Available online at www.sciencedirect.com
SCIENCE DIRECT®
Process Biochemistry 39 (2004) 1159–1165
PROCESS BIOCHEMISTRY
www.elsevier.com/locate/procbio

Kinetic models for nitrification inhibition by ammonium and nitrite in a suspended and an immobilised biomass systems

Julián Carrera [a,*], Irene Jubany [a], Lorena Carvallo [b], Rolando Chamy [b], Javier Lafuente [a]

[a] *Chemical Engineering Department, Universitat Autónoma de Barcelona, 08193 Bellaterra, Barcelona, Spain*
[b] *Biochemical Engineering Department, Universidad Católica de Valparaíso, General Cruz 34, Valparaiso, Chile*

Received 26 March 2003; accepted 12 June 2003

Abstract

Ammonium oxidation to nitrite nitratation and nitrite oxidation to nitrate (nitratation) were studied in a suspended biomass system (SBS) and an immobilised biomass system (IBS). Nitritation and nitratation rates at several substrate concentrations were obtained through respirometry. Process kinetics were evaluated by comparing different substrate inhibition models. The applied statistical criteria prove that the Aiba equation is the best model to describe nitritation inhibition by ammonium in the SBS and the IBS whereas the Haldane equation is the best model to describe nitratation inhibition by nitrite in both systems. The ratios between the kinetic coefficients in both systems suggest that the IBS coefficients are influenced by internal mass transfer in the biofilm. Moreover, the small difference in these coefficient ratios in the nitritation and the nitratation processes suggests that the distribution of the ammonium-oxidising and the nitrite-oxidising biomasses in the biofilm is homogeneous.

Keywords: Nitrification; Kinetic parameters; Substrate inhibition; Suspended and immobilised biomasses; Wastewater treatment

1. Introduction

The biological nitrogen removal (BNR) process is the most common methodology for removing ammonium from municipal wastewater, but this is not the usual treatment for high-strength ammonium wastewater, where physical–chemical systems such as stripping are more frequently used. Nevertheless, from an environmental and economical point of view, the BNR could be an interesting methodology for treating high-strength ammonium wastewater [1]. The BNR process includes two steps: oxidation of ammonium to nitrate or nitrification and reduction of nitrate to nitrogen gas or denitrification. The BNR of high-strength ammonium wastewater has an important operational problem; the inhibition by substrate of the nitrification process. Nitrification is a two-step reaction. Firstly, ammonium is oxidised to nitrite by ammonium-oxidising biomass. This process is called nitritation and its stoichiometry is:

$$NH_4^+ + 3/2O_2 \rightarrow NO_2^- + 2H^+ + H_2O \quad (1)$$

Secondly, nitrite is oxidised to nitrate by nitrite-oxidising biomass. This process is called nitratation and its stoichiometry is:

$$NO_2^- + 1/2O_2 \rightarrow NO_3^- \quad (2)$$

Both biomasses are inhibited by their own substrates: ammonium and nitrite. Frequently, the Haldane model has been used in modelling the inhibition of nitrification by substrate [2–4]:

$$r = \frac{r_{max} S}{K_S + S + \frac{S^2}{K_{IH}}} \quad (3)$$

Nevertheless, other models can be considered to describe inhibitions by substrate. Edwards [5] proposed the following kinetic model for describing the inhibition by substrate:

* Corresponding author. Tel.: +34-93-581-2141; fax: +34-93-581-2013.
E-mail address: julian.carrera@uab.es (J. Carrera).

