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# INHIBITORY EFFECT OF A SURFACTANT ON PURE CULTURES OF A FILAMENTOUS AND A FLOC FORMING MICRO-ORGANISM

A. CARAVELLI[1], L. GIANNUZZI[1] AND N. ZARITZKY[1,2]*

[1]Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA), Facultad de Ciencias Exactas, Universidad Nacional de La Plata-CONICET, 47 y 116, La Plata (1900) Argentina
[2]Facultad de Ingeniería, Universidad Nacional de La Plata, 115 y 48, La Plata (1900) Argentina

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**ABSTRACT**

Activated sludge is the most widely used biological process for wastewater treatment. Inorganic and organic compounds are removed by a biotic community in the aeration basin. Problems of these systems are loss of settleability and poor sludge compaction due to excessive growth of filamentous micro-organisms. The filamentous bulking can be controlled by the addition of chemical agents. Strong oxidants, such as chlorine, are utilized to eliminate filamentous bacteria; however, these substances also tend to attack floc-forming bacteria and to cause process breakdown. Besides, chlorine may become hazardous owing to the formation of chemical products as chloramines. Surfactant addition constitutes an interesting alternative for the control of filamentous bulking. In this work the effect of a surfactant Triton X-100 (octylphenol ethoxylate), on the respiratory activity (RA) of pure cultures of a filamentous (*Sphaerotilus natans*) and a floc-former micro-organism (*Acinetobacter anitratus*) was evaluated. In the concentration range tested (60-220 mg $l^{-1}$), the surfactant was observed to exhibit high RA specific inhibition of the filamentous micro-organism with no significant effect on the floc-forming bacteria. Light microscopy observations showed that the surfactant induced cell lysis, leaving only empty sheaths in the case of filamentous micro-organisms. A kinetic equation to predict the microbial RA fraction of a *S. natans* pure culture as a function of surfactant concentration and contact time was proposed. The effect of Triton X-100 on the inactivation of pure cultures of both micro-organisms was compared to that of chlorine. Triton X-100 results were adequate to eliminate filamentous bacteria emerging as an alternative for filamentous bulking treatment.

Keywords: *Sphaerotilus natans*, Triton X-100, chlorination, respirometry, filamentous bulking.

## INTRODUCTION

The activated sludge process is the prevalent technology for biological treatment of wastewaters [1].

Solids separation determines the efficiency of the overall process. The process is based on the formation of bioflocs which involves microbial adhesion and aggregation of micro-organisms. The filamentous micro-organisms act as the backbone around which the floc-forming bacteria aggregate [2]. However, problems often appear which lead to poor settling and sludge compaction owing to excessive growth of filamentous bacteria. This phenomenon referred to as filamentous bulking is an important ecological and technical problem that leads to a discharge of suspended solids in receiving water bodies and operating problems in the activated sludge systems [3].

The filamentous bacteria *Sphaerotilus natans*, is a very common micro-organism in activated sludge systems that can cause severe bulking problems [2].

The filamentous bulking can be controlled by specific or non-specific methods. In all cases it is important to avoid an excessive damage of the floc-forming micro-organisms. Specific methods are applied to eliminate the causes responsible for growth of filamentous micro-organisms. As specific actions, selectors were implemented [4] along with modifications to influent, plant configuration, and operating conditions [1]. However, system modifications are sometimes impossible due to limitations in plant design, characteristics and system capacity.

Addition of chemical agents is one of the simplest forms of non specific bulking control; strong oxidants as chlorine, hydrogen peroxide, and ozone are utilized to

eliminate filamentous bacteria [5]. However, these substances also tend to damage floc-forming bacteria and to cause process breakdown. Chlorination was one of the first methods used to control filamentous bulking [6] and is still used [7,8]. However, chlorine is not selective, since it affects both filamentous and floc-forming micro-organisms, and, it is known that chlorine addition to effluents may become hazardous due to the formation of chemical products as chloramines, which are considered carcinogenic. Other authors [9] have determined the kinetics of chlorine action on a filamentous micro-organism (*Sphaerotilus natans*) and on one species of floc-former (*Acinetobacter anitratus*). It was observed that in pure cultures the floc-forming bacteria are 2 to 3 times more sensitive to chlorine than the filamentous micro-organism.

Studies on the application of chemicals with selective bactericide action are interesting to attain effective control of filamentous bulking without affecting plant performance when adequately dosed. Among other options, the addition of chemical surfactants can be an interesting method due to their ability to lysate filamentous bacteria; however few reports are found in the literature on this subject. Lytic activity tests with different anionic and non-ionic surfactants on *S. natans* and activated sludges with bulking problems were carried out elsewhere [10]. One of the advantages with regard to the use of surfactants is their low cost; some of them are harmless and can be easily mineralized by micro-organisms. Modern synthetic surfactants are generally considered to be biodegradable [11], and thus, when properly used can be useful to solve bulking problems without affecting floc-forming micro-organisms [10]. Notwithstanding, the use of surfactants to control bulking could favor foam formation; which can be controlled by using silicone-based antifoaming agents.

