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Influence of C/N ratio on autotrophic biomass development in a sequencing batch reactor

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Abstract

Experimental limitations on biomass assignment in wastewater treatment units lead to an oversimplified description of the complex competing biological system. Different microorganisms are usually considered as a single entity expressed as suspended volatile solids measured by gravimetry. This work proposes a method to access autotrophic biomass fraction in a carbon/nitrogen removal system using a reduced order model. This approach allows for the determination of the autotrophic biomass fraction in response to changes in feed concentration and operational strategies in a sequencing batch reactor. The results herein presented clearly demonstrate the influence of operating conditions and batch scheduling in the resultant autotrophic fraction responsible for processes bioconversions in such a competing system. The linear relationship obtained between the autotrophic fraction and the ratio C_{org}/N of the load can be used for biomass assignment as well as for the development of on-line estimators.

Keywords: Batch processing; Bioreactors; Biological nitrogen removal; Autotrophic; Modelling; Wastewater treatment

1. Introduction

Activated sludge processes typically employ suspended cells as biocatalysts. Practical limitations on biomass assignment, either due to sampling or analysis techniques, usually led to the determination of its concentration by the traditional gravimetric methods (volatile suspended solids). This procedure considers biomass as a unique entity not distinguishing between active and inert or even between autotrophic and heterotrophic cells. The problem of such oversimplified analysis appears when the input of toxicants, changes on operation or non-steady states responses are expected [1] as happens in a sequencing batch reactor. Additionally, the competition for available substrates is the main driving force that determines community structure and the diversity of biological populations that can coexist [2].

To overcome the limitations of this approach, this work proposes to determine a relationship that expresses the dependence between the autotrophic and heterotrophic biomass fractions and the loading changes in the C_{org}/N ratio in a sequencing batch reactor dealing with biological nitrogen and carbon removal. These systems develop a complex biomass structure since they consist of a nitrification step, where autotrophic organisms under aerobic conditions act while the heterotrophic bacteria perform carbon oxidation. This nitrification step is followed by a denitrification that is carried by heterotrophic bacteria under anoxic conditions responsible for converting nitrate to molecular nitrogen.

Since the design of such systems is closely related to process kinetics, which depends on the microbial population, wastewater composition and a wide variety of physical and chemical parameters [3], the methodology herein employed is based on the estimation of the most relevant state variables with the aid of a reduced order model previously proposed by Souza [4] for the experimental set-up used. This approach allows the quantification of the key chemical constituents and their removal rates as well as the estimation of the relation between hetero- and autotrophic concentrations with changes in the system load. Such a relationship would permit the prediction of changes in the biomass community that produce modifications in system performance.

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Table 1
Synthetic wastewater composition

Component	Concentration (mg/L)
Glucose	0.0 for $C_{org}/N = 0.0$
	25.1 for $C_{org}/N = 0.5$
	50.3 for $C_{org}/N = 1.0$
	149.0 for $C_{org}/N = 3.0$
	370.0 for $C_{org}/N = 7.5$
	400.0 for $C_{org}/N = 8.0$
	450.0 for $C_{org}/N = 9.0$
	547.0 for $C_{org}/N = 11.0$
NH_4Cl	76.1
$Na_2HPO_4\cdot 12H_2O$	46.2
NaCl	10.1
KCl	4.7
$CaCl_2\cdot 2H_2O$	4.7
$MgSO_4\cdot 7H_2O$	16.7
$NaHCO_3$	243.3
Na_2CO_3	162.2
$FeCl_3\cdot 6H_2O$, $ZnSO_4$, $MnSO_4\cdot H_2O$ and $CuSO_4$	<0.2

2. Experimental approach

2.1. Experimental methodology

2.1.1. Microorganisms and synthetic wastewater composition

Biomass characteristics were determined as 4200 mg/L for total suspended solids, 1300 mg/L as fixed suspended solids and volatile solids equal to 2900 mg/L, gently provided by PETROBRAS—Petróleo Brasileiro S.A. Synthetic wastewater composition is presented in Table 1. For biomass maintenance, trace elements were added in lower concentrations. The pH in the bioreactor (around 7.5) was maintained through sodium bicarbonate buffer addition. Aeration was supplied to assure dissolved oxygen concentrations around 5.5–6.0 ppm.

2.1.2. On-line measurements and data acquisition

A microcomputer for supervisory control, a feeding system, an agitation device and an oxygen supply were interfaced with a sequencing batch reactor. A multi-loop controller was employed for temperature, level, pH, dissolved oxygen and redox potential measurements. Commercial supervisory control software (FIX-DMACS MMI, Version 6.2, Intellution Inc.) was available for real-time database management and on-line display of process variables, as shown in Fig. 1.

