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KINETIC MODELLING OF *PSEUDOMONAS DENITRIFICANS* GROWTH AND DENITRIFICATION UNDER AEROBIC, ANOXIC AND TRANSIENT OPERATING CONDITIONS

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Abstract—The transient growth characteristics of *Ps. denitrificans* to changes from anoxic to aerobic conditions and vice-versa were studied and an appropriate mathematical model was developed. This kinetic model adequately describes the behavior of the denitrifying bacterium under strictly anoxic, strictly aerobic and transient conditions of growth. Dissolved oxygen exhibited an inhibitory effect on the activity of the enzymes associated with denitrification. Each step of the denitrification pathway was affected differently by dissolved oxygen concentration. Nitrate reduction was the least sensitive step, while reduction of N_2O and/or NO was almost completely inhibited by dissolved oxygen. Very long lag phases were observed following an anoxic to aerobic shift, whereas denitrification was immediately initiated following an aerobic to anoxic shift.

Key words—denitrification, *Ps. denitrificans*, kinetics, transient, enzyme inhibition.

NOMENCLATURE

b_1 = nitrate inhibition coefficient on the growth yield (mg cell dry wt·l·mmol glutamate^{-1}·mg NO_3^--N^{-1})
b_2 = maximum inhibition coefficient of nitrite on the growth yield (mg cell dry wt·mmol glutamate^{-1})
b_3 = half saturation constant of nitrite inhibition on the growth yield (mg NO_2^--N·l^{-1})
c_o = dissolved oxygen concentration (mg·l^{-1})
c_o^* = dissolved oxygen concentration at equilibrium with the gas phase (mg·l^{-1})
K_{I1} = nitrate inhibition constant on the aerobic growth rate (mg NO_3^--N·l^{-1})
K_{I2} = nitrite inhibition constant on the aerobic growth rate (mg NO_2^--N·l^{-1})
K_{I3} = nitrite inhibition constant on the adaptability parameter (mg NO_2^--N·l^{-1})
K_{In1} = nitrate inhibition constant on the nitrite reduction rate (mg NO_3^--N·l^{-1})
K_{Io1} = oxygen inhibition coefficient on the nitrite reduction (mg·l^{-1})
K_{Io2} = oxygen inhibition coefficient on the nitrite reduction through an energy producing pathway (mg·l^{-1})
K_{Io3} = oxygen inhibition coefficient on the nitrite reduction through a non energy producing pathway (mg·l^{-1})
$k_L\alpha$ = volumetric mass-transfer coefficient (h^{-1})
K_{n1} = nitrate half saturation constant (mg NO_3^--N·l^{-1})
K_{n2} = nitrite half saturation constant (mg NO_2^--N·l^{-1})
K_o = oxygen half saturation constant (mg·l^{-1})
K_s = glutamate half saturation constant for aerobic growth (mM)
K_{s1} = glutamate half saturation constant for nitrate reduction (mM)
K_{s2} = glutamate half saturation constant for nitrite reduction (mM)
n_1 = nitrate concentration (mg NO_3^--N·l^{-1})
n_2 = nitrite concentration (mg NO_2^--N·l^{-1})
s_g = organic carbon (glutamate) concentration (mM)
t = reaction time (h)
v_{an} = specific non growth associated nitrite utilization rate for nitrite reduction to nitrogen gas (h^{-1})
v_{n2} = maximum specific non growth associated nitrite utilization rate for nitrite reduction to nitrogen gas (h^{-1})
x = cell mass concentration (mg cell dry wt·l^{-1})
Y_{n1} = growth yield coefficient for nitrate reduction to nitrite (mg cell dry wt·mg NO_3^--N^{-1})
Y_{n2} = growth yield coefficient for nitrite reduction to nitrogen gas (mg cell dry wt·mg NO_2^--N^{-1})
Y_{sn1} = yield coefficient for glutamate utilization under nitrate reduction conditions (mmol glutamate·mg NO_3^--N^{-1})
Y_{sn2} = yield coefficient for glutamate utilization under nitrite reduction conditions (mmol glutamate·mg NO_2^--N^{-1})
$Y_{x/o}$ = growth yield coefficient based on oxygen utilized (mg cell dry wt·mg oxygen^{-1})
$Y_{x/s}$ = growth yield coefficient based on glutamate utilized (mg cell dry wt·mmol glutamate^{-1})
$Y_{o/s}^{max}$ = maximum oxygen utilization coefficient based on glutamate (mg oxygen·mmol glutamate^{-1})
$Y_{x/o}^{max}$ = maximum growth yield coefficient based on oxygen utilized (mg cell dry wt·mg oxygen^{-1})
$Y_{x/s}^{max}$ = maximum growth yield coefficient based on glutamate utilized (mg cell dry wt·mmol glutamate^{-1})
μ_{an1} = specific growth rate for nitrate reduction to nitrite (h^{-1})
μ_{an2} = specific growth rate for nitrite reduction to nitrogen gas (h^{-1})
μ_{m1} = maximum specific growth rate for nitrate reduction to nitrite (h^{-1})
μ_{m2} = maximum specific growth rate for nitrite reduction to nitrogen gas (h^{-1})
μ_{max} = maximum aerobic specific growth rate (h^{-1})
$\mu_{t,aer}$ = target aerobic specific growth rate (h^{-1})
μ_{aer} = aerobic specific growth rate (h^{-1})
α_{max1} = maximum adaptability parameter for aerobic to aerobic transitions (h^{-1})
α_{max2} = maximum adaptability parameter for anoxic to aerobic transitions (h^{-1})