The objectives of the present work were: a) to evaluate the effect of the surfactant Triton X-100 on the respiratory activity of pure cultures of a filamentous (*Sphaerotilus natans*) and a floc-forming micro-organism (*Acinetobacter anitratus*); b) to develop a mathematical model that describes the decay of microbial respiratory activity of *S. natans* as a function of surfactant concentration and contact time, and c) to compare the effect of Triton X-100 with that of chlorine on the inactivation of pure cultures of both micro-organisms.

## MATERIALS AND METHODS

### Bacterial Strains and Culture Conditions

The ATCC # 29329 strain of *Sphaerotilus natans* was used as filamentous micro-organism, whereas the floc-former strain (*Acinetobacter anitratus*) was isolated from an activated sludge wastewater treatment plant (laboratory scale). Biomass concentrations were determined as chemical oxygen demand (COD) and transformed to volatile suspended solids (VSS) units using a calibration curve. Pure culture of these micro-organisms were grown in separate experiments using a bioreactor (volume = 1 l) operated with a continuous culture regime at 30ºC, pH= 7, dissolved oxygen concentration ≥ 2 mg $O_2$ $l^{-1}$ and dilution rates (D= flow rate (l $h^{-1}$)/reactor volume (V)) ranging between 0.085 and 0.227 $h^{-1}$ for *S. natans* and between 0.115 and 0.430 $h^{-1}$ for *A. anitratus*. Inactivation tests were carried out with pure cultures of the micro-organisms under steady state conditions.

The composition of the medium used in the bioreactor for micro-organism culture was as follows: citric acid monohydrate, 3480 mg $l^{-1}$; $(NH_4)_2SO_4$, 1000 mg $l^{-1}$; $MgSO_4.7H_2O$, 400 mg $l^{-1}$; $CaCl_2.2H_2O$, 50 mg $l^{-1}$; $KH_2PO_4$, 250 mg $l^{-1}$; $Na_2HPO_4.12H_2O$, 1000 mg $l^{-1}$; vitamin B12, 100 µg $l^{-1}$; $FeSO_4.7H_2O$, 15 mg $l^{-1}$; $ZnSO_4.7H_2O$, 5 mg $l^{-1}$; $MnSO_4.H_2O$, 3 mg $l^{-1}$; $CuSO_4.5H_2O$, 0.75 mg $l^{-1}$; $CoCl_2.6H_2O$, 0.15 mg $l^{-1}$; $(NH4)_6Mo_7O_{24}.4H_2O$, 0.5 mg $l^{-1}$; $BO_3H_3$, 0.1 mg $l^{-1}$; IK 0.1 mg $l^{-1}$.

### Inactivation Tests Using Triton X-100

Inactivation tests were carried out using Triton X-100 (octylphenol ethoxylate, $C_{14}H_{22}O(C_2H_4O)_n$) (Sigma Chemical Co), a non-ionic biodegradable detergent synthetized from octylphenol polymerized with ethylene oxide; which presents an average molecular weight of 625 and 9.5 ethylene oxide units (mean value) per molecule. Triton X-100 is soluble in $H_2O$ and organic solvents, and is usually employed for solubilization of cell membranes.

Inactivation tests were carried out in a 1 l glass flask under magnetic stirring and controlled temperature (30ºC). A culture volume of 300 ml, corresponding to each strain, was poured into the flask followed by variable volumes of Triton solution (10 g $l^{-1}$) to obtain surfactant concentrations ranging from 60 to 220 mg $l^{-1}$. Aliquots of 20 ml were extracted from the flask at different contact times (5-20 minutes), the effect of the surfactant on the micro-organisms was evaluated by measuring their oxygen uptake rate using a respirometric technique. Separate tests were carried out with each micro-organism.

Different biomass concentrations were used ranging from 300 to 1200 mg VSS $l^{-1}$ which were obtained by dilution with the culture medium mentioned above. Doses of surfactant ($D_0$, ratio between surfactant concentration and biomass concentration) ranged between 50 and 730 mg Triton g $VSS^{-1}$. By this method, it was possible to determine whether micro-organism inactivation as a function of time was affected by the biomass concentration present in each test. Experiments were carried out in duplicate.

### Chlorine-Based Inactivation Tests

Inactivation tests for both strains using chlorine were carried out under the same operating conditions of stirring and temperature as applied in the experiments using the surfactant. Three hundred milliliters collected from the bioreactor under steady state conditions were transferred to a glass flask; different volumes of a stock solution of sodium hypochlorite (NaClO) were added. Initial chlorine

concentration ($C_o$) ranged between 1.05 to 14.50 mg $Cl_2$ $l^{-1}$, which corresponded to initial doses ($D_o$, ratio between chlorine concentration and biomass concentration) ranging from 1.81 to 27.2 mg $Cl_2$ g $VSS^{-1}$ for *S. natans*. For *A. anitratus*, initial chlorine concentration was between 0.56 and 6.45 mg $Cl_2$ $l^{-1}$ equivalent to doses ranging between 2.05 to 9.15 mg $Cl_2$ g $VSS^{-1}$.

At different contact times (1 to 40 min) 20 ml samples were taken from the glass flask to assess the effect of chlorine on the micro-organisms by using a respirometric technique. All experimental conditions were tested in duplicate.

