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GROWTH KINETICS OF THE FILAMENTOUS MICROORGANISM *SPHAEROTILUS NATANS* IN A MODEL SYSTEM OF A FOOD INDUSTRY WASTEWATER

E. M. CONTRERAS[1]*, L. GIANNUZZI[1] and N. E. ZARITZKY[1,2]

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

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Abstract—Bulking by *Sphaerotilus natans* has been attributed to several factors such as low dissolved oxygen in the aeration basin, wastes with high C:N ratios and phosphorus limitation; however, the occurrence of bulking has been reported in fruit, vegetable, meat and poultry wastewaters in which the ratio C:N is variable.

Growth of *S. natans* was analyzed in a model system of a food industry wastewater (potato processing waste) that was characterized by HPLC determining that citric acid was the most important identified component. The effect of several carbon sources on *S. natans* growth was also studied; different C:N ratios were tested in a continuous culture system (chemostat). This strain grew in a mineral medium with citric acid as a sole carbon source, in spite of the contradictory results found in literature. Chemostat studies showed that the medium was carbon-limited when C:N ratios <19 mgCOD (mgN-NH_3)$^{-1}$. Monod kinetic growth coefficients, determined for this strain in chemostat were: maximum specific growth rate, $\mu_{max}=0.301$ h^{-1}; Monod constant, $K_S=4.6$ mgCOD l^{-1}; true biomass growth yield, $Y^T_{X/S}=0.490$ mgVSS (mgCOD)$^{-1}$; endogenous decay rate, $k_d=0.011$ h^{-1} and maintenance coefficient, $m_S=0.022$ mgCOD (mgVSS)$^{-1}$ h^{-1}. The obtained parameters were compared with literature data and the effect of glucose and citric acid as carbon sources was discussed; these parameters are useful in modeling the growth of *S. natans* in potato processing wastewaters (or in other effluents under carbon-limiting conditions) especially when citrate is the main component and can be used to control filamentous bulking by metabolic or kinetic selection. © 2000 Elsevier Science Ltd. All rights reserved

Key words—filamentous bulking, food industry wastewater, kinetic coefficients, *Sphaerotilus natans*

NOMENCLATURE

D	dilution rate (h^{-1})
k_d	endogenous decay rate (h^{-1})
K_S	Monod constant (mgCOD l^{-1})
m_S	observed biomass maintenance coefficient (mgCOD (mgVSS)$^{-1}$ h^{-1})
q_S	specific rate of substrate utilization (mgCOD (mgVSS)$^{-1}$ h^{-1})
q_{Smax}	maximum specific rate of substrate consumption (mgCOD (mgVSS)$^{-1}$ h^{-1})
R_S	rate of substrate consumption (mgCOD l^{-1} h^{-1})
S	output substrate concentration (mgCOD l^{-1})
S_o	input substrate concentration (mgCOD l^{-1})
X	biomass concentration (mgVSS l^{-1})
$Y_{X/S}$	observed biomass growth yield (mgVSS (mgCOD)$^{-1}$)
$Y^T_{X/S}$	true biomass growth yield (mgVSS (mgCOD)$^{-1}$)
μ	specific growth rate (h^{-1})
μ_{max}	maximum specific growth rate (h^{-1})

INTRODUCTION

Food processing industries usually discharge large volumes of wastewater characterized by high chemical oxygen demand (COD) or biological oxygen demand, large amounts of total suspended solids and various inorganic constituents including nitrogen and phosphorus. The high organic load in the processing wastewater creates a pollution problem to water quality when discharged to rivers and lakes (Karim and Sistrunk, 1985). In the processing of vegetables, large amounts of waste in the form of peelings and starch as well as some sugars and proteins are released into the waste stream. With the increasing costs of pollution abatement and costly municipal surcharges, food processors are forced to find alternative methods in pre-treatment of wastewater prior to discharge for secondary treatment or other treatment systems (Karim and Sistrunk, 1984).

The activated sludge process is a widely used method for treating industrial and domestic waste-

*Author to whom all correspondence should be addressed.

water. One of the most common and serious problems in the operation of activated sludge plants is the inability to separate solids from treated effluents in the clarifiers. One particular solid separation problem, known as filamentous bulking, is caused by excessive growth of filamentous microorganisms beyond the confines of activated sludge flocs into the bulk solution (Sezgin *et al.*, 1978).

Bulking by *Sphaerotilus natans* has been attributed to several factors such as low dissolved oxygen in the aeration basin, treatment of wastes with high carbon to nitrogen (C:N) ratios and phosphorus limitation (Richard *et al.*, 1985; Jenkins, 1992; Jenkins *et al.*, 1993). However, the occurrence of bulking by *S. natans* has been reported in potato processing wastewater treatment plants (Eikelboom, 1977), meat and poultry wastewater (Strom and Jenkins, 1984), and fruit processing wastewater and brewery (ATV Working Group 2.6.1., 1989), in which the ratio C:N is variable.