0032-9592/$ – see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0032-9592(03)00214-0

$$r = r_{max}\left(\exp\left(-\frac{S}{K_{IE}}\right) - \exp\left(-\frac{S}{K_S}\right)\right) \quad (4)$$

Edwards [5] also suggested that substrate inhibition can be described by a modification of the equation proposed by Aiba et al. [6]. This equation was previously proposed for product inhibition of alcoholic fermentation:

$$r = \frac{r_{max}S}{K_S + S}\exp\left(-\frac{S}{K_{IA}}\right) \quad (5)$$

Luong [7] proposed the application of substrate inhibition to the microorganism growth, describing butanol inhibition on yeast growth with the following kinetic model:

$$r = \frac{r_{max}S}{K_S + S}\left(1 - \left(\frac{S}{S_m}\right)\right)^n \quad (6)$$

in which, r, substrate uptake rate (g N m^{-3} min^{-1}); r_{max}, maximum substrate uptake rate (g N m^{-3} min^{-1}); S, substrate concentration (g N m^{-3}); K_S, half-saturation coefficient (g N m^{-3}); K_{IH}, Haldane inhibition coefficient (g N m^{-3}); K_{IE}, Edwards inhibition coefficient (g N m^{-3}); K_{IA}, Aiba inhibition coefficient (g N m^{-3}); S_m, substrate concentration above which net growth ceases (g N m^{-3}); n, Luong coefficient.

There are some articles comparing different kinetic models to express growth kinetics of microorganisms inhibited by substrate. Meriç et al. [8] tested their own kinetic model and several models from other authors with *P. putida* and *T. cutaneum* growing with phenol as a substrate. Han and Levenspiel [9] tested their own kinetic model and others in the growth of a mixed culture with *n*-pentane. Velizarov and Beschkov [10] studied the inhibition of *Gluconobacter oxydans* by glucose. Tsuneda et al. [11] found that the Haldane model with an endogenous coefficient is the best kinetic model to describe BOD_5 removal in a three-phase fluidized bed. Tanyolaç et al. [12] analysed some growth kinetic models including a death factor, in order to find the best fit to corresponding data of inhibition of nitrification by ammonium sulphate. Tanyolaç et al. found that the best model to express the kinetic behaviour of the micro-organisms was the Monod model, including a death factor. Nevertheless, there are no references evaluating different kinetic models for substrate inhibition of both nitrification steps: nitritation and nitratation. Furthermore, there are no references comparing the kinetics of both nitrification steps in suspended and immobilised biomasses with the same operational conditions.

The aim of this work was to determine statistically, the best kinetic model for nitritation inhibition by ammonium and nitratation inhibition by nitrite in a suspended biomass system (SBS) and an immobilised biomass system (IBS) with the same operational conditions. Respirometry is the methodology chosen to quantify nitritation and nitratation rates at different ammonium and nitrite concentrations, respectively.

2. Materials and methods

2.1. Experimental set-up

SBS was an activated sludge system with three aerobic reactors (27 dm^3 each) and a settling tank. Each aerobic reactor has in-line sensors (DO, pH, ORP, temperature) connected to probe controllers. The IBS was an acrylic concentric-tube airlift reactor of 3.25 dm^3. A three-phase separator was located at the top of the reactor in order to retain biofilm particles. Biofilm was developed on small-suspended particles of 0.3 mm mean diameter. Internal mixing of wastewater and biofilm particles, as well as an efficient aeration was provided by the airflow coming through a porous glass diffuser at the bottom of the reactor. Both nitrification systems were maintained with the same operational conditions for 2 months before the respirometric experiments. Temperature and pH of both systems were maintained at 23 ± 0.5 °C and 7.5 ± 0.1 while the nitrogen loading rate was fixed in both systems at 0.1 g N–NH_4^+ g VSS^{-1} d^{-1}. The biomass concentration of SBS and IBS systems were 0.9 and 7.3 g VSS dm^{-3}, respectively.