Microscopy Observations of Micro-Organisms

Microscopy observations were carried out under phase contrast illumination at 1000X magnification using a Leica DMLB microscope fitted with a photo camera [1, 9].

Respirometric Tests

The effects of the tested chemical agents on the micro-organisms were evaluated by respirometry. This technique is based on the relationship between the oxygen uptake rate by a given micro-organism (OUR) and its biomass concentration (X) according to the following expression:

$$OUR = q_{O_2} X \quad \text{(i)}$$

where $q_{O2}$ is the specific oxygen uptake rate of the tested micro-organism (mg $O_2$ g $VSS^{-1}$ $h^{-1}$).

The OUR is proportional to the biomass concentration bearing respiratory activity ($X_r$) that in a certain manner can be considered as a function of cell viability. Thus, the respirometric technique allows the fraction of micro-organisms respiratory activity (FR) to be calculated by means of the following equation:

$$FR = \frac{OUR}{OUR_o} = \frac{X_r}{X_{ro}} \quad \text{(ii)}$$

where $OUR_o$ corresponds to the control sample (before the addition of the chemical agent), $X_{ro}$ is the initial biomass concentration with respiratory activity in the control sample, whereas OUR and $X_r$ are the corresponding values after the chemical treatment.

The respirometer consisted of a 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 microprocessor at the frequency of 1 Hz. The OUR was measured by placing 20 ml aliquots of pure culture (with a known biomass concentration) in the respirometer. The aliquots were obtained from the bioreactor operating at steady state. A 1 ml aliquot of citric acid solution (3.5 wt/vol%, pH= 7) was added as oxidizable substrate and the mixture was aerated. When aeration stopped, a linear decrease in dissolved oxygen concentration was determined; the slope of this line corresponded to $OUR_o$ of the control sample.

For inactivation tests with chlorine and Triton, 20 ml aliquots of the culture were transferred from the glass flask to the respirometer and the OUR corresponding to each of the chemical agents employed was determined.

Statistical Analysis

Linear and non linear regressions were obtained using Sigma Plot 2.0 software. Analysis of variance was done using Systat software. The Bartlett test to analyze variance homogeneity was applied in each case.

## RESULTS AND DISCUSSION

Effect of Triton X-100 on Filamentous and Floc-forming Micro-organisms

In all experiments, the fraction of micro-organisms respiratory activity as a function of time (FR curves) was obtained for both *S. natans* and *A. anitratus* after Triton X-100 addition; the curves were independent ($P > 0.05$) of the biomass concentrations tested.

Figure 1 shows the FR fraction of a pure culture of the floc-forming micro-organism *A. anitratus* as a function of time for a Triton concentration treatment of 120 mg $l^{-1}$. As can be observed, FR remained high even after 5 min of contact with the surfactant, keeping values of 0.9 approximately, showing that floc-forming micro-organisms are not strongly affected by Triton X-100.

FR curves as a function of time for a pure culture of the filamentous micro-organism *S. natans* and a Triton concentration of 60 mg $l^{-1}$ are also shown in Figure 1. After 5 min contact time with the surfactant, a FR value of 0.6, lower than in the case of the floc-forming micro-organism, was obtained, showing that *S. natans* is more sensitive to the surfactant action than *A. anitratus*.

The inhibition of the respiratory activity for both micro-organisms depended significantly ($P < 0.05$) on the Triton concentration used. Figure 2 shows FR curves as a function of time for pure cultures of *S. natans* and *A. anitratus* exposed to Triton X-100 concentrations in the range of 60-220 mg $l^{-1}$, and biomass concentrations ranging between 300 and 1200 mg VSS $l^{-1}$.

When exposed to a Triton concentration of 80 mg $l^{-1}$ *A. anitratus* maintained relatively high FR values (0.85) even after 5 min contact time with the surfactant. This value became slightly lower (FR= 0.75) for a surfactant concentration of 220 mg $l^{-1}$. In contrast, *S. natans* exposed to a Triton concentration of 80 mg $l^{-1}$ showed a gradual inactivation as a function of time, reaching a FR value of 0.25 after 20 min contact time with the surfactant. For a higher