There are two strategies to control filamentous bulking: nonspecific control methods and biological methods. The first group covers remedial actions that only treat the consequences or symptoms of the elevated occurrence of filamentous microorganisms in activated sludge (e.g. disinfectant addition). The effects of these methods are temporary and the actions should be repeated after a certain period. The second group (biological methods) comprises preventative actions to suppress the growth of filamentous microorganisms to such an extent that they do not hinder sedimentation and compaction, and selectively support the growth of floc-forming microorganisms. This task can be fulfilled in two ways. The first one is to create conditions under which the rate of substrate utilization and growth is significantly higher for floc-formers than for filaments, and they are referred to as kinetic selection. The second possibility is to block the metabolic pathways of substrate utilization to filamentous microorganisms and thus to stop their growth. These methods are referred to as metabolic selection. In activated sludge plants both kinds of selection are frequently combined as it occurs in plants with selectors to control filamentous bulking (Cech *et al.*, 1985; Jenkins *et al.*, 1993; Wanner, 1994). Modeling of *S. natans* growth is useful in the control of filamentous bulking by metabolic or kinetic selection. To understand the causes of the filamentous microorganisms' outgrowth in activated sludges, the growth kinetics of the causative organisms should be known. Available kinetics data from literature for the filamentous microorganism *S. natans* are limited to systems containing glucose as carbon source (Richard *et al.*, 1985). However, there are many wastewater systems where glucose may not be the main carbon source such as municipal, chemical, and food industry wastewaters. Previous studies (Contreras *et al.*, 1999) revealed that citric acid was an important carbon source in a potato processing industry wastewater and glucose was present in small quantities; however it is known that microorganisms have different maximum growth rates and growth yields depending on the substrate supplied (Babel *et al.*, 1993).

The objective of the present study was to characterize growth kinetics of *S. natans* in a model system of a food industry wastewater (potato processing plant) by the evaluation of: (i) the wastewater composition; (ii) wastewater carbon sources usage for growth by *S. natans*; (iii) the influence of different C:N ratios on *S. natans* growth tested in a continuous culture system (chemostat); and (iv) the kinetic growth coefficients (maximum specific growth rate, Monod constant, true biomass growth yield, endogenous decay rate, and maintenance coefficient) measured in chemostat.

MATERIALS AND METHODS

Production of the wastewater model system

The model system of a food industry wastewater was obtained by dipping 3000 g of potato cubes 1-cm side in 7 l of tap water at room temperature (Bertola *et al.*, 1999). Samples were drawn and filtered by Millipore HA 45 μm membranes and stored at -20°C for later analysis. Chemical oxygen demand (COD) was determined by a commercial kit (COD Reactor Model 456000, method 8000, Hach Corp., Loveland, USA). pH was evaluated with a pHmeter Hach EC30. Organic acids were analyzed by HPLC (Shimadzu) with an AMINEX HPX-87H-Biorad column. Operating conditions were: H_2SO_4 0.009 N as mobile phase, 0.7 ml min^{-1} flow, 580–600 psi, between 58 and 62°C (Picha, 1985). Glucose concentration was measured with a commercial kit (Wiener Lab.). Total Kjeldahl nitrogen (TKN) was measured with a Büchi equipment (Büchi Laboratoriums-Technik, Flawil, Switzerland).

Carbon compound usage for growth by S. natans ATCC ♯29329

S. natans ATCC ♯29329 was obtained from American Type Culture Collection. Cultures were maintained on Casitone–Glycerol–Yeast autolysate (CGY) agar slants at 4°C and subcultured at 6-month intervals (Dondero *et al.*, 1961). Biochemical tests were performed in solid media using Petri dishes with minimum saline media containing 1.5% agar to which different carbon sources were added: citric acid, glucose or acetic acid. The composition of the medium per liter was: carbon source (citric acid, glucose or acetic acid) 2400 mgCOD; $(NH_4)_2SO_4$ 2000 mg; $MgSO_4.7H_2O$ 400 mg; $CaCl_2.2H_2O$ 50 mg; KH_2PO_4 500 mg; $Na_2HPO_4.12H_2O$ 2000 mg; 1 ml of M1 solution, 1 ml of M2 solution, 100 μg of vitamin B_{12}. M1 solution contained (in g/100 ml): $FeSO_4.7H_2O$ 1.5, $ZnSO_4.7H_2O$ 0.5, $MnSO_4.H_2O$ 0.3, $CuSO_4.5H_2O$ 0.075, $CoCl_2.6H_2O$ 0.015, citric acid 0.6. M2 solution contained (in g/100 ml): $(NH_4)_6Mo_7O_{24}.4H_2O$ 0.05, BO_3H_3 0.01, KI 0.01. pH was adjusted to 7 ± 0.1 with NaOH. Sterilization was done under autoclave at 121°C for 15 min. Vitamin B_{12} was sterilized by filtration with a Millipore HA 0.45 μm membrane prior to being added to the sterilized medium. The same medium without any carbon source was used as negative control of growth. The inoculum was grown in a 0.1% peptone broth and was incubated for 48 h at 30°C. Plates were incubated for 48–72 h at 30°C.

