X_0 (Fig. 3). Each point of the curves corresponds to the average of two runs of a given experimental condition (X_0) thus 8 experiments were performed for *S. natans* and 12 experiments for strain E932; results are shown in Table 1. Within the assayed VSS concentration range (0.2–1.2 gVSS l^{-1}) the obtained results indicated that

chlorine decay rate due to *S. natans* was 2–4 times faster than strain E932. It should be stressed that the proposed model is for particulate material irrespectively of the biomass respiratory activity.

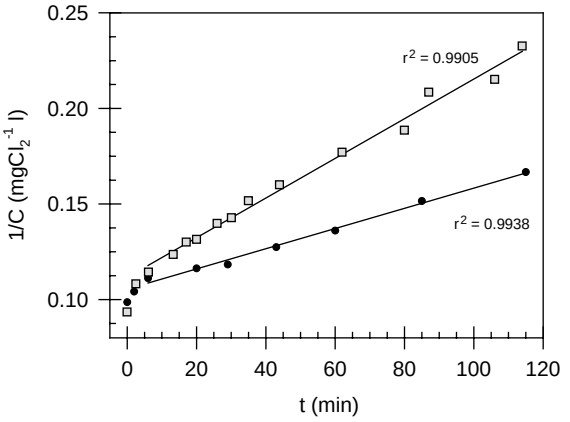

Fig. 2. Relationship between $1/C$ and time (t) for two typical experimental conditions. Lines correspond to the second-order model for chlorine decay at long contact times.

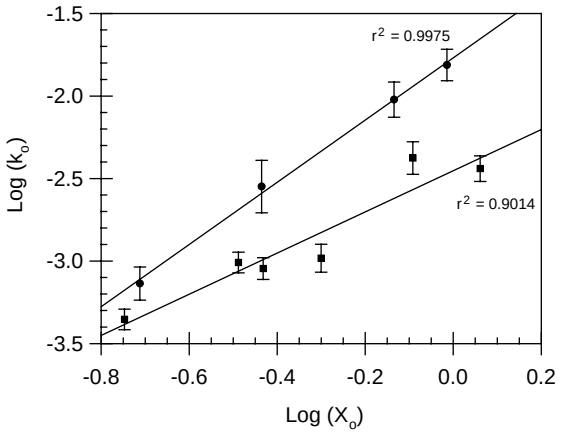

Fig. 3. Effect of initial biomass concentration (X_0) (gVSS l^{-1}) on the apparent second-order chlorine decay constant (k_0) at long contact times. (●) *S. natans*, (■) strain E932. Bars indicate the standard deviation. Lines correspond to the regression equation (Eq. (4)).

3.2. Chlorine decay at short times and inactivation of micro-organisms

Fig. 4 shows the effect of chlorine on the respiring fraction (F_R) of *S. natans* and strain E932 for different initial chlorine doses (D_0) as a function of time (t). For low D_0 values, F_R decreased with time until a constant value is reached. However, for higher D_0 respiration activity rapidly decreased reaching almost zero values. Similar results were previously described for *Escherichia coli* [47], *Yersinia enterocolitica* and *Klebsiella pneumoniae* [48] treated with chlorine dioxide and for *K. pneumoniae* treated with chloramines [49].

In complex systems such as the used in the present work, chlorine may react with different biomass components. It is very difficult to identify these components experimentally, as well as to determine their concentrations and their particular kinetic reactions with chlorine [29]. The model proposed in the present work considers that chlorine consumption depends on the presence of readily oxidized organic matter (Z) reacting at short contact times and on other compounds that react slowly and whose amount is directly proportional to the total biomass X_0; Z corresponds to the organic matter forming the biomass but it is not related specifically with micro-organism respiratory activity. Accordingly, a kinetic model that considers both active (respiring) biomass (X_R) and chlorine (C) decay rates was proposed as follows:

$$\frac{dX_R}{dt} = -k_R X_R C, \tag{5}$$

$$\frac{dZ}{dt} = -k_z Z C, \tag{6}$$

$$\frac{dC}{dt} = -\alpha_z k_z Z C - k X_0^n C^2, \tag{7}$$

where k_R is the biomass respiratory activity decay coefficient, k_z is the decay constant of the Z component and α_Z is a stoichiometric coefficient which represents chlorine uptake per Z mass unit.