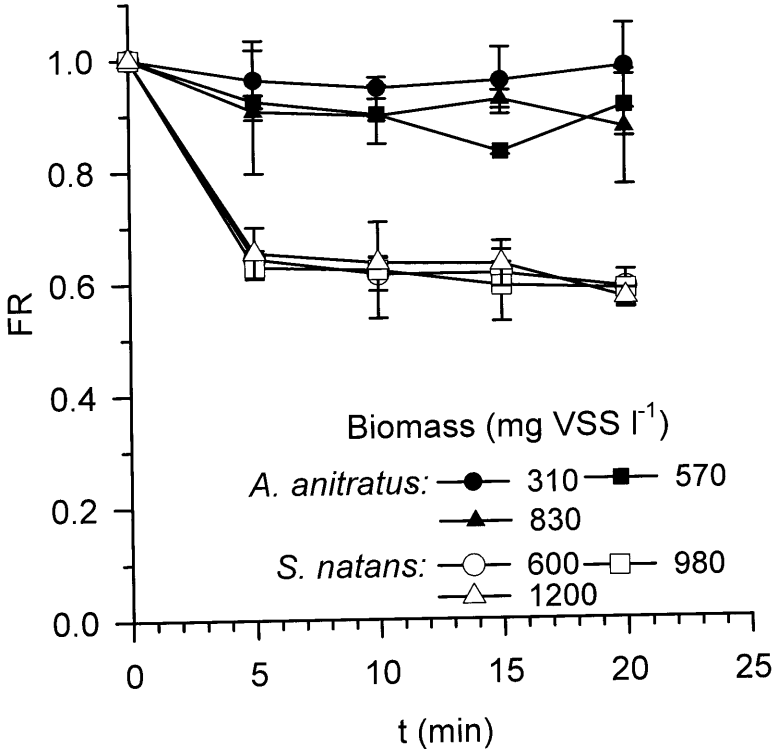


Figure 1. Respiratory activity fraction (FR) of the floc-forming *A. anitratus* and filamentous *S. natans* micro-organisms as a function of contact time with Triton X-100 for different biomass concentrations (mg VSS $l^{-1}$). *A. anitratus* was treated with a Triton X-100 concentration of 120 mg $l^{-1}$, and *S. natans* with a Triton X-100 concentration of 60 mg $l^{-1}$.

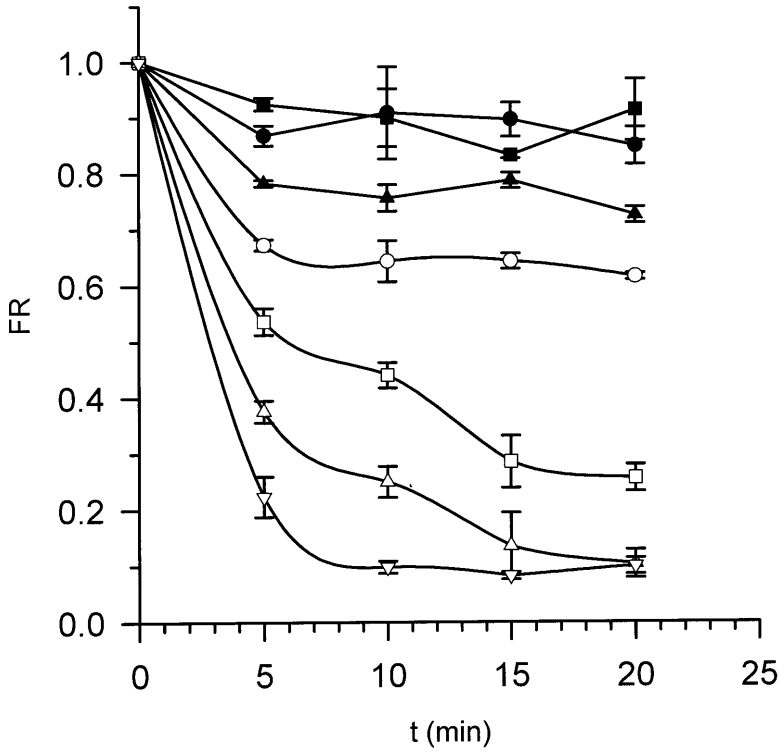


Figure 2. Effect of Triton X-100 concentration on the respiratory activity fraction (FR) as a function of time for the floc-forming and the filamentous micro-organisms. Triton X-100 concentration (mg $l^{-1}$) for *A. anitratus*: (●) 80, (■) 120, (▲) 220 (mg $l^{-1}$), and for *S. natans*: (O) 60, (□) 80, (Δ) 100, (∇) 120 (mg $l^{-1}$).

Triton concentration (120 mg $l^{-1}$), FR of *S. natans* decreased to 0.1 for an exposure time of 10 min, after which the fraction remained constant. Under the same conditions, the floc-forming micro-organism was less affected (FR= 0.85) by the surfactant in the range of the assayed biomass concentration (300-1200 mg VSS $l^{-1}$). For a lower Triton X-100 concentration (60 mg $l^{-1}$) FR values for *S. natans* decreased to 0.65 after 5 min contact, meanwhile floc-forming micro-organisms were unaffected by this concentration. Thus, *S. natans* was very sensitive to Triton in the concentration range tested, while the floc-forming micro-organism *A. anitratus* was less affected, even at relatively high surfactant concentrations (220 mg $l^{-1}$).

Light microscopy observation showed that cell lysis was induced by the surfactant Triton X-100 leaving only empty sheaths, when *S. natans* was exposed to a concentration of 120 mg $l^{-1}$ for 30 and 60 min.

A possible explanation for the different susceptibility of both micro-organisms to the surfactant was given elsewhere [10], where it was considered that strong surfactants (SDS and Triton X-100) cause collapse in the phospholipid bilayer that surrounds cell surface in gram-negative filamentous bacteria. However, many floc-forming bacteria, as is the case of *A. anitratus*, are gram-negative and less affected by these surfactants. Against this evidence, the same authors reported that many filamentous micro-organisms (*S. natans*, among others) exhibit a sheath which would protect bacteria cells; cell membrane in these bacteria would be weaker than in bacteria having no sheath.