Chemostat studies

Culture broth. Culture broth for chemostat studies was identical to the previously described solid medium but without agar. Monohydrate citric acid (MCA) was the only carbon source. For a total culture volume of 10 l, sterilization was carried out in an autoclave at 121°C for 45 min.

Influence of C:N ratio. In any study involving microorganism growth in a chemostat it is important to determine the limiting substrate. The limiting substrate is determined by the relative amounts of the nutrients that are present in the culture media. In the present study *S. natans* growth was analyzed in a chemostat using different C:N ratios in the previously described medium. Citrate concentration was maintained at 2400 mgCOD l^{-1} and ammonium sulfate concentration varied from 100 to 2000 mg l^{-1} (20–400 mgN-NH_3 l^{-1}); in all cases dilution rate was 0.20 h^{-1}.

Chemostat operation. Figure 1 shows the continuous flow reactor used in the experiments; the chemostat has a 1-l reactor with a working volume $V = 0.8$ l.

S. natans innocula consisted of cells from agar slant. The chemostat was operated as a batch for several hours until growth of *S. natans* was observed; then, pumping of fresh medium was begun. Tested dilution rates (D) ranged between 0.02 h^{-1} and 0.30 h^{-1}. The system was considered to run under steady-state conditions after operating for a period of at least five residence times (Pirt, 1975). Operating temperature was 20 ± 1°C; an aerator provided culture aeration. Oxygen transfer coefficient (Kla) was measured by the sulfite method (Eckenfelder and Ford, 1970) and depending on stirring rate, Kla values between 70 h^{-1} and 90 h^{-1} were obtained; aeration was enough to maintain dissolved oxygen concentration above 4 mgO_2 l^{-1}.

Chemostat studies allowed to determine growth kinetics coefficients. Classical steady state methods were used to determine the Monod equation coefficients: maximum specific growth rate (μ_{max}), half saturation coefficient for citric acid (K_S), endogenous coefficient (k_d), true biomass growth yield ($Y^T_{X/S}$) and specific maintenance energy (m_S) (Pirt, 1975).

Mass balances for microorganisms and limiting substrate in the chemostat under steady state conditions were considered and the following expressions were obtained:

$$\mu - k_d - D = 0 \tag{1}$$

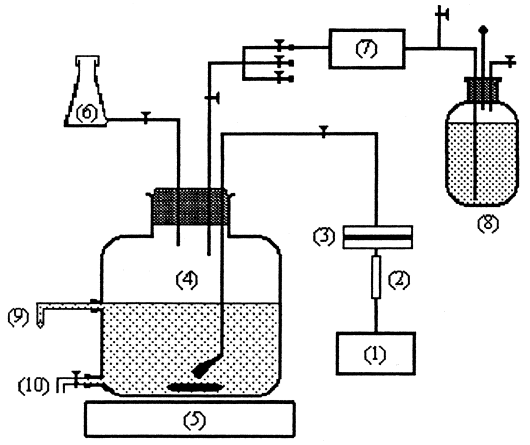

Fig. 1. Scheme of the chemostat system used for the experiments: (1) aeration pump; (2) cotton pre-filter; (3) 0.45 μm Millipore AP double filter; (4) bioreactor; (5) magnetic stirrer; (6) vitamins and inoculum reservoir; (7) peristaltic pump; (8) culture broth reservoir; (9) overflow output; (10) sampling line.

$$D(S_o - S) - R_s = 0 \tag{2}$$

where μ is the specific growth rate (h^{-1}); k_d the endogenous decay rate (h^{-1}); D the dilution rate (h^{-1}); S_o and S are the input and output substrate concentration (mgCOD l^{-1}); and R_S is the rate of substrate consumption (mgCOD l^{-1} h^{-1}).

The true biomass growth yield ($Y^T_{X/S}$, mgVSS (mgCOD)$^{-1}$) can be defined as:

$$Y^T_{X/S} = \frac{\mu X}{R_S} \tag{3}$$

where X is the biomass concentration (mgVSS l^{-1}) under steady state conditions. Combining equations (1)–(3) the following was obtained:

$$q_S = \frac{D(S_o - S)}{X} = \frac{D}{Y^T_{X/S}} + \frac{k_d}{Y^T_{X/S}} \tag{4}$$

where q_S is the specific rate of substrate utilization (mgCOD (mgVSS)$^{-1}$ h^{-1}). The observed biomass growth yield can be calculated as:

$$Y_{X/S} = \frac{X}{S_o - S} \tag{5}$$

Combining equations (4) and (5) the relationship between observed and true biomass yield was obtained:

$$Y_{X/S} = Y^T_{X/S}\frac{D}{D + k_d} \tag{6}$$

The maintenance coefficient m_S (mgCOD (mgVSS)$^{-1}$ h^{-1}) (Pirt, 1975) was calculated according to:

$$m_S = \frac{k_d}{Y^T_{X/S}} \tag{7}$$

Monod equation parameters (Monod, 1949) were also calculated according to the following equation:

$$q_S = q_{Smax}\frac{S}{K_S + S} \tag{8}$$

where q_{Smax} is the maximum specific rate of substrate consumption (mgCOD (mgVSS)$^{-1}$ h^{-1}) and K_S the Monod constant (mgCOD l^{-1}).

Values of S_o, S and X were measured at different D; equations (6)–(8) were used to calculate kinetic and stoichiometric constants. The value of μ_{max} was calculated with equation (3) considering $q_S = q_{Smax} = R_{Smax}/X$.

Washout and batch methods were used to evaluate μ_{max} and k_d (Pirt, 1975) for comparison purposes. Increasing D to a D_L value higher than μ_{max} the culture is washed-out; since substrate increases it can be assumed that μ equals μ_{max}. In the washout method if D_L is constant, the mass balance for microorganisms in the reactor can be integrated and the following expression is obtained:

$$\ln(X) = \ln(X_0) + (\mu_{max} - k_d - D_L)t \tag{9}$$

where X and X_0 are the biomass concentration at time t and zero, respectively. Equation (9) shows biomass variation inside the reactor with respect to time. Thus, measuring biomass or any related property such as optical density with time, the difference ($\mu_{max}-k_d$) was calculated. The determination of ($\mu_{max}-k_d$) was carried out on a culture at its exponential phase using the washout method. Feed flow rate was adjusted to obtain a $D_L = 0.50$ h^{-1} and culture absorbancy was measured.

Parameters μ_{max} and k_d were estimated individually in batch experiments. If microorganisms are growing under

Table 1. Composition of the wastewater model system[a]

t (h)	pH	COD	TKN	P_T	Glc	Oxalic	Citric	Malic	Lactic	Acetic	COD:N:P
2.5	6.4	2288	260	34	149	19	350	121	n.d.	n.d.	100:11.4:1.5
6.5	6.6	2840	292	44	197	19	436	176	136	n.d.	100:10.3:1.5
20.5	6.5	5412	574	48	6	27	890	109	753	157	100:10.6:0.9

[a]All quantities (except pH) are expressed as mg l^{-1}. n.d.: not detected.

exponential phase, then, the following is obtained:

$$\ln(X) = \ln(X_0) + (\mu_{max} - k_d)t \quad (10)$$

When substrate is exhausted μ equals 0 and equation (10) leads to:

$$\ln(X) = \ln(X_0) - k_d t \quad (11)$$

From a culture of *S. natans* under steady state at $D = 0.20\ h^{-1}$, mixed liquor was drawn enough so that only 200 ml culture remained in the reactor. Then, 600 ml of fresh broth was added and the reactor run under batch conditions; optical density was measured throughout the experiment.

Measurements. Biomass concentration was determined by volatile suspended solids (VSS) (APHA, 1989). pH values were assessed by a Hach electrode model EC30. Citric acid concentration was measured with a commercial kit (Boehringer Mannheim) using an UV-enzymatic method. Monohydrate citric acid (MCA) was converted into COD units using the conversion factor 0.69 mgCOD $(mgMCA)^{-1}$ according to the combustion stoichiometry. COD was determined as previously described. N-NH_3 was determined by a Hach equipment (method 10031). All measurements were made on filtered effluent with 45 μm Millipore HA membrane. Optical density measurements were carried out with a spectrophotometer DU 650 Beckman at 620 nm. All determinations were done at least in duplicates and results were expressed as the average value.

Gram, Neisser and poly-β-hydroxybutyrate (PHB) staining were performed according to the techniques described by Jenkins *et al.* (1993). Microscopy observations of preparations were made using light microscopy with a Leitz microscope, model Ortholux II (Germany).

Statistical analysis. Data fits were done by non linear regression analysis using a statistical software (Systat 5.02 for Windows). The Systat software calculates the set of parameters with the lowest residual sum of squares and their 95% confidence interval. It also provides for each data fit, the sum of squares, the degree of freedom (DF) and the mean square due to the regression and due to the residual variation.