Eq. (5) describes the decay of the biomass respiratory activity due to chlorination, Eq. (6) represents the decline of the Z component by chlorination and Eq. (7) describes chlorine decay due to reaction with Z

Table 1
Model parameters calculated by Eqs. (4) and (10) for pure cultures of *S. natans* and strain E932

Parameter	*S. natans*	Strain E932	Technique
k (l^{n+1} $mgCl_2^{-1}mgVSS^{-n}min^{-1}$)	0.0170 (0.0017)[a]	0.0039 (0.0006)	Chlorine decay
n (dimensionless)	1.89 (0.10)	1.28 (0.17)	
$\alpha_z f_z$ ($mgCl_2$ $(gVSS)^{-1}$)	11.9 (0.8)	4.5 (0.1)	Respirometry
K_R ($mgCl_2 l^{-1} min)^{-1}$	0.070 (0.005)	0.142 (0.006)	
k_z ($mgCl_2 l^{-1} min)^{-1}$	0.065 (0.009)	0.832 (0.066)	

[a] Standard deviation between parenthesis.

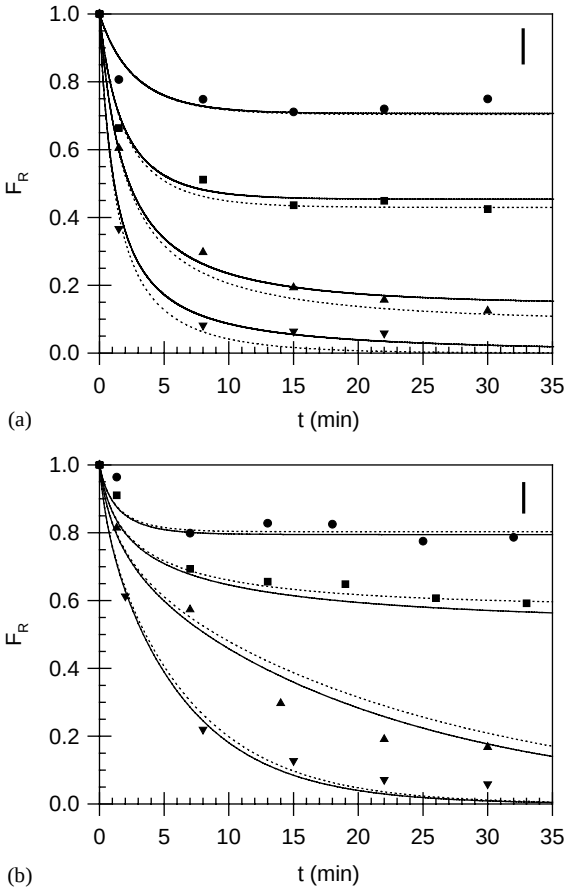

Fig. 4. Microbial respiring fraction (F_R) as a function of time (t) for different initial chlorine doses (D_0, $mgCl_2$ $(gVSS)^{-1}$) (a) *S. natans*: (●) 4.1, (■) 6.5, (▲) 9.5, (▼) 18.2. (b) strain E932: (●) 3.3, (■) 4.4, (▲) 5.2, (▼) 8.0. (—) numerical solution, (- - -) analytical solution (Eq. (10)). Bars in the upper right corner indicate the mean standard deviation.

(first term of the equation, at short contact times) and chlorine decay at longer times (second term). When Z decreases (Eq. (6)), the first term of Eq. (7) tends to zero and chlorine decay rate is represented by a second-order kinetics expressed by the second term of Eq. (7) in agreement with the previously described chlorine decay experiments. At short times (up to 5–10 min), experimental data showed that chlorine decay was mainly interpreted by the first term of Eq. (7) and the second term could be neglected. In these conditions, the analytical solution of the equations system led to the following expressions:

$$F_R = \frac{OUR}{OUR_0} = \frac{X_R}{X_{R0}} = \left(\frac{Z}{Z_0}\right)^{k_R/k_z}, \tag{8}$$

$$\frac{Z}{Z_0} = \frac{(C_0 - \alpha_z f_z X_0)e^{\alpha_z f_z k_z X_0 t}}{C_0 e^{k_z C_0 t} - \alpha_z f_z X_0 e^{\alpha_z f_z k_z X_0 t}}, \tag{9}$$