Experimental data of *S. natans* inactivation as a function of time, for different concentrations of Triton (60-120 mg $l^{-1}$) and biomass (300-1200 mg VSS $l^{-1}$), were represented by a first order kinetic equation:

$$\frac{dX_r}{dt} = -k\ X_r \qquad \text{(iii)}$$

where $X_r$ is the biomass concentration with respiratory activity, and k the biomass decay coefficient. Triton concentration was maintained constant over each experiment.

Integrating Eq. (iii) and combining with Eq. (ii), results in the expression:

$$\ln FR = -k\ t \qquad \text{(iv)}$$

Experimental data of the effect of surfactant concentrations (60, 80 and 100 mg $l^{-1}$) on the inhibition of *S. natans* respiratory activity as a function of contact time, and the satisfactory agreement of Eq. (iv) is shown in Figure 3a. Decay coefficients (*k*) were obtained for different surfactant concentrations from the slopes of the linear regressions (Eq. (iv)).

Values of k were plotted as a function of surfactant concentration in Figure 3b and the following equation was fitted to the experimental data:

$$k = a\ (e^{b\ C} - 1) \qquad \text{(v)}$$

where C is the Triton concentration in mg $l^{-1}$.

The non linear regression fit ($r^2$= 0.81) led to the following parameters in Eq. (v): a= 0.01623 $min^{-1}$ (D= 0.00421 $min^{-1}$) and b= 0.02295 (mg Triton)$^{-1}$ l (D= 0.00222 (mg Triton)$^{-1}$ l). Eq. (v) calculated the decay coefficient *k* of the respiratory activity of the filamentous micro-organisms as a function of Triton concentration; subsequently the introduction of this coefficient in Eq. (iv) made it possible to predict FR values of *S. natans* as a function of both, time and surfactant concentration, FR being independent of biomass concentration.

The results obtained in the present work related to microbial inactivation by using Triton X-100 are in agreement with those presented elsewhere [10]. It was reported that applying a surfactant concentration of 50 mg $l^{-1}$ to a *S. natans* suspension in buffer solution (0.1 M $KH_2PO_4$, pH= 7), optical density at 600 nm decreased by 50%. When the surfactant concentration increased to 100 mg $l^{-1}$, activated sludge settling properties improved noticeably, producing lysis of filamentous micro-organisms Type 1701 and Type 021N, without considerably affecting the respiratory activity of the floc-forming micro-organisms.

In the present research a surfactant concentration of 100 mg $l^{-1}$ Triton X-100 inactivated the respiratory activity of the filamentous micro-organism *S. natans* by 90%, whereas its effect on the activity of the floc-former *A. anitratus* was only 15%.

Biosurfactants and some synthetic surfactants as Triton X-100 do present lytic activity on some types of filamentous micro-organisms resulting in their total destruction, despite the mechanism by which filamentous bacteria are selectively lysated is not totally understood [10].

## Effect of Chlorine on Filamentous and Floc-forming Micro-Organisms

The inhibition achieved by using the Triton X-100 surfactant was compared to the traditional use of the chlorination method. As an example, Figure 4a shows FR as a function of time for a pure culture of *S. natans* with different biomass concentration (1.15 and 0.35 g VSS $l^{-1}$), treated with a single initial chlorine concentration ($C_o$= 6.9 mg $Cl_2$ $l^{-1}$), which led to initial doses ($D_o$) of 6.0 and 19.71 mg $Cl_2$ g $VSS^{-1}$. Figure 4a also shows data of a pure culture of *A. anitratus* with biomass concentrations of 0.72 and 0.42 g VSS $l^{-1}$ treated with an initial chlorine concentration of 3.3 mg $Cl_2$ $l^{-1}$, which led to initial doses of 4.58 and 7.85 mg $Cl_2$ g $VSS^{-1}$.