RESULTS AND DISCUSSION

Wastewater model system

The composition of the wastewater model system used in the present study was analyzed during storage time and important variations were found (Table 1). For times up to 6.5 h, the wastewater composition was the result of the diffusion of substances like carbohydrates and organic acids that are naturally present in potatoes (Talburt and Smith, 1987). At 20.5 h a qualitative change in the wastewater composition was observed. Microorganisms utilized the organic matter to grow and metabolic products such as acetic and lactic acids were observed in the system. Nevertheless, the pH value was 6.4–6.6 and the COD:N:P ratio remained approximately constant at 100:10:1. A nutrient ratio of 100:5:1 is considered as the ideal one for carbon removal (Winkler, 1996). The experimental COD:N:P ratio between 2.5 h and 20.5 h indicates an excess of nitrogen while phosphorus was balanced with respect to the carbon source. Among the identified wastewater components, citrate was

the most important followed by other organic acids and glucose.

Carbon compound usage for growth by S. natans ATCC ♯29329

Biochemical testing showed that all assayed carbon compounds could sustain *S. natans* growth. However, in glucose medium *S. natans* grew slower and yielded less cellular material than in media with acetate or citrate. These results are in accordance with reports by other authors. Kämpfer *et al.* (1995) analyzed qualitative and quantitatively the nutritional requirements of *S. natans* in a batch system without agitation finding good growth in a medium containing 0.2–0.5 g l^{-1} of acetate but poor growth with glucose concentrations between 0.2 and 2 g l^{-1}. Mulder (1989) found that *S. natans* used citrate as a sole carbon source; however, Richard *et al.* (1985) classified citrate as a compound not used by the microorganism. This discrepancy may be due to the study of different strains. Four strains of *Sphaerotilus* sp. were isolated by Pellegrin *et al.* (1999) from slimes collected in different paper mill factories. They reported pronounced physiological differences between the isolates and *S. natans* DSM 6575(T) (ATCC ♯13338), DSM 565 (ATCC ♯29329) and DSM 566 (ATCC ♯29330) with respect to their ability to metabolize complex polysaccharides, sugars, polyalcohols, or organic acids.

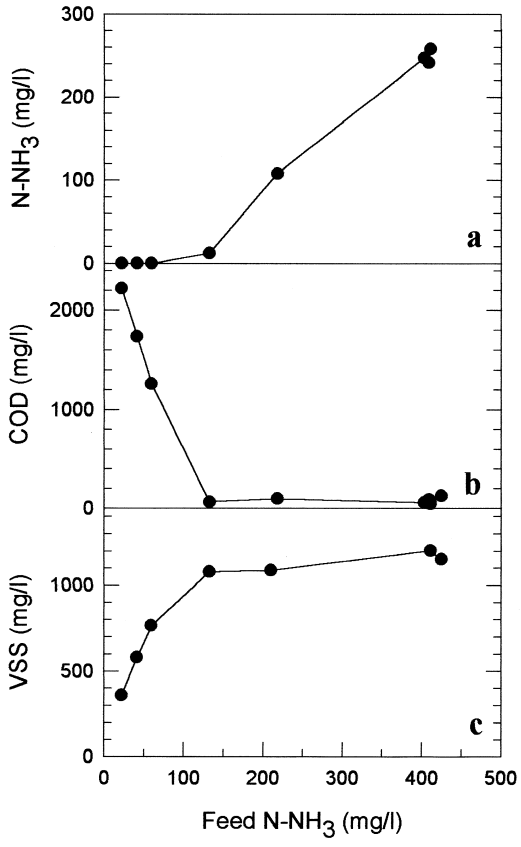

Fig. 2. Effect of $N\text{-}NH_3$ concentration in the feed stream on: (a) effluent $N\text{-}NH_3$ concentration; (b) COD; and (c) VSS. In all cases, citric acid concentration was held constant at 2400 mgCOD l^{-1}.

Influence of C:N ratio on S. natans ATCC ♯29329 growth in chemostat

S. natans growth was analyzed in a chemostat using different C:N ratios. In all cases, dilution rate was 0.20 h^{-1} and citrate concentration was held at 2400 mgCOD l^{-1}.

For nitrogen concentrations in the feed stream between 20 and 130 mgN-NH_3 l^{-1} (C:N > 19 mgCOD $(mgN\text{-}NH_3)^{-1}$), complete nitrogen utilization was observed (Fig. 2a), soluble COD decreased as nitrogen was added to the medium (Fig. 2b) while VSS increased (Fig. 2c). These results indicate that the culture was grown under nitrogen limiting conditions. However, when nitrogen concentration varied from 130 to 400 mgN-NH_3 l^{-1} (C:N < 19 mgCOD $(mgN\text{-}NH_3)^{-1}$), nitrogen was detected in the effluent, COD utilization was almost total and VSS were constant showing a limiting carbon source condition. Richard *et al.* (1985) found that *S. natans* reduced soluble COD to low levels up to a feed C:N ratio of 21; however soluble COD concentrations increased at greater feed C:N ratios. These results are in accordance with most of the previous studies in pure and mixed cultures and in sludge systems which showed that when the ratio C:N < 20 mgCOD $(mgN\text{-}NH_3)^{-1}$, the system is carbon limited.