2.1.3. Off-line measurements

All off-line measurements followed the standard methods for the examination of water and wastewater [5] and were determined three times. Samples were analyzed for NH_4^+ and NO_3^- concentrations, chemical oxygen demand (COD) and volatile solids. For substrate concentration determinations, samples were treated by centrifugation and filtration processes for solids separation.

2.1.4. Experimental strategies

Table 2 describes the operating parameters and batch scheduling for the experiments herein employed. For C_{org}/N ratios from 0.0 to 1.0 no denitrification step was conducted. For this batch schedule a 2 h symmetric pulse fill phase was employed and a 4 h aerobic phase was conducted for carbon oxidation and nitrification step, as described in Coelho et al. [6]. For the C_{org}/N ratios range of 3.0–11.0, the total batch time was carried on as three symmetric pulses for reactor fill (30 min each) and subsequently 30 min of anoxic phase and

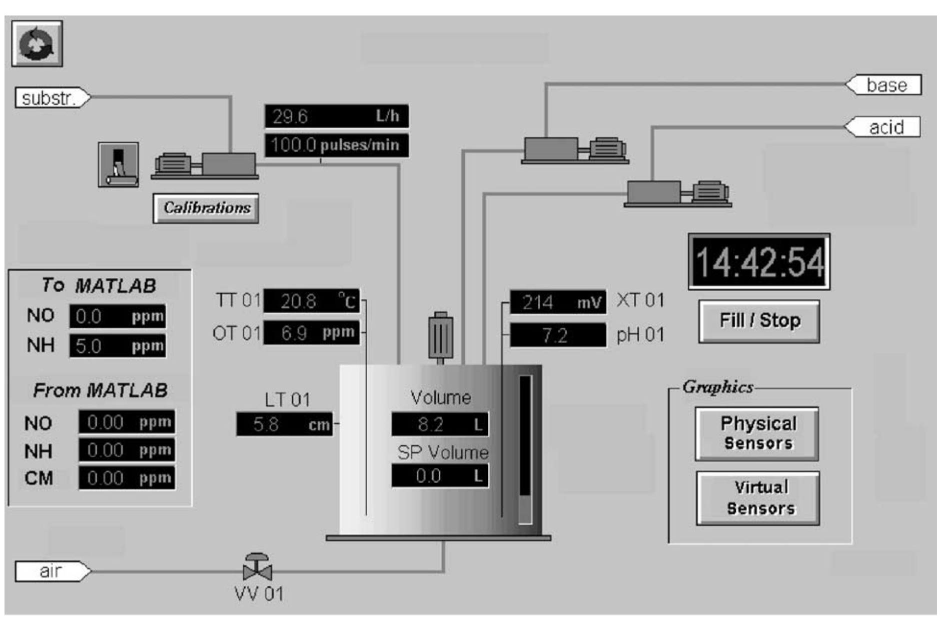

Fig. 1. Supervisory control: measurements and operation coordinates.

Table 2
Operational conditions

C_{org}/N ratio	0.0–1.0
Feed ammoniacal concentration	25.8 ppm
Wastewater volume treated	30 L
Initial reactor volume	5 L
Feed flow rate	15 L/h
Fill phase	2 h
Anoxic phase	0 h
Aerobic phase	4 h
Total batch time	6 h
C_{org}/N ratio	3.0–11.0
Feed ammoniacal concentration	25.8 ppm
Wastewater volume treated	15 L
Initial reactor volume	5 L
Feed flow rate	10 L/h
Fill phase	1.5 h
Anoxic phase	1.5 h
Aerobic phase	9 h
Total batch time	12 h

Table 3
Removal rates[a] for different C/N loading

C_{org}/N ratio	NH_4^+ (%)	S_S (%)
0.0	100.0	–
0.5	64.5	73.2
1.0	98.0	95.6
3.0	69.8	92.0
7.5	89.1	94.5
8.0	92.7	99.2
9.0	98.9	98.7
11.0	99.6	100.0

[a] Determined in respect of feed concentration and final batch values.

3 h of aerobic one, according to Souza [7]. Between each change on C_{org}/N load, a period of at least 2 weeks for microbial adaptation at the new condition before experimental determinations was used. During the microbial adaptation, carried as a 24 h single batch, periodical analyses were performed to guarantee homogenous removal rates.