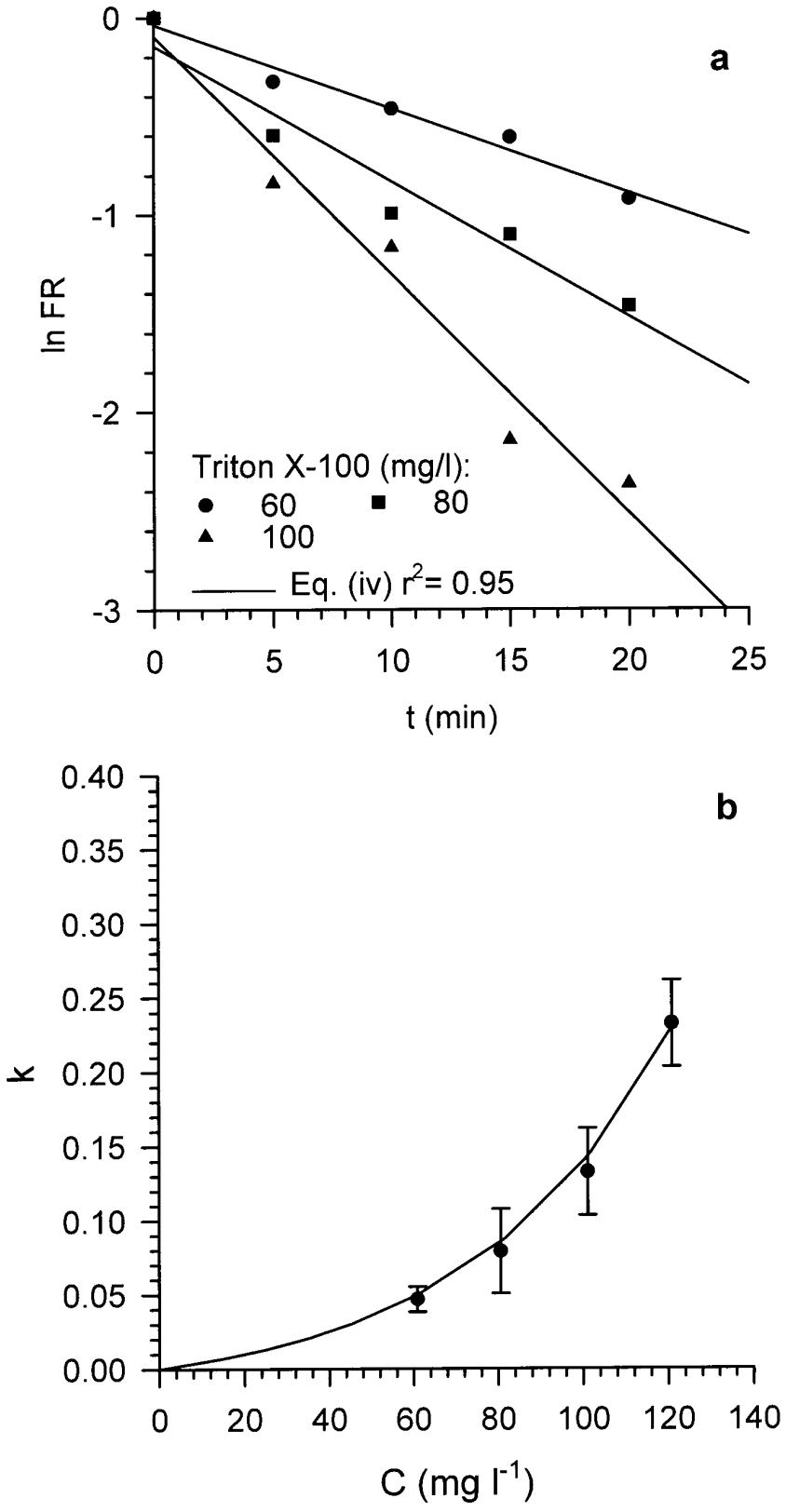


Figure 3. *S. natans* inhibition by Triton X-100 surfactant. a) Effect of time (t) and concentration (C) on respiratory activity fraction (FR) of *S. natans* (biomass concentration ranging between 300-1200 mg VSS $l^{-1}$). b) Respiratory activity decay coefficient (k) for *S. natans* as a function of Triton X-100 concentration (C, mg $l^{-1}$).