2.2. Respirometry

The respirometer consisted of a glass vessel of 0.3 dm^3 internal volume with three ports at the top of the respirometer for insertion of a DO probe (WTW, Cellox 325), a pH probe and the injection of the test compounds. A magnetic stirring bar and a stirring plate provided internal mixing of mixed liquor and biofilm particles. Aeration was provided through a porous glass diffuser at the bottom of the respirometer. The respirometric tests of both systems were carried out at the same biomass concentration (0.3 g VSS dm^{-3}). For every respirometric test in the SBS, 0.1 dm^3 of mixed liquor were obtained from the second aerobic reactor, and diluted with water in the respirometer until 0.3 dm^3. For every respirometric test in the IBS, 12 cm^3 of settled biofilm nitrifying particles were added to the respirometer, and diluted with water until 0.3 dm^3. Both systems were kept without substrate for 5 h before every respirometric experiment in order to establish endogenous respiration, evaluated as a constant oxygen uptake rate (OUR). Temperature was kept at 23 ± 0.5 °C and pH at 7.5 ± 0.1 using 2 M NaOH. Once the mixed liquor reached saturation, substrate was added to the test liquor. DO depletion was monitored for 3 min and the OUR was determined twice for every substrate concentration, considering as statistical error two times the standard deviation.

2.3. Analytical methods

The analysis of volatile suspended solids (VSS) and ammonium were done using the methodology described in APHA's standard methods [13]. The analyses of nitrite (NO_2^-) and nitrate (NO_3^-) were done by capillary electrophoresis using a WATERS Quanta 4000E CE. The

Table 1
Results in the selection between the several kinetic models employed for the fitting of experimental data of nitritation

Model		SBS					IBS				
		R^2_{adj}	F	Coeff	Confidence limits	P	R^2_{adj}	F	Coeff	Confidence limits	P
Haldane	r_{max}			0.56	0.09(16%)	0.0001			0.19	0.02(11%)	0.0001
	K_S	0.808	28	13	5(39%)	0.03	0.937	84	33	10(30%)	0.009
	K_{IH}			384	154(40%)	0.03			1910	597(31%)	0.01
Aiba	r_{max}			0.51	0.06(12%)	0.0001			0.18	0.02(11%)	0.0001
	K_S	0.816	30	11	4(36%)	0.015	0.935	81	28	8(29%)	0.006
	K_{IA}			725	159(22%)	0.0008			3057	671(22%)	0.001
Luong	r_{max}			0.5	0.1(20%)	0.0009			0.18	0.03(170%)	0.0001
	K_S	0.798	18	*10*	*5*	*0.08*	0.927	48	28	11(39%)	0.02
	S_m			*9160*	*2×10^5*	*0.97*			*3.2×10^6*	*1.8×10^9*	*1*
	n			*12*	*287*	*0.97*			*1061*	*1.3×10^6*	*1*
Edwards	r_{max}			0.46	0.05(11%)	0.0001			0.158	0.01(6%)	0.0001
	K_S	0.777	24	14	4(29%)	0.0017	0.922	66	37	7(19%)	0.0007
	K_{IE}			800	197(25%)	0.0017			3796	993(26%)	0.004

Coefficients values in *italics* do not pass the *P criterion.*

electrolyte used was a WATERS commercial solution. The conditions of the analysis were: temperature of 20 °C, 15 kV from a negative source, indirect UV detection at 254 nm and 5 min of analysis.

3. Results and discussion

3.1. Nitritation inhibition by ammonium

Nitritation rates were obtained from the OUR measurements with ammonium sulphate as substrate. These OUR measurements were the sum of the oxygen consumed simultaneously in the ammonium oxidation to nitrite and the nitrite oxidation to nitrate. If the ammonium is completely oxidised to nitrate, 75% of the oxygen consumption is due to the ammonium oxidation to nitrite and 25% is due to nitrite oxidation to nitrate (Eqs. (1) and (2)). Consequently, the nitritation rate could be calculated with the 75% of the oxygen consumption. However, the nitrite oxidation to nitrate is inhibited by high ammonium concentrations [14,15]. Therefore, it is necessary to quantify the nitratation inhibition by ammonium to calculate the oxygen consumption due to nitritation. A batch experiment was carried out to quantify this inhibition. A initial ammonium concentration of 1000 mg N–NH_4^+ dm^{-3}, at 23 °C and pH of 7.5, was added in the respirometer with aeration and the ammonium and nitrite concentrations were monitored throughout the experiment. These concentrations show that only 30% of the nitrite formed was oxidised to nitrate while the ammonium concentration in the respirometer was greater than 50 mg N–NH_4^+ dm^{-3}. When the ammonium concentration decreased below this value, all the nitrite formed was oxidised to nitrate. With this result, it was possible to calculate the percentage of the OUR corresponding to the nitrite oxidation, and consequently to calculate the nitritation rate.