2.2. Experimental results

Experimental data obtained for different C_{org}/N load ratios were analyzed. Fig. 2 clearly displays a microbial adaptation with changes in the C_{org}/N load. Regarding the nitrogen removal, lower levels of ammoniacal nitrogen were found in the reactor with increasing C_{org}/N ratio. This is related with the nitrate formation and removal, presented in Fig. 3, where lower levels of this pollutant are also observed at the beginning of each batch and during the entire experiment with increasing C_{org}/N ratio. For higher C_{org}/N ratios, no significant differences in removal rates for readily biodegradable substrate concentration (S_S), determined as chemical oxygen demand, could be observed as shown in Fig. 4. This indicates a rapid consumption of this organic material in initial stages of the batch, almost immediately after the filling phase.

Although some authors describe a negative influence of the increasing loading organic carbon content on biological nitrogen removal, this was not observed in the studied systems. Contrary to the initially expected an increase in nitrification rate with an increase in organic loading rate was found till C_{org}/N ratio of 8.0, as reported in Table 3. Similar results were observed by Gupta and Gupta [3,8] for a

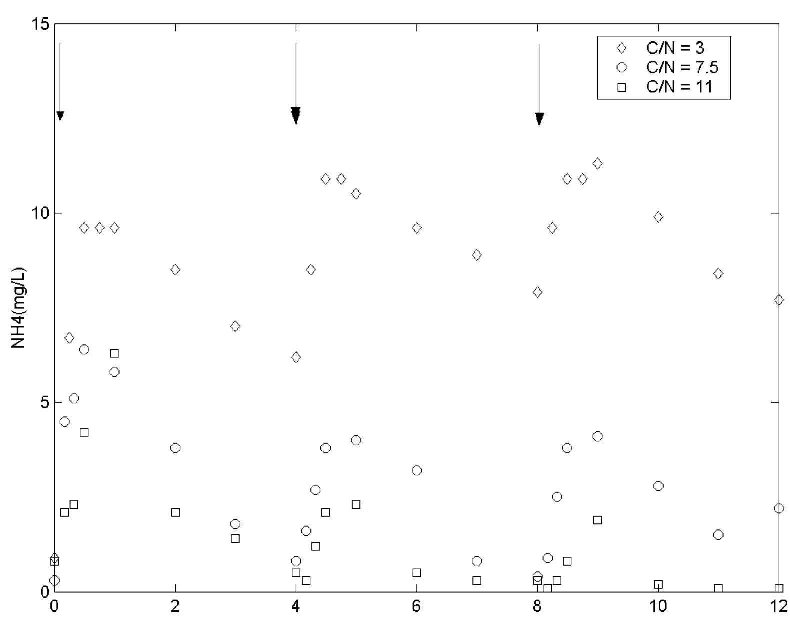

Fig. 2. Ammoniacal nitrogen kinetic data for distinct C_{org}/N conditions in a SBR (arrows indicate initial feed step).

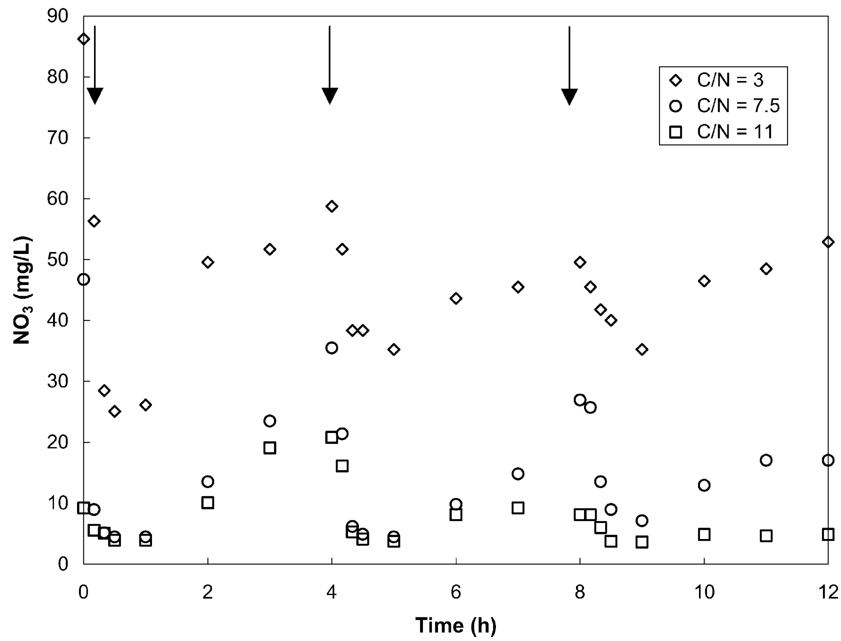

Fig. 3. Nitrate kinetic data obtained for distinct C_{org}/N conditions in a SBR (arrows indicate initial feed step).