INTRODUCTION

Biological denitrification combined with nitrification is one of the most feasible and cost effective

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processes for nitrogen removal from wastewaters, both municipal and industrial. Considered more economical than conventional activated sludge treatment followed by chemical or physio-chemical nitrogen removal, the biological nitrogen removal methods have been extensively used all over the world as an effective means to attain effluent nitrogen requirements.

Complete nitrogen removal from wastewaters can be accomplished using several different process configurations. Almost all of the most recently installed systems are of the single sludge pre-denitrification type, which means that their principle of operation is the recirculation of the nitrified mixed liquor to the anoxic basins in order to achieve denitrification, or the utilization of appropriate aeration patterns in the tanks in order to secure the existence of anoxic zones or periods. Biological nitrogen removal systems, with suspended-growth nitrification–denitrification, can be classified (Rittman and Langeland, 1985; Henze, 1991) to four general types of process configurations according to their principle of operation: (a) Recirculation systems (Bardenpho, UCT); (b) Tank alternation systems (Bio-Denitro); (c) Spatial alternation systems (Oxidation ditches); (d) Temporal alternation systems (SBRs, intermittent aeration in continuous-flow reactors).

In any one of the above systems the denitrifying bacteria are alternately exposed to aerobic conditions (where oxygen serves as terminal electron acceptor) and anoxic conditions (where nitrogen oxides can serve as terminal electron acceptors).

Initially, denitrification was considered to be a strictly anoxic process (Payne, 1973; Knowles, 1982). This idea was supported by many researchers who have found that very low dissolved oxygen concentrations can cause almost immediate and complete cessation of denitrifying activity in cells which are dispersed (not aggregated): 0.13 mg/l (Nelson and Knowles, 1978), 0.2 mg/l (Focht and Chang, 1975) and less than 0.1 mg/l (Krul, 1976). However, in certain bacterial species, denitrification has been accepted to occur, although at a lower rate, under aerobic conditions (Meiberg *et al*., 1980; Robertson and Kuenen, 1984; Lloyd *et al*., 1987). In any case, the possibility of denitrification under aerobic conditions and the extent of oxygen inhibition on the denitrifying cells are of interest from physiological, ecological and economic point of view. Kugelman *et al*. (1991), for instance, have claimed that aerobic denitrification may be a significant mechanism to account for aeration tank nitrogen losses they have observed in sequential anoxic–aerobic activated sludge systems.