Determination of kinetic parameters and biomass yield

Based on obtained results, *S. natans* growth kinetics were studied in a chemostat using a medium with monohydrate citric acid (MCA) as the only carbon source (2400 mgCOD l^{-1}). A concentration of 400 mgN-NH_3 l^{-1} corresponding to a C:N ratio of 6 mgCOD $(mgN\text{-}NH_3)^{-1}$, ensured nitrogen in excess. Thus, kinetic parameters correspond to a carbon limited medium.

The typical *S. natans* ATCC ♯29329 appearance in this culture medium is shown in Fig. 3a, b. Relatively long filaments (10–100 × 1.3–1.6 μm) composed of round-ended, Gram-negative and Neisser-negative, rod-shaped cells contained in a clear tightly fitting sheath were observed. Cells contained sudanophilic granules, PHB, observed even under carbon limiting conditions.

Although the culture broth contained a relatively strong buffer system (0.01 M PO_4^{-3}), microorganisms increased the pH of the medium. The pH in the outlet stream was 8.4 ± 0.2, with a decreasing trend as D increased (Fig. 4a). The observed increase of pH indicated proton-consuming reactions in the metabolic breakdown of citrate. This behavior was also observed in some lactic acid bac-

teria utilizing citrate as a sole carbon source (Ramos *et al.*, 1994; Magni *et al.*, 1996).

Figure 4b shows the effect of dilution rate (D) on the biomass and substrate concentrations. As D increased, VSS decreased. Additionally, citric acid concentration in the reactor increased above $D = 0.25\ h^{-1}$. At D values higher than $0.30\ h^{-1}$ a continuous washout was observed and steady state could not be reached. The effect of D on the observed yield ($Y_{X/S}$) is shown in Fig. 5. As D increased, $Y_{X/S}$ increased up to a limiting value. The true growth yield ($Y^{T}_{X/S}$) and the endogenous decay rate (k_d) were calculated according to equation (6). Figure 6 shows the effect of the substrate concentration on the specific rate of substrate utilization (q_S). Monod equation parameters were calculated according to equation (8).

Kinetic parameters and yield coefficients for *S. natans* obtained in this work using the three previously described methods (chemostat, washout and batch culture) were compared to available literature data (Table 2). Substrate concentration was expressed as COD to allow a valid comparison with data from Richard *et al.* (1985). Original data from