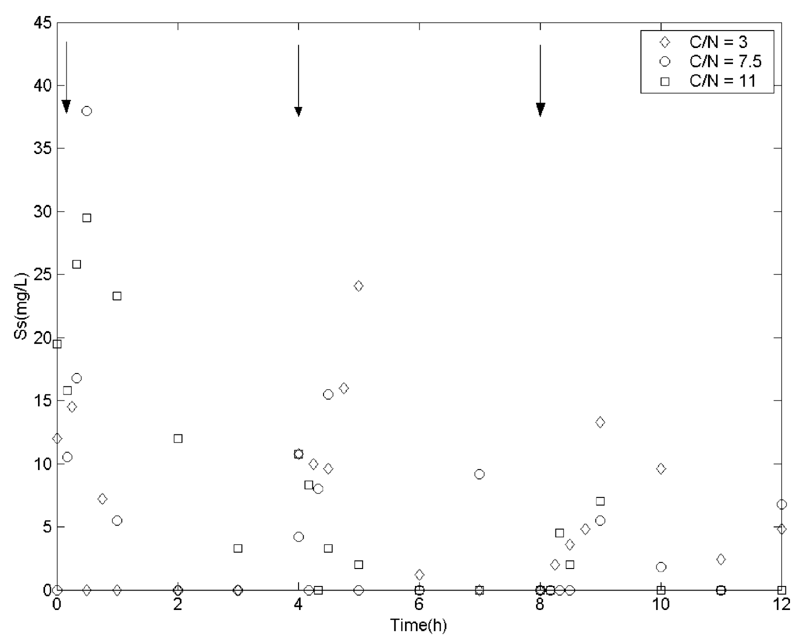

Fig. 4. Carbon oxidation data obtained for distinct C_{org}/N conditions in a SBR (arrows indicate initial feed step).

mixed culture on an aerobic rotating biological contactor (RBC).

A similar behavior was obtained when increasing C_{org}/N ratios from 0.5 to 1.0, even for a different operational strategy (Fig. 5). An increase in both nitrogen and carbon removal was achieved, as reported in Table 3. This behavior seems to be related to different states of the microbial community developing in each condition, especially in a cyclic, non-steady state system as the SBR.

3. Modelling approach

3.1. Modelling methodology

An analysis of the relationship between autotrophic growth and process kinetics under different C_{org}/N loading was performed applying a mathematical model to determine the autotrophic fraction (f_{BA}) responsible for biological conversion in a sequencing batch reactor. The

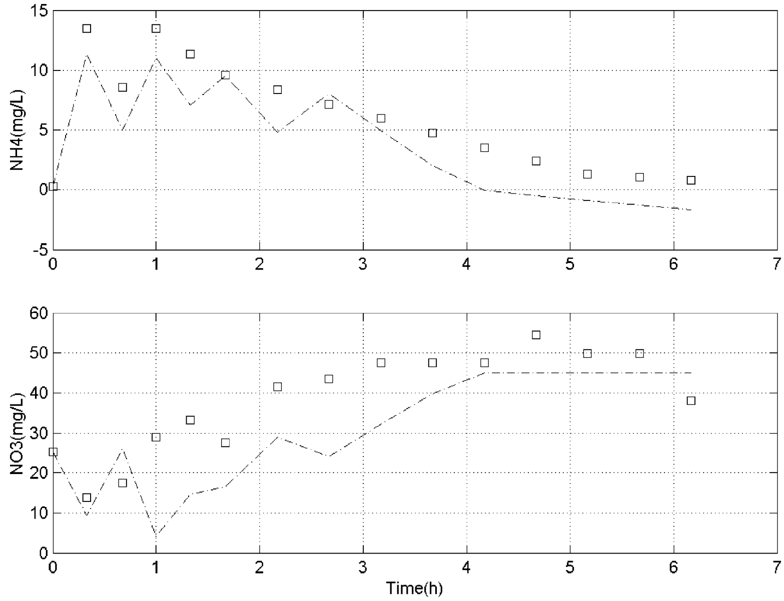

Fig. 5. Experimental data (□) and model prediction (– – –) for C_{org}/N ratio = 1.0.

employed model incorporates both fill and reaction phases, including fundamental phenomena such as cellular maintenance, carbonaceous and ammoniacal nitrogen removal, nitrate formation, considered as semi-associated to cellular growth, and its consumption in anoxic conditions by the heterotrophic metabolism.