Denitrifying bacteria reduce one or both ionic oxides (nitrate and nitrite) to nitrogen gas through the production of the following intermediates as proposed initially by Payne (1973) using organic substrate(s) for energy (electron donor) and cell synthesis:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \qquad (1)$$

Since each of the above nitrogen compounds can serve as a terminal electron acceptor instead of oxygen, another issue of great importance is the type of inhibition that oxygen exerts on denitrification, i.e. whether it is on the synthesis or on the activity of the denitrifying enzymes and which of the sequential steps of the denitrification process [equation (1)] are the most affected by oxygen. It has been long known that oxygen has two modes of operation: repression of enzyme synthesis and inhibition of enzyme activity, but assessment of the critical oxygen concentrations for each inhibition mechanism has not been extensively made. For three of the cases, in which denitrifying enzyme synthesis has been studied [*Thiobacillus denitrificans* (Justin and Kelly, 1978), *Hyphomicrobium* X (Meiberg *et al*., 1980), and *Ps. stutzeri* (Körner and Zumft, 1989)], the synthesis of these denitrifying reductases is permitted at higher dissolved oxygen concentrations than the oxygen threshold concentrations for their activity. Thus, an important design and operating consideration is the time required for the denitrifiers to activate or deactivate their denitrifying enzymes (reductases) at the end of the electron transport respiratory chain. Surprisingly, little information is available on these important reaction step changes for activated sludge wastewater systems. Payne and Riley (1969) showed that 40 min was required for an aerobically grown culture to shift to maximum activity under anoxic conditions. Nelson and Knowles (1978) found that *Azospirillum* required a 2-h lag period to shift metabolism to nitrate reduction. However, numerous laboratory experiments with mixed denitrifying cultures and usual observations on activated sludge wastewater treatment plants have not revealed such lag adaptation periods as a result of the overlapping which occurs on the individual microorganisms' behavior.

Batchelor (1982) used a multiple Monod type kinetic model to include the inhibiting effect of dissolved oxygen concentration on denitrification rates. The *Activated Sludge Model No. 2* (ASM2) of IAWQ (1995) is also based on this approach. Most of the kinetic models presented so far, either dynamic or steady-state (Brenner and Argaman, 1990; Henze *et al*., 1987; Van Haandel *et al*., 1982), have been developed by conducting experiments with mixed population systems such as activated sludge. Because of the interactions between various microbial species and substrates, macroscopic microbial behavior varies with time thus rendering reproducibility of kinetic results virtually impossible. Although the "real world" of wastewater treatment is reproduced in such experiments, the wide application of these models for design and development of optimal operating strategies for wastewater treatment plants WWTPs encounters great risks

and could potentially lead to failure due to the limited ability of the models to predict the system performance. Therefore, an understanding of the fundamental principals and mechanisms involved in denitrification is the key for reliable modelling and successful design.

Kornaros *et al*. (1996) have recently studied the growth of a representative denitrifier (*Pseudomonas denitrificans*) under anoxic conditions and developed a mathematical model capable of describing with sufficient accuracy the nitrate and nitrite reduction rates, the cell growth and the organic carbon utilization rates. In the sequel, Kornaros and Lyberatos (1997) studied the behavior of the same denitrifier under aerobic conditions of growth in a defined synthetic medium, in the presence of nitrates and/or nitrites. The authors developed a kinetic model which is able to predict accurately the biomass growth and the consumption rates of organic carbon and oxygen in the presence of various concentrations of nitrate, nitrite, both or neither of them.

The purpose of this work was to study the dynamic characteristics of the same denitrifier during the transient phases from aerobic to anoxic and from anoxic to aerobic conditions, which are observed in all the biological nutrient removing wastewater treatment systems, and to develop an appropriate kinetic model describing these transient characteristics. Of course, the proposed model should reduce to the previously developed models for purely aerobic and purely anoxic conditions.

MATERIALS AND METHODS

Organism

Pseudomonas denitrificans (ATCC 13867) was used in this study. The organism was maintained on peptone-yeast extract agar slants at 4°C.

Culture media

The basic medium used for the agar slants and the synthetic liquid medium used for the growth of the bacterium was the same as reported previously (Kornaros *et al*., 1996). L-glutamic acid was used as the sole carbon, energy and nitrogen source. The amounts of L-glutamic acid and nitrate or nitrite were added to the basic synthetic medium according to the desirable condition of growth.