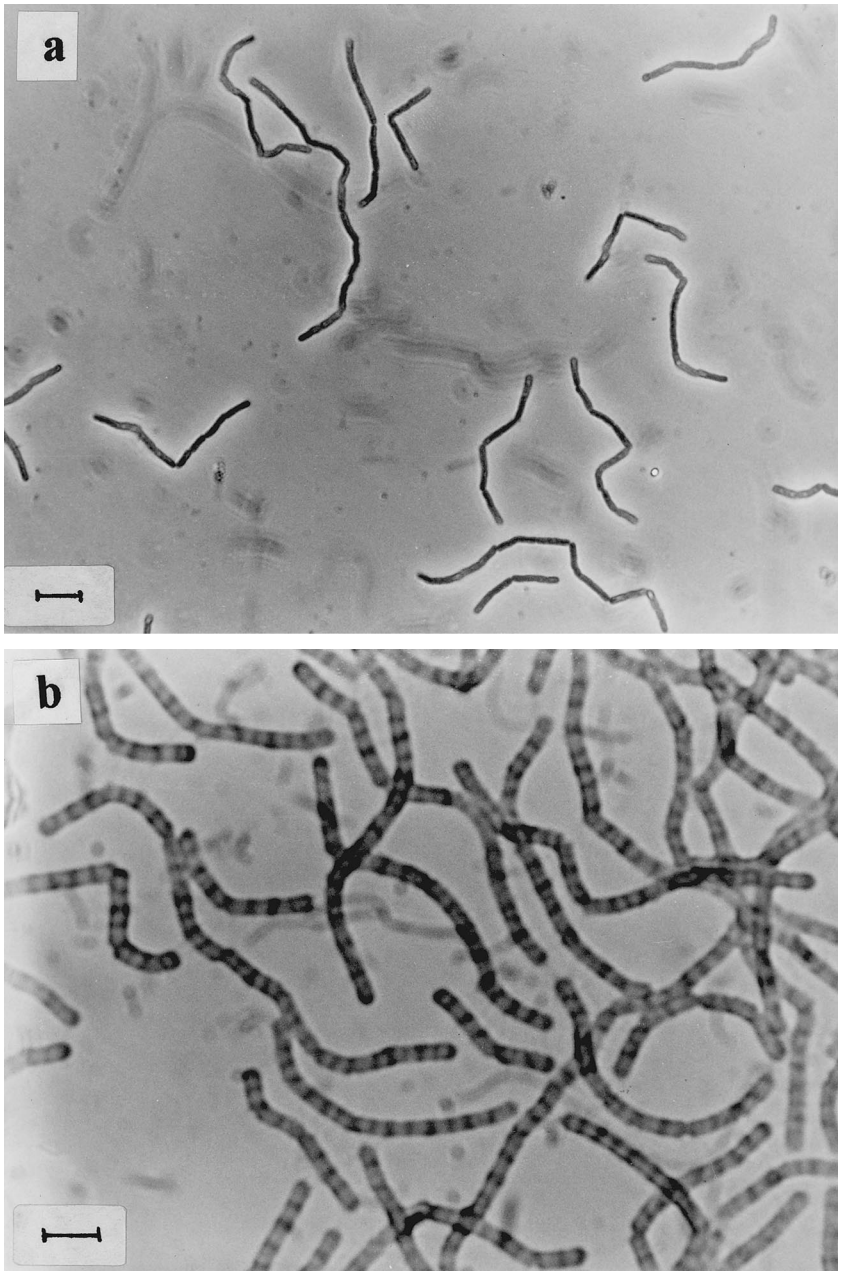

Fig. 3. Micrographs of *S. natans* ATCC ♯29329 in the used culture media. (a) 500 ×, phase contrast. Bar 50 μm. (b) 1000 ×, direct illumination, PHB staining. Bar 20 μm.

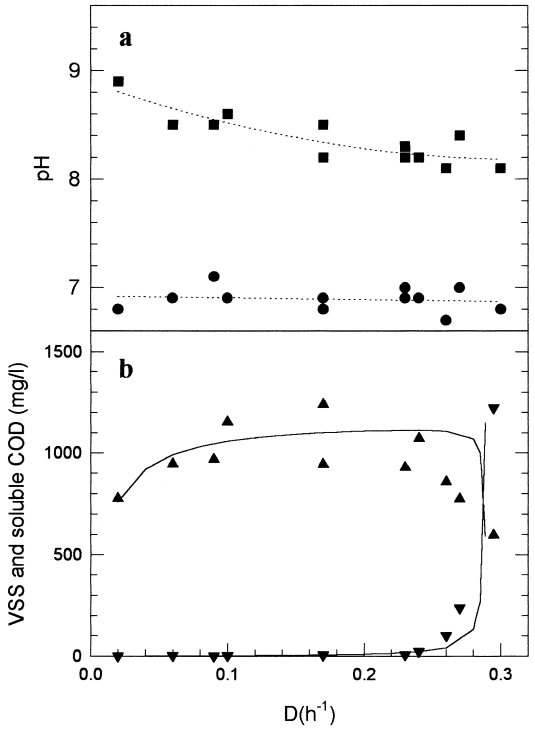

Fig. 4. Effect of dilution rate (D) on (a) ● pH of the feed stream, ■ pH of the effluent stream, (– – –) regression line; and (b) ▲ VSS, ▼ citric acid, (—) model prediction; model parameters are shown in Table 2.

Richard *et al.* (1985) were obtained with glucose (Glc) as a sole carbon source; the conversion factor applied to these values was 1.07 mgCOD $(\text{mgGlc})^{-1}$ according to oxidation stoichiometry. No available data about kinetic studies of *S. natans* with citrate as a sole carbon source were found in literature.

The values of μ_{max} for *S. natans* obtained in the present work by the different applied techniques are slightly higher than those corresponding to glucose (found in literature). Besides, K_S value for citrate (4.6 mgCOD l^{-1}) was approximately half of the glucose value (10.7 mgCOD l^{-1}) and indicates a higher affinity of *S. natans* for citrate than for glucose. Both factors contributed to increase growth rate of *S. natans* in citrate compared to glucose that is in agreement with findings in solid media. The obtained value of the endogenous decay rate k_d (0.011 h^{-1}) was significantly higher than that of literature (0.003 h^{-1}). Culture conditions such as temperature, pH, ionic strength and medium composition have a high influence on k_d or m_S values (Postgate and Hunter, 1962, 1964; Fieschko and Humphrey, 1984). The medium used in this work had a relative strong ionic strength due to the high ammonium, phosphate and sulfate concentrations; additionally, it should be considered the antimicrobial effect of citric acid due to its chelating capacity (Wiley, 1994). These factors may explain the higher obtained values for m_S and k_d.

On a COD basis, the values of $Y^T_{X/S}$ for *S. natans* that was grown in a medium with glucose or citrate as carbon sources were very similar. $Y^T_{X/S}$ can also be expressed on substrate molar basis. While for a glucose medium, biomass yield was 95.4 gVSS per mole of glucose consumed, a yield of 70.6 gVSS per mole of citrate consumed was observed in the present study. This difference may be attributed to the lower oxidation state of carbon in glucose compared to that in citric acid. The aerobic ATP production from glucose oxidation is 38 ATP/mole while from citric acid oxidation is 27 ATP/mole. Additionally, oxidation state of citrate carbon is higher than biomass carbon. Therefore, electrons coming from citrate oxidation must reduce citrate carbon to produce biomass and they are unavailable for ATP formation. These factors may explain the lower obtained values of $Y^T_{X/S}$ for *S. natans* in a medium with citrate with respect to glucose.