Simplifications adopted in the model formulation include constant temperature and pH, absence of inhibitory effects and nutritional limitations, homogeneous biomass and constant kinetics parameters. Since during reactor operation, no significant biomass growth was detected, i.e., a constant ratio between volatile suspended solids (VSS) and total suspended solids (TSS) was found, biomass growth was not considered in the modelling following what was done previously by Julien et al. [9] and Boaventura et al. [10]. This experimental observation indicates a quasi steady-state condition for total biomass reactor concentration, resulting probably from similar growth and death rates. Since this was observed for the total population, a constant fraction of autotrophic cells during the entire batch is assumed. The period for sludge adaptation used, described in the Section 2.1.4, also supports this simplification.

This model considers substrates as exclusively non-particulate. Oxygen concentration was determined on-line and the corresponding mass balance was disregarded. The fundamental phenomena described by the model are expressed by Eqs. (1)–(6). The model allows the inference of variable profiles that are difficult to be measured on-line.

$$\frac{dV}{dt} = F \tag{1}$$

$$\frac{dX_{BA}}{dt} = -\frac{F}{V} f_{BA}(X_{BA} + X_{BH}) \tag{2}$$

$$\frac{dX_{BH}}{dt} = -\frac{F}{V}(1 - f_{BA})(X_{BA} + X_{BH}) \tag{3}$$

$$\frac{dS_{NH}}{dt} = \frac{F}{V}(S_{NHf} - S_{NH}) - \mu_A\left(\frac{S_{NH}}{S_{NH} + K_{NH}}\right) \times \left(\frac{S_{OD}}{S_{OD} + K_{OD}}\right) f_{BA}(X_{BA} + X_{BH}) \tag{4}$$

$$\frac{dS_{NO}}{dt} = \frac{F}{V}(S_{NOf} - S_{NO}) + Y_{NO}\left(\frac{S_{NH}}{S_{NH} + K_{NH}}\right) \times \left(\frac{S_{OD}}{S_{OD} + K_{OD}}\right) f_{BA}(X_{BA} + X_{BH}) - \eta m_H\left(\frac{S_S}{S_S + K_S}\right)\left(\frac{S_{NO}}{S_{NO} + K_{NO}}\right) \times \left(\frac{K_{OD}}{K_{OD} + S_{OD}}\right)(1 - f_{BA})(X_{BA} + X_{BH}) \tag{5}$$

$$\frac{dS_S}{dt} = \frac{F}{V}(S_{Sf} - S_S) - m_H\left(\frac{S_S}{S_S + K_S}\right)(1 - f_{BA})(X_{BA} + X_{BH}) \times \left[\left(\frac{S_{OD}}{S_{OD} + K_{OD}}\right) + \eta_g\left(\frac{K_{OD}}{S_{OD} + K_{OD}}\right) \times \left(\frac{S_{NO}}{S_{NO} + K_{NO}}\right)\right] \tag{6}$$

Model variables and parameters are defined as: F: volumetric flow rate; f_{BA}: autotrophic microorganism fraction; K_{NH}: ammonium saturation coefficient; K_{NO}: nitrate saturation coefficient; K_{OD}: dissolved oxygen saturation coefficient; K_s: saturation coefficient for carbonaceous substrate;

m_H: heterotrophic cellular maintenance; V: reactor volume; S_{NH}: ammonium concentration; S_{NO}: nitrate concentration; S_{OD}: dissolved oxygen concentration; S_S: readily biodegradable substrate concentration (determined as COD); t: time; Y_{NO}: nitrate yield; X_{BA}: autotrophic biomass concentration; X_{BH}: heterotrophic biomass concentration; μ_A: specific growth rate of autotrophic microorganisms; η: correction factor for autotrophic microorganisms in anoxic conditions; η_g: correction factor for heterotrophic microorganisms in anoxic conditions. Subscript f refers to feed condition.

Parameter estimation was conducted with MATLAB *Optimization Toolbox* (The Mathworks Inc.) by the minimization of squared residual error between experimental and estimated values (Eq. (7)) through Nelder and Mead type simplex search method.

$$\text{minimize} \sum_{i}^{n_e} \sum_{j}^{n_p} ([S_{NH}]_{exp} - [S_{NH}]_{calc})^2 + ([S_{NO}]_{exp} - [S_{NO}]_{calc})^2 \quad (7)$$

where n_e is the number of experiments and n_p the number of data-points obtained in each experiment. Subscripts exp and calc refers to experimental and calculated data, respectively.