Several kinetic models were adjusted to the nitritation rates obtained at different ammonium concentrations during the experiments. The objective was to find the best kinetic model to describe quantitatively the kinetic behaviour of the nitritation process, in SBS and IBS systems. Experimental data were fitted to each model using the SIGMAPLOT®8.0 software [16]. The most appropriate kinetic models were selected based on statistical criteria. Table 1 shows the results of the selection between kinetic models, adjusted to the experimental nitritation data. Fig. 1 shows the experimental data and model predictions for the nitritation rate in both systems.

Firstly, the P value criterion was applied. The P value is the probability of being wrong in concluding that the coefficient is not zero. The smaller the P value, the greater the probability that the coefficient is not zero. Traditionally when $P<0.05$, it can be concluded that the independent variable can be used to predict the dependent variable [16]. Table 1 shows that the Luong coefficients were greater than 0.05 for the SBS and the IBS, Therefore, the Luong model was not valid to describe the nitritation inhibition by ammonium in both systems.

Secondly, the adjusted R^2 (R^2_{adj}) and the F statistic criteria were considered to choose the best kinetic model. R^2_{adj} is a measure, related to the certainty between the experimental data and the proposed model, (considering the number of independent variables, which reflects the degrees of freedom), Larger R^2_{adj} values (close to 1) indicate that the equa-