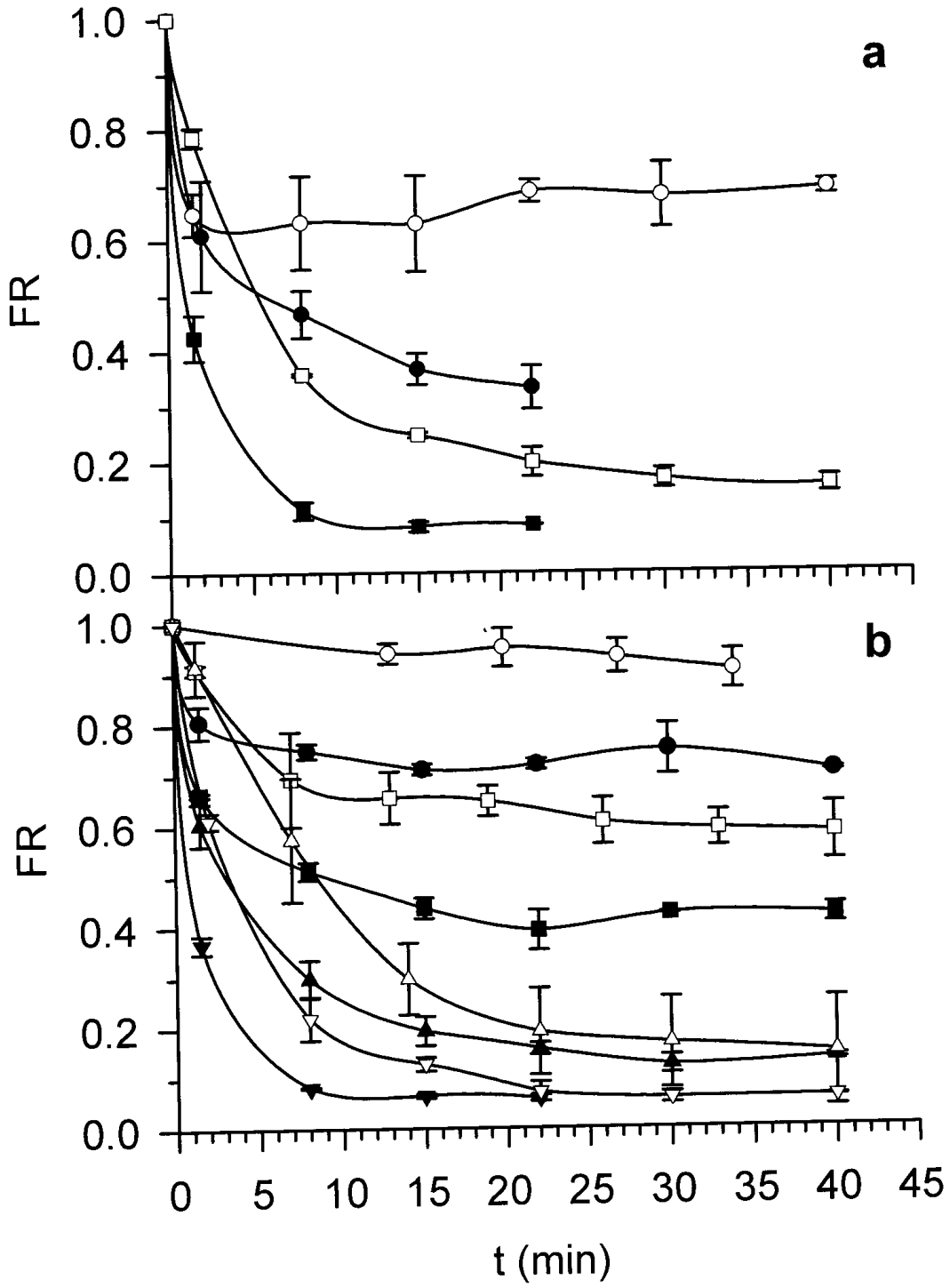


Figure 4. Fraction of bacterial respiratory activity (FR) as a function of time (t) for different initial chlorine doses ($D_o$, mg $Cl_2$ g $VSS^{-1}$). a) *S. natans* treated with an initial chlorine concentration ($C_o$)= 6.9 mg $Cl_2$ $l^{-1}$, corresponding to initial chlorine doses ($D_o$): (●) 6.0, (■) 19.71 (mg $Cl_2$ g $VSS^{-1}$); *A. anitratus* treated with an initial chlorine concentration= 3.3 mg $Cl_2$ $l^{-1}$, corresponding to initial chlorine doses: (O) 4.58, (□) 7.85 (mg $Cl_2$ g $VSS^{-1}$). b) *S. natans* treated with $C_o$ ranging between 1.05-14.50 mg $Cl_2$ $l^{-1}$, corresponding to $D_o$: (●) 4.1, (■) 6.5, (▲) 9.5, (▼) 18.2 (mg $Cl_2$ g $VSS^{-1}$); *A. anitratus* treated with $C_o$ ranging between 0.56-6.45 mg $Cl_2$ $l^{-1}$, corresponding to $D_o$: (O) 2.3, (□) 4.4, (Δ) 5.2, (∇) 8.1 (mg $Cl_2$ g $VSS^{-1}$). Bars indicate standard deviation of experimental data.

For each microbial strain, the application of a given initial chlorine concentration was observed to produce significant different ($P < 0.05$) inhibition curves (Figure 4a). For $C_o$= 6.9 mg $Cl_2$ $l^{-1}$ applied to *S. natans*, a respiratory activity inhibition of about 65% (FR= 0.35) was attained after 22 min contact time with chlorine, when the biomass concentration was 1.15 g VSS $l^{-1}$, which corresponded to a dose of 6.0 mg $Cl_2$ g $VSS^{-1}$. For a higher dose (19.71 mg $Cl_2$ g $VSS^{-1}$), that is when biomass concentration decreased to 0.35 g VSS $l^{-1}$, a rapid inhibition of *S. natans* was observed and reached a FR value of 0.1 after 8 min contact time (90% inhibition).

For *A. anitratus*, a $C_o$ value of 3.3 mg $Cl_2$ $l^{-1}$ led to almost 40% inhibition (FR= 0.6) after 2 min contact time when the dose applied was relatively low (4.58 mg $Cl_2$ g $VSS^{-1}$), whereas for a higher dose (7.85 mg $Cl_2$ g $VSS^{-1}$) the microbial respiratory activity rapidly decreased to FR value of 0.16 (contact time= 30 min), which represented an inhibition of 84% (Figure 4a).

The kinetic equations required to describe results of chlorine action on both microbial strains were more complex than for Triton X-100. The model, which includes three coupled differential equations corresponding to: 1) microbial inactivation, 2) readily organic matter oxidation and 3) chlorine decay, was reported in a previous work [9].

Figure 4b shows the fraction of bacterial respiratory activity (FR) as a function of time for pure cultures of *S. natans* and *A. anitratus* treated with different initial chlorine doses. These results allow the comparison of the sensitivity of both micro-organisms to this oxidant agent.