3.2. Modelling results

Table 4 reports the values for the model parameters proposed by Souza [4] and estimated based on data retrieved on the experimental set-up used in this work. These parameters were here used for the determination of the autotrophic microorganism fraction, f_{BA}. Using the reduced order model, an autotrophic fraction was determined for each C_{org}/N ratio analyzed. The profiles obtained for the different C_{org}/N ratios are compared with experimental data in Figs. 6–10. It is patent in the results presented in these figures that both operational strategies employed were successful in reducing pollutant concentrations leading to final effluents obeying Brazilian discharge limits for both ammoniacal nitrogen and nitrate. Additionally, it is possible to verify through Figs. 6–10 that the feed strategy employing symmetric pulses produces lower concentrations inside the reactor minimizing inhibitory effects.

Table 4
Model parameters

Parameter	Value
K_{OD} (mg O_2/L)	1.463
K_{NH} (mg NH_4^+/L)	0.059
Y_{NO} (h^{-1})	0.219
μ_A (h^{-1})	0.072
m_H (h^{-1})	2.494
K_S (mg DQO/L)	0.826
η ([mg NO_3^-] [mg DQO]$^{-1}$)	01.543
K_{NO} (mg NO_3^-/L)	117.83
η_g	0.123

The autotrophic microorganism fractions, f_{BA}, obtained are presented in Fig. 11a and b as function of the C_{org}/N ratio. A linear relationship between f_{BA} and C_{org}/N load was obtained. Although the increase in f_{BA} with C_{org}/N load was at first unexpected, it reflects the increase in the nitrification rate previously discussed and can be explained through the operating schedule applied for SBR cycle. Since the feed was carried out in three different steps along whole batch and is followed by a denitrification step (an anoxic regime), the heterotrophic biomass responsible for nitrate removal will use this carbon source added to the system. However the carbon source was almost completely used and no significant

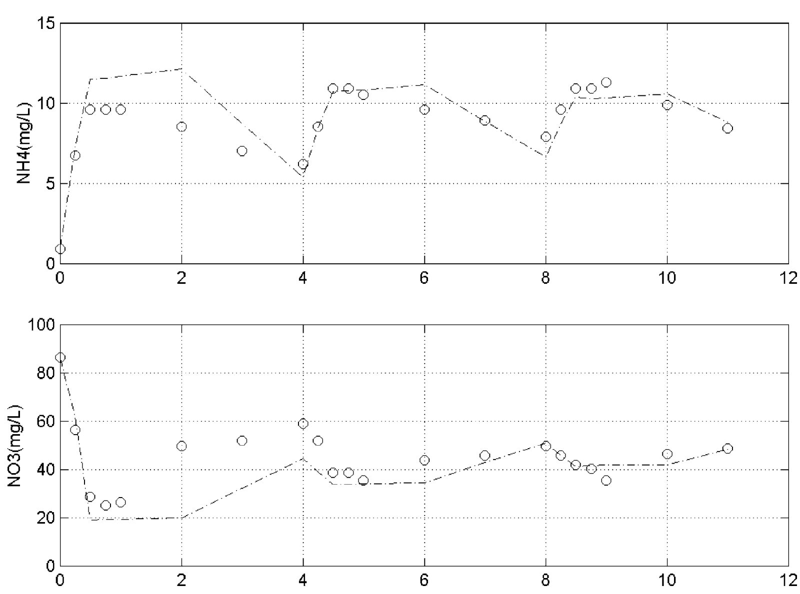

Fig. 6. Experimental data (○) and model prediction (– – –) for C_{org}/N ratio = 3.0.

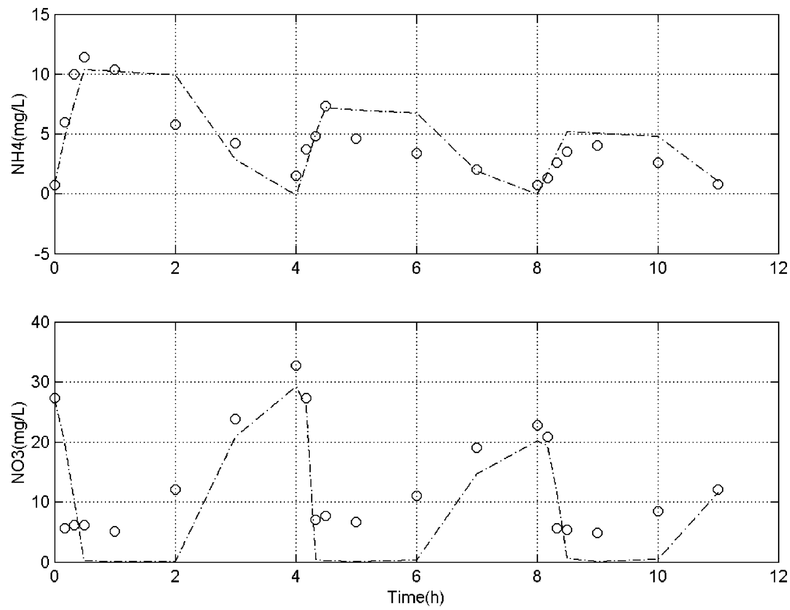

Fig. 7. Experimental data (○) and model prediction (– – –) for C_{org}/N ratio = 7.5.