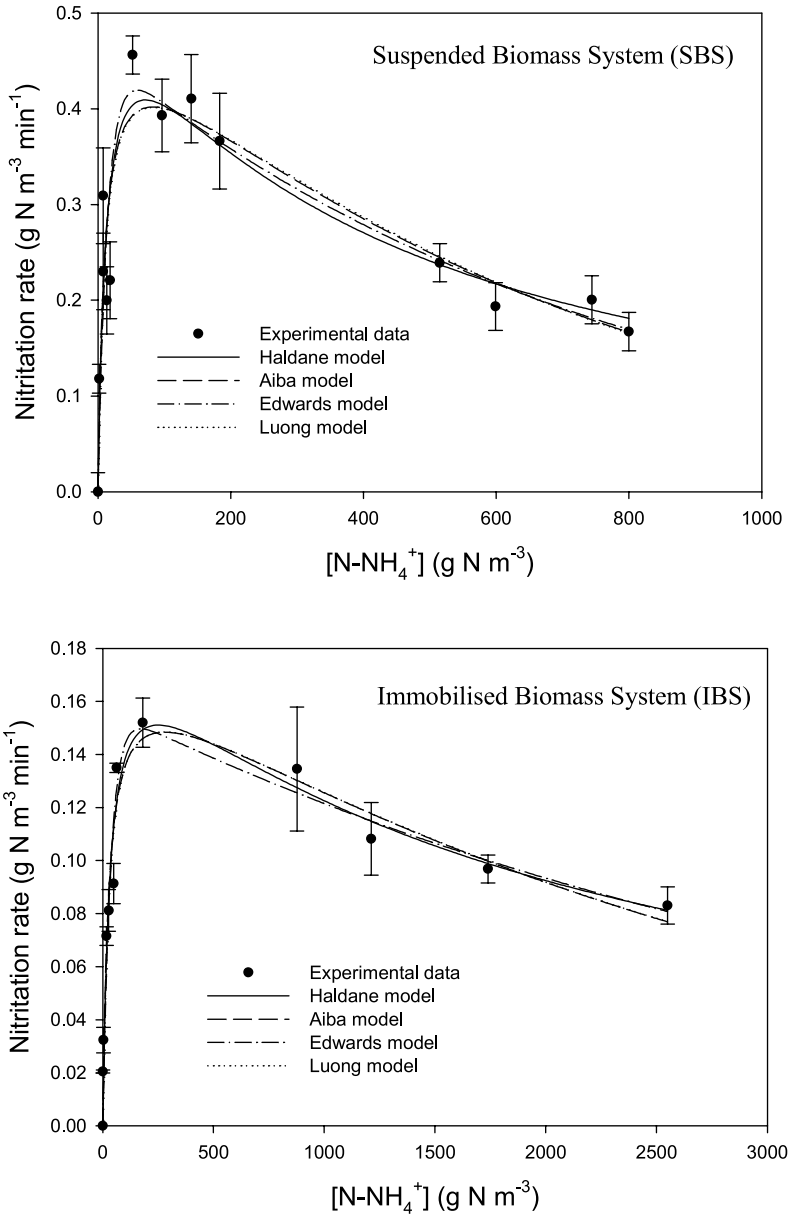

Fig. 1. Experimental data and model predictions of the nitritation rate for the SBS and the IBS.

tion is a good description of the relation between the independent and dependent variables. Aiba and Haldane models had similar values of R^2_{adj} in both systems: 0.808 and 0.816 for the SBS and, 0.937 and 0.935, respectively, for the IBS. The Edwards model had smaller R^2_{adj} values, compared with the Aiba and Haldane models, The F test statistic gauges the contribution of the independent variables in predicting the dependent variable. If F is a large number, it is possible to conclude that the independent variables contribute to the prediction of the dependent variable. If F is around 1, it can be concluded that there is no association between the variables. Table 1 shows that Haldane and Aiba models have similar F values in both systems: 28 and 30, respectively, for the SBS and 84 and 81, respectively, for the IBS. Edwards model had smaller values than Aiba and Haldane models.

These results show that there were no large differences between the Aiba and Haldane models fitting. Besides, both are good kinetic models to describe nitritation inhibition by ammonium, in the SBS and the IBS. However, the confidence limits of the coefficients of the Aiba model were smaller than the coefficients of the Haldane model. These results could lead to the conclusion that the Aiba model is more sensitive than the Haldane model.

3.2. Nitratation inhibition by nitrite

Nitratation rates were obtained from the OUR measurements with sodium nitrite as substrate. The nitratation rates were calculated from the oxygen consumed in nitrite oxidation to nitrate and the stoichiometry of the reaction (Eq. (2)).