Experimental system

All the batch experiments were performed in a 2.5-liter Virtis Omni-Culture bench-top fermentor with working volume of 2 liters. The suspension medium was continuously stirred at a constant rate of 500 rpm during all the runs. The temperature of the fermentor was automatically controlled at 2.5 ± 0.1°C. The DO was monitored via a steam sterilizable galvanic (Johnson&Borkowski) type oxygen electrode, whereas a steam sterilizable combined electrode (Ingold, Type 465) was used for pH monitoring. The pH in the fermentor was controlled at 7.1 with a pH-controller (Virtis) using an aqueous solution of 1N hydrochloric acid. Air was supplied to the fermentor in order to attain aerobic conditions, whereas high purity argon was used for excluding air and securing anoxic conditions.

Culture methods

Inoculum from stock culture maintained on the agar slants was transferred to a 1000-ml Erlenmeyer flask containing 400 ml of the synthetic liquid medium and cultivated under aerobic or anoxic conditions (depending on the experiments) in a shaker bath for approximately 36 h at 25°C. A portion of the preculture medium was transferred to the fermentor at 10% of the working volume after the cells had reached the late exponential stage.

Analytical methods

Growth was monitored by measuring at regular intervals the absorbance of samples at 550 nm. Optical density was converted to dry cell mass via a calibration curve. The liquid samples after filtration with a 0.22 micron membrane filter were analyzed for nitrate, nitrite and glutamate concentrations through the methodologies described at Kornaros *et al*. (1969). In addition to those methods, nitrate and nitrite concentrations were also determined by ion chromatography on a model DX300 gradient system (Dionex Corp. Sunnyvale, Calif.) using an AS11 column and a CDM-3 conductivity detector. After the injection of a 25-μl appropriately diluted sample, the chromatogram was developed within 11 min using a NaOH eluent at a flow rate of 2.0 ml/min. The retention time for nitrate was 5.3 min and for nitrite 3.4 min. The separation of nitrate and nitrite from other ions which were present in the samples was achieved by running a gradient method tailored for the particular samples, where selectivity, resolution and total run time were optimized.

MODEL DEVELOPMENT

Pseudomonas denitrificans as a facultative aerobic bacterium, can grow under both anoxic and aerobic conditions (Kornaros *et al*., 1996; Kornaros and Lyberatos, 1997). As a denitrifier, the bacterium under anoxic conditions uses nitrates and nitrites as terminal electron acceptors and L-glutamic acid as a sole carbon, nitrogen and energy source. The anoxic model that was developed by Kornaros *et al*. (1996) was proved capable of accurately describing the behavior of the denitrifier under strictly anoxic conditions.

Under aerobic conditions, *Ps. denitrificans* uses oxygen as terminal electron acceptor (Kornaros and Lyberatos, 1997). Since nitrates and nitrites are expected to be present in the aerobic basins of all activated sludge plants for wastewater treatment of either municipal or industrial wastes, the effect of their presence in the culture medium was investigated and was properly accounted for in the kinetic model that was developed.

Transient characteristics of growth under aerobic conditions

When aerobically grown cells of *Ps. denitrificans* in a culture medium without nitrates and nitrites were transferred into a fresh medium also lacking in concentrations of any nitrogenous alternative electron acceptors, no lag phase in the culture's growth rate was observed. In fact, such an immediate response was expected, since no enzymatic or any other type of physiological changes were

needed by the cells in order to continue their growth in the new environment where the basic culture medium was the same as before. This was also the case (no lag of growth) for the transition of aerobically grown cells in the absence of nitrates and nitrites, to an aerobic environment where the culture medium contained various concentrations of nitrates (from 0 to 400 mg NO_3^--N/l). However, their presence during the subsequent growth affected the maximum specific cell growth rate and the cell yield. On the contrary, when cells of *Ps. denitrificans* aerobically grown, in a culture medium free from alternative respiratory substrates (NO_3^-, NO_2^-, N_2O etc.) besides oxygen, were transferred into a fresh medium with the same concentrations of mineral salts and organic carbon but different nitrite concentrations (from 0 to 313 mg NO_2^--N/l) different lag periods in the adaptation of the cultures were observed (Fig. 1). An extensive description of all the above aerobic experiments can be found in Kornaros and Lyberatos (1997). The difference in the length of these lag phases is exclusively dependent upon the nitrate concentration of each culture medium. This type of response of the denitrifying cells can be attributed to the fact that the bacterial cells need time to adjust their metabolism in utilizing oxygen as terminal electron acceptor while at the same time synthesize and/or maintain adequate levels of reductase(s) for the competing denitrification pathway. Although the activity of denitrifying reductases is seriously inhibited by high dissolved oxygen concentrations, the bacterial cells are prepared for immediate use of nitrites under the common condition of declining oxygen concentration. The delay for adaptation that became evident in the case of nitrites and not in the case of nitrates also suggests that, in the cells of *Ps. denitrificans*, the synthesis of nitrate reductase is separately, and probably differently, regulated than that of nitrite reductase or NO/N_2O reductases.