biodegradable organic carbon remains in the reactor for the aerobic phase, as shown in Fig. 4. Since heterotrophic growth rate is usually higher in aerobic than in anoxic or anaerobic conditions, the results reflect the insignificant growth of these species under batch conditions. Gupta and Gupta [3] obtained similar results in a RBC where the first reactor stage was responsible for most of carbon consumption at almost all hydraulic retention times studied.

The increase in the autotrophic organisms fraction with the C_{org}/N load encountered in this work is further supported by Atlas [2] as he states that, "different environmental conditions will favor the competitive success of populations with adaptive features. Often a well-adapted population will become dominant, especially under stable environmental conditions. Greater diversity is favored by unstable (fluctuating) environmental conditions. Specially,

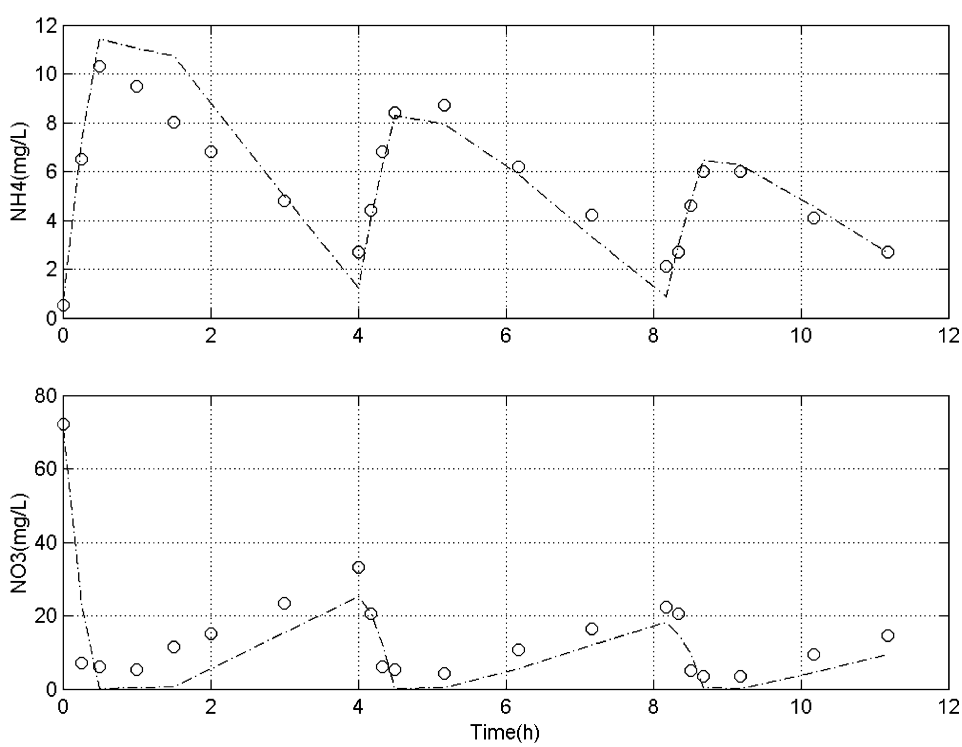

Fig. 8. Experimental data (○) and model prediction (– – –) for C_{org}/N ratio = 9.0.