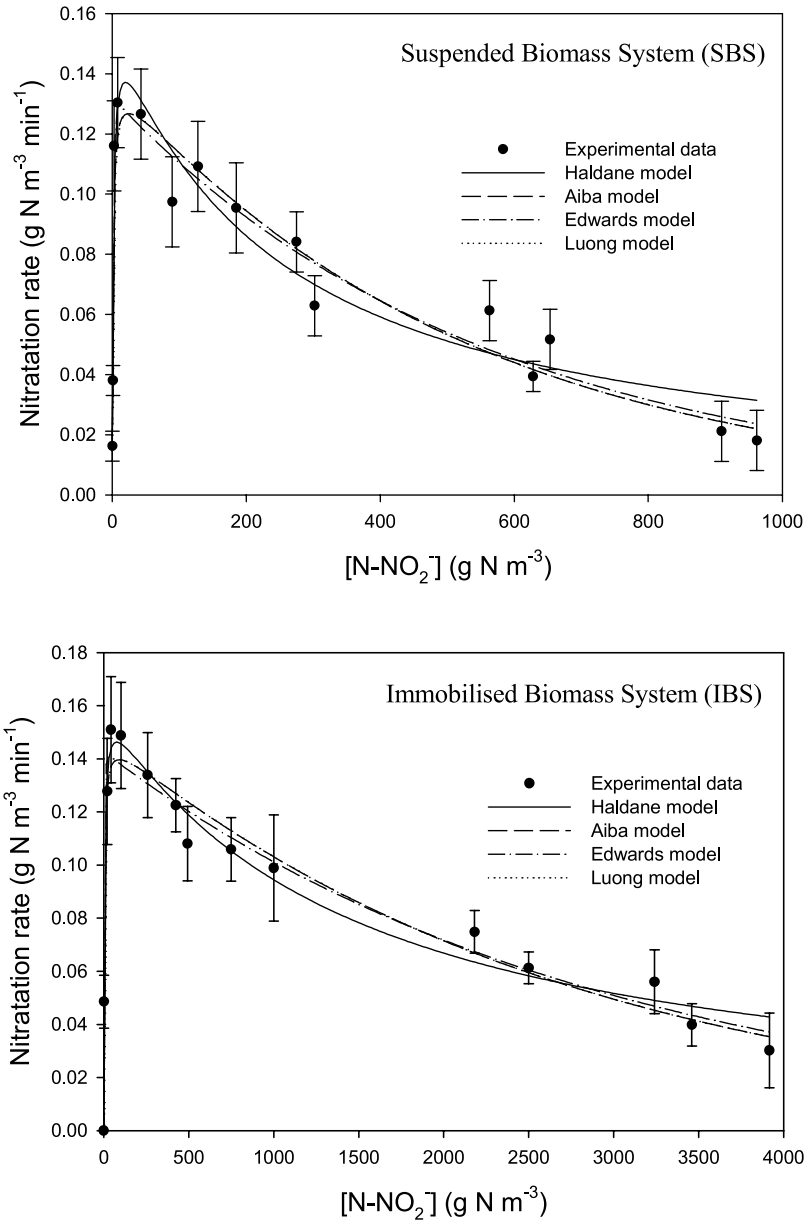

Fig. 2. Experimental data and model predictions of the nitratation rate for the SBS and the IBS.

Experimental data of nitratation rate versus nitrite concentration were fitted to the same kinetic models as the nitritation process to obtain the best quantitative description of the nitratation process in the SBS and the IBS. Experimental results were fitted to each model using SIGMAPLOT®8.0 software [16]. Fig. 2 shows the experimental data and model predictions of the nitritation rate for both systems. Table 2 shows the results achieved in the discrimination among the several kinetic models employed for the fitting of experimental data of nitratation.

Table 2 shows that, based on P criterion, the Luong model was not statistically valid to describe the nitratation inhibition by nitrite in the SBS but was suitable to describe this inhibition in the IBS. However, despite the statistical meaning of the coefficients of the Luong model, these coefficients have no biochemical meaning. For example, S_m is the parameter of the Luong model that predicts the substrate concentration above which net growth ceases. The adjusted S_m (1.2×10^7 g N–NO_2^- m^{-3}) has no biochemical meaning, Besides, the Haldane model had the greatest R^2_{adj} (0.896 and 0.982) and F values (66 and 283) for the SBS and the IBS, respectively.

These results show that the best kinetic model for describing nitratation inhibition by nitrite in the SBS and the IBS was the Haldane model.

Finally, a death factor was included to the selected kinetic model and compared with the experimental results. Some authors found that the best kinetic model for substrate

Table 2
Results in the selection between the several kinetic models employed for the fitting of experimental data of nitratation