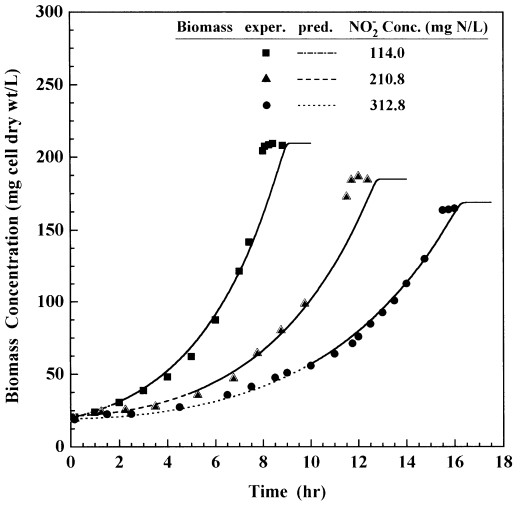

Fig. 1. Inhibitory effect of nitrite on growth. Experimental and theoretical profiles of biomass concentrations for the aerobic growth of *Ps. denitrificans* in a batch culture under carbon-limited conditions in the presence of various nitrite concentrations. Dash and dot lines represent the lag phases and solid lines the exponential growth curves.

In order to describe mathematically the observed lag phases in the case of nitrites, a differential equation (2) proposed by Wang and Stephanopoulos (1984) was used for the estimation of the specific growth rate:

$$\frac{d\mu_{aer}}{dt} = \alpha \cdot (\mu_{t,aer} - \mu_{aer}), \text{ if } \mu_{t,aer} > \mu_{aer} \quad \text{and,} \quad (2)$$

$$\mu_{aer} = \mu_{t,aer}, \text{ if } \mu_{t,aer} \leq \mu_{aer} \quad (3)$$

where $\mu_{t,aer}$ is the specific growth rate described by equation (4):

$$\mu_{t,aer}(s_g, c_o, n_1, n_2) = \mu_{max} \frac{s_g}{K_s + s_g} \frac{c_o}{K_o + c_o} \frac{K_{I1}}{K_{I1} + n_1} \frac{K_{I2}}{K_{I2} + n_2} \quad (4)$$

and α is an "adaptability" parameter that measures the culture's ability to adapt to environmental changes. The smaller the magnitude of the parameter α, the slower is the response of the growth rate to a change in an environmental condition which influences the "target" aerobic specific growth rate ($\mu_{t,aer}$). The incorporation of such a delay model has the great advantage, against a structured model that could have been developed instead, that it is very simple and it only adds one extra parameter to the above aerobic model, the "adaptability" parameter (α). Equation (3) simply describes the expected rapid response of the specific cell growth rate to substrate limitation.

As was mentioned above and as can also be seen in Fig. 1, the higher the value of nitrite concentration in the culture medium, the longer the lag phase of growth and the lower the value of α. Therefore, the following functionality was employed to describe the inhibitory effect of nitrites on the culture's adaptability α:

$$\alpha = \alpha_{max\,1} \frac{K_{I3}}{K_{I3} + n_2} \quad (5)$$

For each one of the three batches which were carried out (Fig. 1), a separate value of the "adaptability" parameter α was evaluated from the slopes of the lines $-\ln(\mu_{t,aer} - \mu_{aer})$ vs time according to equation 2 which are shown in Fig. 2. The best values of α for the batches with 114.0, 210.8 and 312.8 mg NO_2^--N/l were found to be 0.43, 0.28 and 0.20 h^{-1} respectively. In the sequel, the values of maximum "adaptability" parameter α_{max1} and nitrite inhibition coefficient K_{I3} were determined by rewriting equation 5 in the form:

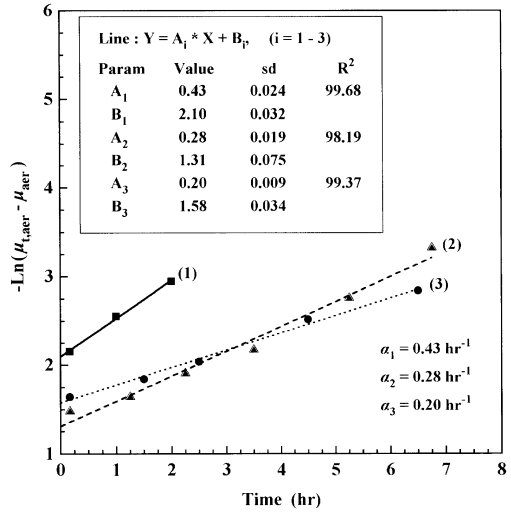

Fig. 2. $-\mathrm{Ln}(\mu_{t,aer}-\mu_{aer})$ vs time for the data obtained from the batch aerobic cultivations of *Ps. denitrificans* in the presence of various nitrite concentrations.

$$\frac{1}{\alpha}=\frac{1}{\alpha_{\max 1}}+\frac{1}{\alpha_{\max 1}K_{I3}}\cdot n_2 \qquad (6)$$

From a plot of $1/\alpha$ vs n_2 (Fig. 3), the best values of α_{max1} and K_{I3} were found to be $1.30\ h^{-1}$ and 57.2 mg NO_2^--N/l respectively.

Transitions of cultures from anoxic to aerobic conditions

Many nitrogen removal systems alternate exposure of the denitrifiers to anoxic and aerobic conditions. Therefore, an important design and operating consideration is the time required by the microorganisms to activate and deactivate their denitrifying enzyme system at the end of the electron transport respiratory chain during the transition from one environment to the other.

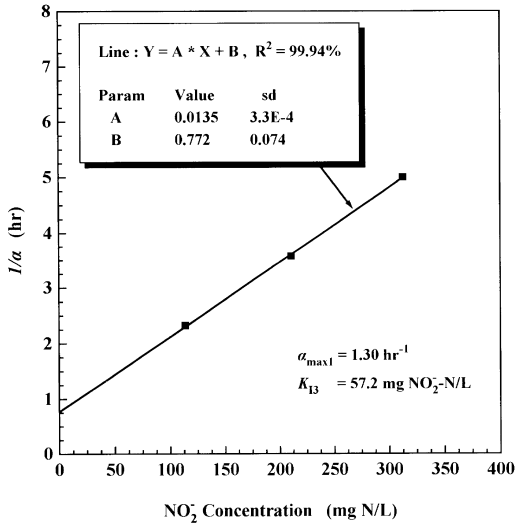

Fig. 3. $1/\alpha$ vs nitrite–nitrogen concentration according to equation (6). Evaluation of the maximum adaptability parameter α_{max1} and nitrite inhibition coefficient K_{I3} from the intercept and the slope of the line.

In order to investigate the behavior of *Ps. denitrificans* during the transition from anoxic to aerobic conditions and examine the effect of oxygen on the denitrification as well, a batch experiment was conducted. An actively denitrifying culture of *Ps. denitrificans* was changed from anoxic to aerobic conditions and the response of the culture was monitored (Fig. 4). At the mid-exponential phase of the anoxic growth a step change on the aeration of the culture was imposed. Air was supplied inside the reactor with a flowrate of 1.4 l/min. Nitrite production from the nitrate reduction immediately stopped after the change, and the nitrite and nitrate concentrations remained unchanged as long as air was supplied to the system. Following the aeration change, the growth of *Ps. denitrificans* was continued, but without observing an appreciable increase in the specific growth rate, as was expected under aerobic conditions. Finally, the cell growth stopped due to glutamate (organic carbon) exhaustion.