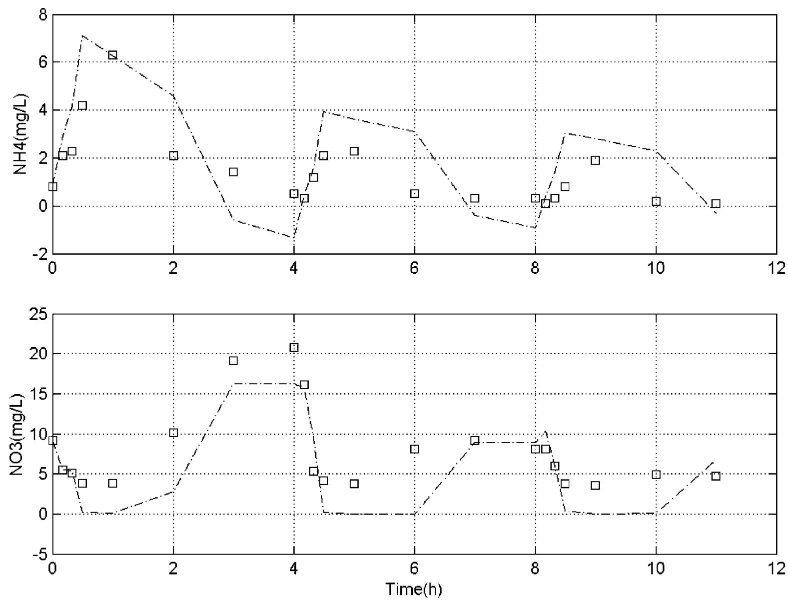

Fig. 9. Experimental data (☐) and model prediction (– – –) for C_{org}/N ratio = 11.0.

under low nutrient conditions a more diverse community of slowly growing bacteria is favored". A sequencing batch reactor, such as used in this work, is not a stable environment and the rapid carbon consumption observed in the batch schedule applied produces a low nutrient condition on the system leading to an unexpected increase of the autotrophic organisms. Additionally, a rapid consumption of organic carbon leads to a favor environment to autotrophic organisms, not inhibiting its activity.

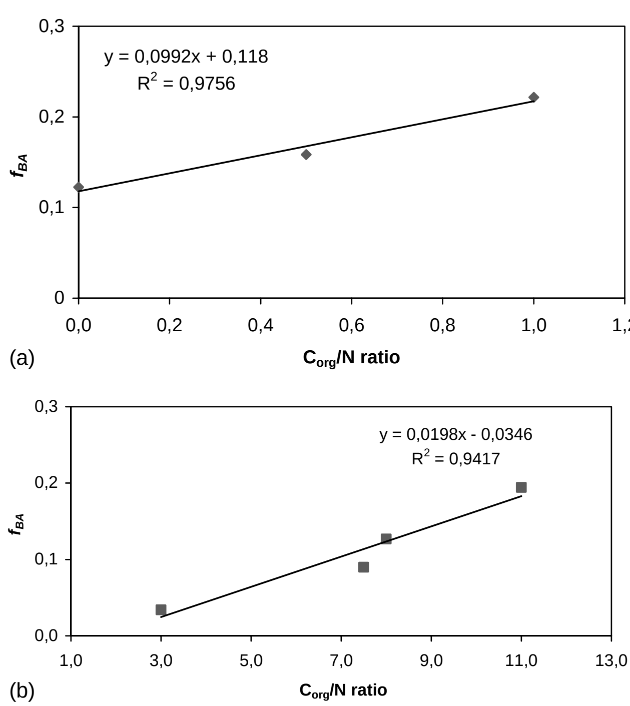

Fig. 10. Relationship between autotrophic fraction and C_{org}/N loading.

When comparing the different operating strategies as those used for C_{org}/N ratios of 0.0–1.0 (Fig. 10a) with the operating strategies used for higher C_{org}/N ratios (Fig. 10b), a similar behavior pattern appears. The higher values for f_{BA} fraction obtained for lower C_{org}/N ratios occur because those experiments were conducted without any denitrification step (Table 2) and with a lower carbon content.

The results of the SBR operating strategies used on this work show that they could be used to conduct the denitrification process, meeting the increasing stringent regulation on effluent nitrogen discharges while affording advantages such as low buffer requirements and no need for external carbon source in the denitrification step eventually resulting on a reduction of the treatment costs. This work establishes the necessity of a well-operated schedule, which permits the utilization of the whole wastewater potential as a nutrient and also the whole potential of the reactor itself.

4. Conclusions

An increase in nitrification rate accomplished by an increase in the organic loading rate led to a linear relationship between the biomass autotrophic fraction, f_{BA}, and the C_{org}/N load ratio, as determined through mathematical modelling. This behavior is explained by the operating schedule applied for the SBR cycle and its inherent characteristics. The relationship here proposed could overcome the usual practical limitations on biomass determination in activated sludge systems, especially in the distinction between the biomass fractions responsible for different catalytical activities such as the autotrophic and heterotrophic microorganisms.

It is shown that an adequate batch schedule for a SBR cycle, using the potential of the wastewater as nutrient for both the autotrophic and heterotrophic biomass, can reach nitrogen removal rates close to 99% and complete carbon removal.

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