where f_z is a proportional factor between the total initial concentration of the components susceptible to react with chlorine (Z_0) and the initial biomass concentration (X_0), $Z_0 = f_z X_0$. By combining Eqs. (8) and (9) the following was obtained:

$$F_R = \frac{OUR}{OUR_0} = \frac{X_v}{X_{v0}} = \left[\frac{(C_0 - \alpha_z f_z X_0)e^{\alpha_z f_z k_z X_0 t}}{C_0 e^{k_z C_0 t} - \alpha_z f_z X_0 e^{\alpha_z f_z k_z X_0 t}}\right]^{k_v/k_z} \tag{10}$$

It should be emphasized that Eq. (10) is the simplified analytical solution of Eqs. (5)–(7) system and is only valid if the second term of Eq. (7) is negligible compared to the first term, that is for short times. In addition, coefficients α_z and f_z always appeared in Eq. (10) as the product $\alpha_z f_z$; this product corresponds to the amount of chlorine consumed ($mgCl_2$) per biomass unit (gVSS). Table 1 shows k_R and k_z coefficients and the product $\alpha_z f_z$ determined by non-linear regression analysis of Eq. (10) with the experimental data (F_R, t, C_0 and X_0); for each tested micro-organisms a good correlation between experimental data and calculated values from Eq. (10) was obtained ($r^2 = 0.9589$ for *S. natans* and $r^2 = 0.9830$ for strain E932).

In order to validate the simplified analytical equation, F_R values calculated with Eq. (10) were compared to those obtained by the numerical solution of the complete proposed model without any simplification (Eqs. (5)–(7)). For both calculation procedures, coefficients of Table 1 were used. Fig. 4 shows that similar results were obtained from both methods for different initial doses; thus, F_R may be estimated by the simplified analytical solution (Eq. (10)). From Eq. (10) it can be demonstrated that for initial chlorine concentrations $C_0 \ll \alpha_z f_z X_0$, the asymptotic F_R at long times ($F_{R\infty}$) results:

$$F_{R\infty} = \left(1 - \frac{C_0}{\alpha_z f_z X_0}\right)^{k_R/k_z} = \left(1 - \frac{D_0}{\alpha_z f_z}\right)^{k_R/k_z}, \tag{11}$$

where $D_0 (= C_0/X_0)$ is the initial chlorine dose ($mgCl_2$ $(gVSS)^{-1}$).

Fig. 5 shows experimental values of F_R corresponding to $t > 30$ min and values of $F_{R\infty}$ calculated with Eq. (11) as a function of D_0. For *S. natans* $F_{R\infty}$ decreased almost linearly as D_0 increased; however, a marked decrease of $F_{R\infty}$ for initial chlorine doses between 4 and 5 $mgCl_2(gVSS)^{-1}$ was observed for strain E932. Eq. (11) allows to understand the physical meaning of the product $\alpha_z f_z$. If the initial critical dose (D_c) is defined as the lowest initial dose that leads to a total inhibition of the micro-organism respiratory activity ($F_{R\infty} = 0$), it can be demonstrated that:

$$D_c = \left(\frac{C_0}{X_0}\right)_{F_{R\infty}=0} = \alpha_z f_z \tag{12}$$

thus, the initial critical dose (D_c) for *S. natans* was 11.9 $mgCl_2(gVSS)^{-1}$ while for E932 strain was 4.5 $mgCl_2(gVSS)^{-1}$ (Table 1). D_c can be interpreted as

the micro-organism resistance to chlorine; accordingly *S. natans* was 2–3 times more resistant to chlorine action than E932 strain.

It should be considered that all the used cultures observed under microscope did not form aggregates nor flocs, thus differences between micro-organisms in their resistance to chlorine is an intrinsic characteristic. The higher resistance of *S. natans* to chlorination should be attributed to the presence of a sheath constituted by proteins, polysaccharides and lipids [2,50,51] which would react with chlorine acting as a protection barrier. Because strain E932 do not have a sheath, its resistance to chlorination is lower.