CONCLUSIONS

Composition analysis of the wastewater model

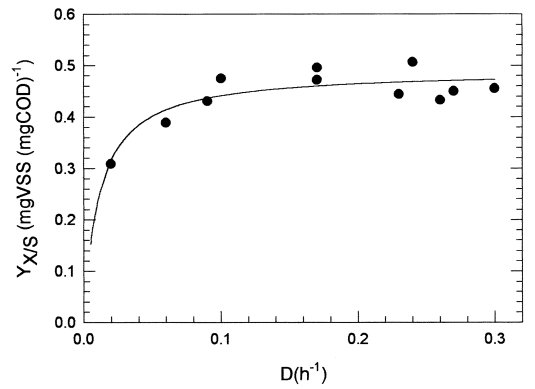

Fig. 5. Effect of dilution rate (D) on the observed biomass growth yield ($Y_{X/S}$) (●) experimental data, (—) equation (6).

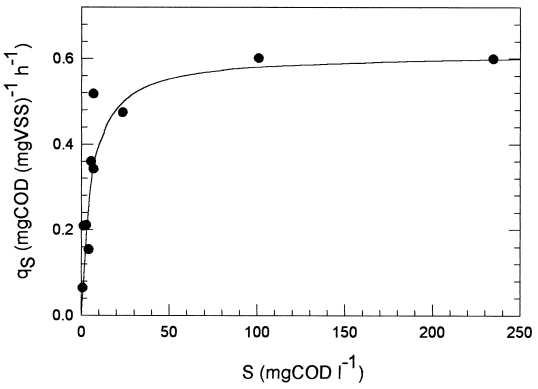

Fig. 6. Effect of substrate concentration (S) on the specific rate of substrate utilization (q_S) (●) experimental data, (—) equation (8).

Table 2. Comparison between bibliographic and obtained data

	S. natans ATCC ♯29329 (present work)	*S. natans* FA3[a] (adapted from Richard *et al.*, 1985)
μ_{max} (h^{-1})[c]	0.301 (0.029)[b]	0.27
μ_{max} (h^{-1})[d]	0.276 (0.010)	–
μ_{max} (h^{-1})[e]	0.288 (0.005)	–
k_d (h^{-1})[c]	0.011 (0.003)	0.003
k_d (h^{-1})[e]	0.010 (0.001)	–
K_S (mgCOD l^{-1})	4.6 (1.4)	10.7
$Y^T_{X/S}$ (mgVSS (mgCOD)$^{-1}$)	0.490 (0.014)	0.50
q_{Smax} (mgCOD (mgVSS)$^{-1}$ h^{-1})	0.614 (0.056)	0.55
m_S (mgCOD (mgVSS)$^{-1}$ h^{-1})	0.022 (0.006)	0.005

[a]Original data were expressed in glucose units. In this table the data were converted into COD units using the factor 1.07 mgCOD (mgGlc)$^{-1}$.
[b]Mean standard error between parentheses.
[c]Chemostat method.
[d]Washout method.
[e]Batch method.

system of a typical potato processing plant showed an excess of nitrogen while phosphorus was balanced with respect to the carbon source. Among the identified wastewater components, citrate was the most important followed by other organic acids and glucose.

Results showed that *S. natans* ATCC ♯29329 can grow in media with glucose or acetate as unique carbon source. *S. natans* can also utilize citrate as a sole carbon source, although literature shows contradictory results.

For feed nitrogen concentrations ranging between 20 and 130 mgN-NH_3 l^{-1} (C:N > 19 mgCOD (mgN-NH_3)$^{-1}$) nitrogen was totally used, residual COD decreased and VSS increased thus indicating a nitrogen limited system; however for a ratio C:N < 19 mgCOD (mgN-NH_3)$^{-1}$ the medium was carbon limited.

A culture medium was formulated containing citrate as sole carbon source with a ratio C:N of 6 mgCOD (mgN-NH_3)$^{-1}$ to ensure nitrogen excess. Monod kinetic growth coefficients determined for this strain in chemostat at the operating conditions were: $\mu_{max} = 0.301$ h^{-1}, $K_S = 4.6$ mgCOD l^{-1}, $Y^T_{X/S} = 0.490$ mgVSS (mgCOD)$^{-1}$, $k_d = 0.011$ h^{-1} and $m_S = 0.022$ mgCOD (mgVSS)$^{-1}$ h^{-1}. These parameters are useful in modeling the growth of *S. natans* in potato processing wastewaters (or in other effluents under carbon-limiting conditions) especially when citrate is the main component and can be used to control filamentous bulking by metabolic or kinetic selection.

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