The observed delay in the adaptation of a denitrifying culture to a subsequent aerobic growth was examined in more detail by carrying out another batch experiment. Cells of *Ps. denitrificans* were grown under completely anoxic conditions in the presence of both nitrates and nitrites. At the mid-exponential phase of growth an aliquot of about 10% of the fermentor's working volume was transferred to the fermentor and immediately the growth was monitored under fully aerobic conditions (Fig. 5). The air supply was from the top of the fermentor at a constant flowrate of 0.5 l/min which resulted to a $k_L\alpha$ value of $9\ h^{-1}$ as was measured by the "off gas method under process conditions" of Van't Riet (1979). The choice of this reduced air flowrate (compared to 1.4 l/min used for exclusively aerobic experiments or intense aerobic conditions) was meant to serve two main purposes: first, to be sufficient for providing and securing aerobic conditions for an initial long aerobic phase and second, to examine the transition from aerobic to microaerobic growth conditions due to the natural decline of dissolved oxygen concentration in the culture medium. Glutamate was used in excess (40 mM) in this experiment in order to avoid growth limitation by carbon throughout the batch. Calculation of the specific growth rate at the first aerobic phase, indicated an extended lag period of about 10 h. According to the above modelling approach, for the appearance of lag phases to cultures exposed to aerobic conditions with various nitrite concentrations, the expected lag phase for the transition from anoxic conditions to an aerobic environment with nitrite concentration 3 mg NO_2^--N/l was much shorter (0.1 h) than the observed

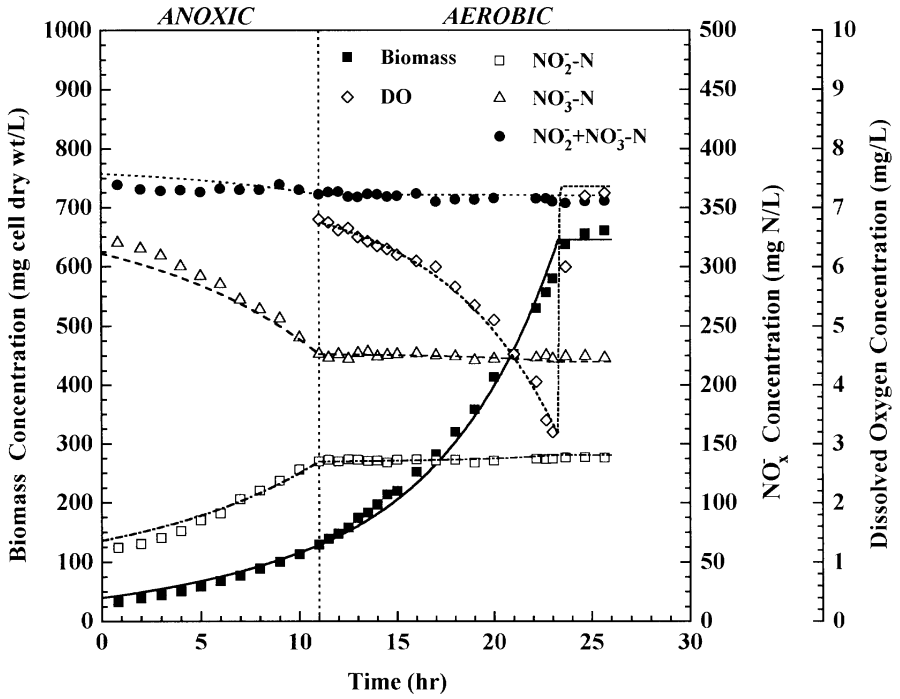

Fig. 4. Response of a batch culture in anoxic conditions to a change to aerobic conditions. Experimental and theoretical profiles of biomass, nitrate-, nitrite- and total nitrogen concentrations for the anoxic and aerobic growth phases and dissolved oxygen concentrations for the aerobic growth phase.