Although floc-forming strain E932 is intrinsically more susceptible to chlorination than the filamentous micro-organism *S. natans*, in an activated sludge system the susceptibility may be different. An activated sludge with severe bulking problems, (corresponding to values of 5 or 6 in the subjective scoring of filament abundance according to Jenkins et al. [2], may be considered as a pure culture of filamentous micro-organisms [52]. In these cases, filamentous micro-organisms have higher exposure to chlorine than floc-forming ones, because filamentous micro-organisms extend from the floc surface towards mixed liquor [6,53]. Thus, the relative position of the different types of micro-organisms in the floc structure (internal for floc-formers and external for filamentous micro-organisms) is an important factor for controlling filamentous bulking by chlorination. Further investigation is needed in order to evaluate both the effect of the relative position and the intrinsic susceptibility contributions in the chlorination of actual filamentous sludges.

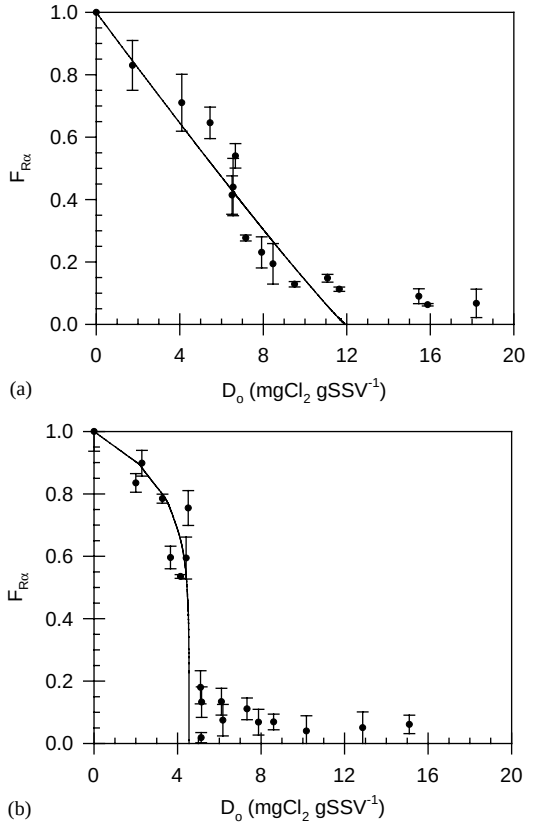

Fig. 5. Asymptotic values of the microbial respiring fraction ($F_{R\infty}$) as a function of initial chlorine dose (D_0). (a) *S.natans*, (b) strain E932. (●) Experimental data, (—) Proposed model (Eq. (11)). Bars indicate the standard deviation.

4. Conclusions

In the present work the chlorine decay kinetics and the decline of the respiratory activity of a filamentous (*S. natans*) or a floc-forming (*A. anitratus*) micro-organism were analyzed and a mathematical model was proposed. Microbial respiratory activity decline was measured using a respirometric technique. From the experiments and model calculations the following conclusions can be drawn:

- The proposed model described satisfactorily both chlorine consumption and microbial respiratory activity decline. The model includes three coupled differential equations corresponding to microbial inactivation, readily organic matter oxidation by chlorine and chlorine decay. Chlorine decay kinetics contains two terms: the first one describes the process at short contact times and depends on the presence of readily oxidized organic matter; the second term depends on other compounds that react slowly and whose amount is directly proportional to the total biomass.
- The rate of chlorine decay depended on both, type and concentration of the micro-organisms in the system. Chlorine consumption rate due to *S. natans* was 2–4 times faster than strain E932.
- Initial critical chlorine doses (the lowest initial dose that leads to a total decay of the micro-organism respiratory activity) were calculated with the proposed model. A higher value was obtained for *S. natans* (11.9 $mgCl_2$ $(gVSS)^{-1}$) than for the floc-former *A. anitratus* (4.5 $mgCl_2$ $(gVSS)^{-1}$). These critical doses indicated that in non-flocculated pure cultures floc-former *A. anitratus* was more susceptible to chlorine action than *S. natans*.

Acknowledgements

Authors gratefully acknowledge the financial support provided by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), the Universidad

Nacional de la Plata, Argentina and by the Project BID 1201 OC-AR PICT 09-04579 (Agencia Nacional de Promoción Científica y Tecnológica, Argentina).

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