Model		SBS					IBS				
		R^2_{adj}	F	Coeff	Confidence limits	P	R^2_{adj}	F	Coeff	Confidence limits	P
Haldane	r_{max}			0.16	0.01(6%)	0.0001			0.162	0.006(40%)	0.0001
	K_S	0.903	66	1.6	0.5(31%)	0.008	0.976	283	4.1	0.9(22%)	0.0006
	K_{IH}			235	54(23%)	0.0009			1407	155(11%)	0.0001
Aiba	r_{max}			0.139	0.009(6%)	0.0001			0.149	0.005(3%)	0.0001
	K_S	0.896	61	1.2	0.4(33%)	0.009	0.968	210	3.1	0.8(26%)	0.0002
	K_{IA}			520	72(14%)	0.0001			2717	218(8%)	0.0001
Luong	r_{max}			0.14	0.01(7%)	0.0001			0.149	0.005(3%)	0.0001
	K_S			1.2	0.5(42%)	0.02	0.965	129	3.1	0.8(26%)	0.0002
	S_m	0.887	38	*1.6×10^6*	*8.7×10^7*	*1*			1.2×10^7	5.1×10^5(4%)	0.0001
	n			*3000*	*5.5×10^5*	*1*			4553	425(9%)	0.0001
Edwards	r_{max}			0.132	0.006(5%)	0.0001			0.143	0.004(3%)	0.0001
	K_S	0.777	59	1.7	0.3(18%)	0.0002	0.970	227	4.3	0.9(21%)	0.0005
	K_{IE}			559	61(11%)	0.0001			2908	226(8%)	0.0001

Coefficients values in *italics* do not pass the *P criterion.*

inhibition included a death factor [11,12]. Nevertheless, no improvement was achieved to the model fitting with this modification. The selected kinetic models for nitritation and nitratation inhibition by substrate in the SBS and the IBS are shown in Table 3.

3.3. Comparison of the kinetic parameters in the SBS and the IBS

The half-saturation and inhibition coefficients for the IBS were larger than the SBS. The average ratios between the coefficients of Haldane, Aiba and Edwards models for the nitritation process in the IBS and the SBS (Table 1) and their standard deviations were:

$$\left[\frac{(K_S)_{IBS}}{(K_S)_{SBS}}\right]_{nitratation} = 2.58 \pm 0.06$$

$$\left[\frac{(K_I)_{IBS}}{(K_I)_{SBS}}\right]_{nitratation} = 4.6 \pm 0.4 \quad (7)$$

The average ratios between the coefficients of Haldane, Aiba and Edwards models for nitratation process in the IBS and the SBS (Table 2) and their standard deviations were:

$$\left[\frac{(K_S)_{IBS}}{(K_S)_{SBS}}\right]_{nitratation} = 2.56 \pm 0.03$$

$$\left[\frac{(K_I)_{IBS}}{(K_I)_{SBS}}\right]_{nitratation} = 5.5 \pm 0.4 \quad (8)$$

These results suggest that the coefficient of the IBS are influenced by the internal mass transfer in the biofilm because the ratios are greater than 1. Therefore, the IBS coefficients include the effect of the substrate consumption and the internal mass transfer. Moreover, the half-saturation coefficients ratio is the same for ammonium-oxidising and nitrite-oxidising biomasses. This fact suggests that the spatial distribution of both biomasses is homogeneous in the biofilm because the internal mass transfer problems of both substrates, ammonium and nitrite, are similar. The homogeneous spatial distribution of both biomasses in the biofilm was suggested by Garrido et al. [17] in a similar IBS. If the spatial distribution of both biomasses would be heterogeneous, for example if the ammonium-oxidising biomass would be in the external part of the biofilm and the

Table 3
Selected kinetic models for the nitritation and nitratation inhibition by substrate in the SBS and the IBS

Process	Biomass system	Selected kinetic model	Parameter values
Nitritation	Suspended	*Aiba*	$r_{max} = 0.51$, $K_S = 11$, $K_{IA} = 725$
	Immobilised	*Aiba*	$r_{max} = 0.18$, K_S=28, $K_{IA} = 3057$
Nitratation	Suspended	*Haldane*	$r_{max} = 0.16$, $K_S = 1.6$, $K_{IH} = 235$
	Immobilised	*Haldane*	$r_{max} = 0.162$, $K_S = 4.1$, $K_{IH} = 1407$

nitrite-oxidising biomass would be in the internal part, the half-saturation coefficients ratio of nitrite-oxidising biomass would be greater than the ammonium-oxidising biomass.