As observed, *A. anitratus* was more sensitive than *S. natans* to chlorine in the range of the concentrations tested. For a dose of 6.5 mg $Cl_2$ g $VSS^{-1}$ *S. natans* became inhibited by 60% (FR= 0.4) after a contact time of 20 min whereas *A. anitratus*, exposed to a lower dose of 5.2 mg $Cl_2$ g $VSS^{-1}$, was inhibited by 85% (FR= 0.15) after being in contact for 30 min. To attain 95% inhibition (FR= 0.05) for the case of *S. natans*, a dose of 18.2 mg $Cl_2$ g $VSS^{-1}$ was applied, whereas the dose required for the floc-forming micro-organism was considerably lower, 8.1 mg $Cl_2$ g $VSS^{-1}$ (Figure 4b).

A general description of the curves showed that at relatively low $D_o$ values (4.1 to 6.5 mg $Cl_2$ g $VSS^{-1}$ for *S. natans* and 2.3 to 4.4 mg $Cl_2$ g $VSS^{-1}$ for *A. anitratus*) FR decreased with time until reaching a constant value, whereas at higher $D_o$ the respiratory activity sharply decreased to FR values close to zero (Figure 4b). Similar results were described for *Escherichia coli* [12], *Yersinia enterocolitica* and *Klebsiella pneumoniae* [13] treated with chlorine dioxide and for *Klebsiella pneumoniae* treated with chloramines [14]. For all the experimental conditions FR remained constant after 30-40 min of contact time with chlorine (Figure 4b).

Oxidants such as chlorine must firstly diffuse through the cell wall [15] to cause inactivation only after vital constituents suffer a certain level of irreversible damage [16]. Chlorine has been verified to exert a wide range of disruptive effects such as oxidative decarboxylation of amino acids [17], inhibition of enzymes involved in intermediary metabolism [18], inhibition of protein biosynthesis [19], damage into chromosomal DNA [20], production of bacterial mutations [21], inhibition of membrane-mediated active transport processes and respiratory activity [18], and uncoupling of oxidative phosphorylation [22]. Besides, oxidant-based inactivation must be considered not to imply cell lysis. In this regard, *E. coli* cells remained morphologically intact after inactivation with a powerful oxidant as ozone, though for high concentrations or extended contact times cell lysis was observed [23].

### Comparison of the Surfactant Triton X-100 and Chlorine Effects on Respiratory Activity of Floc Forming and Filamentous Micro-Organisms

For *S. natans*, respiratory activity inhibitions ranging between 30 to 95% (FR values= 0.05-0.70) were attained when initial chlorine concentrations ranged between 1.05 to 14.50 mg $Cl_2$ $l^{-1}$ for contact times of 22 min. Similar inhibition values on the respiratory activity of *S. natans* (40-90%) were obtained using Triton X-100 with initial concentrations of 60 to 120 mg $l^{-1}$, and contact times of 20 min. Thus, lower chlorine concentrations with respect to Triton X-100 were needed to achieve similar respiratory activity inhibition of *S. natans;* this result might be explained considering the different mechanisms of action of both chemical compounds. For A. *anitratus*, when chlorine was applied at initial concentrations ranging between 0.56 to 6.45 mg $Cl_2$ $l^{-1}$ respiratory activity inhibitions of about 10 to 90% after 20 min of contact time were observed, whereas the inhibition values were lower (ranging between 10 to 25%) when this floc-forming micro-organism was exposed to Triton concentrations in the range of 80-220 mg $l^{-1}$ for the same contact time, thus *A. anitratus* was found to be very more sensitive to chlorine than to the surfactant.

## CONCLUSION

The respiratory activity fraction as a function of time of each micro-organism (*S. natans* and *A. anitratus*) after Triton X-100 addition depended on the Triton concentration but was not affected by the biomass concentration.

The filamentous micro-organism *S. natans* resulted in being most sensitive to the surfactant action than the floc-former *A. anitratus*. In the concentration range tested (60-220 mg $l^{-1}$), the surfactant was observed to exhibit specific inhibition of the respiratory activity on filamentous micro-organism with no significant effect on the floc-forming bacteria. Light microscopy observations showed that the surfactant induced cell lysis, leaving only empty sheaths in the case of filamentous micro-organisms.

A kinetic equation was proposed to predict respiratory activity fraction values of *S. natans* as a function of both time and surfactant concentration and it can be concluded that 100 mg $l^{-1}$ of Triton showed a gradual inactivation as a function of

time, reaching a FR value of 0.10 after 15 min contact time with the surfactant.

In the case of chlorine, respiratory activity fraction for each micro-organism depended on the initial chlorine dose. Both floc-forming and filamentous micro-organisms exhibited diverse sensitivity to chlorine and the surfactant. The floc-forming micro-organism *A. anitratus* was found to be very sensitive to chlorine although little effect was observed when concentrations as high as 220 mg $l^{-1}$ of Triton were used. Conversely, the filamentous micro-organisms *S. natans* was relatively less sensitive to chlorine, and was noticeably susceptible to the surfactant in the range of concentrations tested.

Thus, it can be concluded that bulking control using chemical agents as Triton X-100 acting specifically on filamentous micro-organisms would prevent any deleterious effect on the floc-forming micro-organism community and so on the sludge degradation capacity.

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