4. Conclusion

Four kinetic inhibition models were processed through non-linear regression to represent the inhibitions by substrate of the nitritation and nitratation processes. The statistical criteria proved that the model proposed by Aiba, was the best model to describe ammonium inhibition of the nitritation process, in the SBS and the IBS, whereas the Haldane model was the best model to describe the inhibition by nitrite of the nitratation process in both systems. The ratios between the kinetic coefficients in both systems suggest that the IBS coefficients were influenced by the internal mass transfer in the biofilm. Moreover, the small difference in these ratios in the nitritation and the nitratation processes suggests an homogenous spatial distribution of ammonium-oxidising and the nitrite-oxidising biomasses in the biofilm.

References

[1] Teichgraber B, Stein A. Nitrogen elimination from sludge treatment reject water comparison of the steam-stripping and denitrification processes. Water Sci Technol 1994;30:41–51.
[2] López-Fiuza J, Buys B, Mosquera-Corral A, Omil F, Méndez R. Toxic effects exerted on methanogenic, nitrifying and denitrifying bacteria by chemicals used in a milk analysis laboratory. Enzyme Microb Technol 2002;31:976–85.
[3] Surmacz-Gorska J, Gernaey K, Demuynck C, Vanrollehem P, Verstraete W. Nitrification monitoring in activated sludge by oxygen uptake rate (OUR) measurements. Water Res 1996;30:1228–36.
[4] Sheintuch M, Tartakovsky B, Narkis N, Rebhun M. Substrate inhibition and multiple states in a continuous nitrification process. Water Res 1995;29:953–63.
[5] Edwards VH. The influence of high substrate concentration on microbial kinetics. Biotechnol Bioeng 1970;12:679–712.
[6] Aiba S, Shoda M, Nagatani M. Kinetics of product inhibition in alcohol fermentation. Biotechnol Bioeng 1968;10:845–64.
[7] Luong JHJ. Generalization of Monod kinetics for analysis of growth data with substrate inhibition. Biotechnol Bioeng 1987;29:242–8.
[8] Meriç S, Tünay O, Ali San H. A new approach modelling substrate inhibition. Environ Technol 2002;23:163–77.
[9] Han K, Levenspiel O. Extended Monod kinetics for substrate, product, and cell inhibition. Biotechnol Bioeng 1988;32:430–7.
[10] Velizarov S, Beschkov V. Biotransformation of glucose to free gluconic acid by *Gluconobacter oxydans*: substrate and product inhibition situations. Process Biochem 1998;33:527–34.
[11] Tsuneda S, Auresenia J, Morise T, Hirata A. Dynamic modelling and simulation of a three-phase fluidised bed batch process for wastewater treatment. Process Biochem 2002;38:599–604.
[12] Tanyolaç D, Salih B, Tanyolaç A. Inhibition kinetics of a commercial mixed culture by ammonium sulfate. Biochem Eng J 2001;7:177–82.
[13] APHA. Standard methods for the examination of water and wastewater, 19th ed. Washington D.C.: American Publishers Health Association, 1995.
[14] Anthonisen AC, Loehr RC, Prakasam TBS, Srinath EG. Inhibition of nitrification by ammonia and nitrous acid. J Wat Pollut Control Fed 1976;48:835–52.
[15] Abeling U, Seyfried CF. Anaerobic–aerobic treatment of high-strength ammonium wastewater nitrogen removal. Wat Sci Technol 1992;26:1007–15.
[16] SIGMAPLOT® 8.0 Programming Guide (2002). SPSS Science Marketing Department. SPSS Inc. Chicago, IL 60606-6307, USA.
[17] Garrido JM, van Benthum WAJ, van Loosdrecht MCM, Heijnen JJ. Influence of dissolved oxygen concentration on nitrite accumulation in a biofilm airlift suspension reactor. Biotechnol Bioeng 1997;53:168–78.