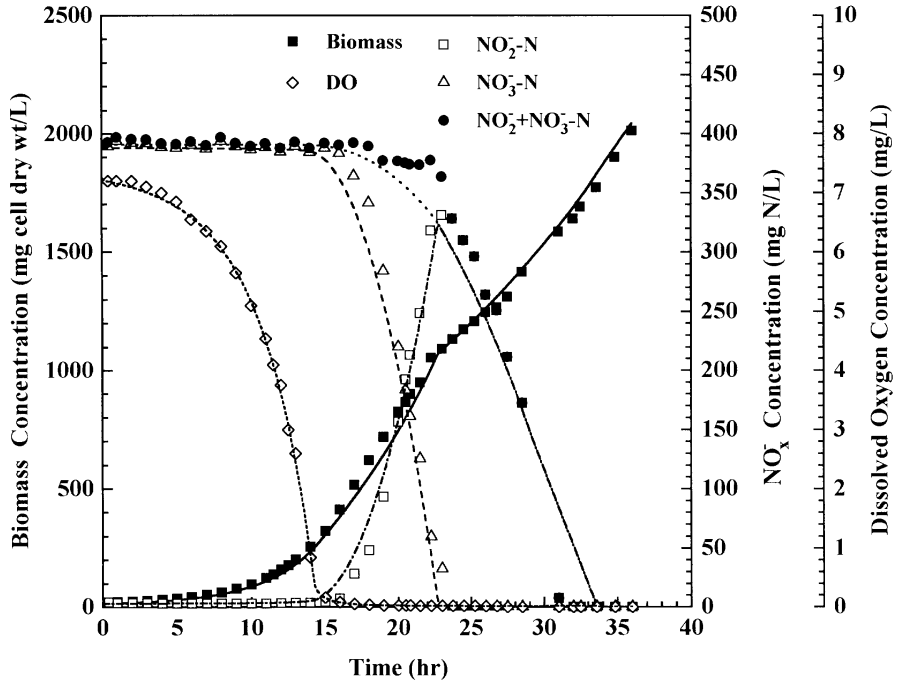

Fig. 5. Experimental and theoretical profiles of biomass, dissolved oxygen, nitrate-, nitrite- and total nitrogen concentrations for the aerobic growth of a batch culture with *Ps. denitrificans* cells previously grown under denitrifying conditions, and natural transition to denitrification under microaerobic conditions.

one. Therefore, a change of the maximum adaptability parameter α_{max1} was considered necessary for describing the effect of this transition on the length of lag phase. An adaptability parameter α was evaluated from a plot of $-\ln(\mu_{t,aer}-\mu_{aer})$ vs time according to equation (2) which is shown in Fig. 6, using the data of the aerobic phase from the last experiment. From the slope of the line in Fig. 6 the value of α was found to be $0.12\ h^{-1}$. A new maximum adaptability parameter α_{max2}, $0.13\ h^{-1}$, was calculated by dividing α by $K_{I3}/(K_{I3}+n_2)$, taking thus into account the existing concentrations of nitrites.

As can also be observed in this last experiment, during the initial aerobic phase of growth, the nitrate and nitrite concentrations remained unchanged although the cells had previously (under the anoxic conditions) a synthesized and active denitrifying respiratory system. However, when the dissolved oxygen concentration fell below 0.2 mg/l denitrifying activity was observed immediately, indicating that the cells had maintained their reductases under aerobic conditions but their function was inhibited by the high concentrations of dissolved oxygen. During the subsequent microaerobic (due to the continued air supply) conditions, very high nitrite concentrations were accumulated in the culture medium. Although nitrite accumulation has been reported by Kornaros *et al.* (1996) during the strictly anoxic growth of *Ps. denitrificans* in the presence of nitrates [attributed to nitrate inhibition on the rest of the denitrification steps of equation (1)], these observed nitrite concentrations were much higher than they were expected. This difference was considered to be due to extra inhibition exerted by oxygen on the denitrification steps from NO_2^- to N_2. Nitrite reduction to nitrogen gas was modeled by Kornaros *et al.* (1996) by using two terms, one associated with energy and thus biomass-yielding and one that is not. The modelling approach that was followed, was based on the fact that nitrite reduction to nitrogen gas proceeds through the parallel formation of two intermediates (NO and N_2O) one of which is not coupled with ATP formation. Regardless of the true reaction mechanism for nitrate reduction to nitrogen gas and in the effort of describing the inhibitory effect of oxygen on the denitrification process, three different oxygen inhibition coefficients (K_{Ioi}) were considered and were incorporated into the overall denitrification model which is a combination of the "anoxic" and "aerobic" model. A batch system with *Ps. denitrificans* cells can thus be defined by the following differential equations:

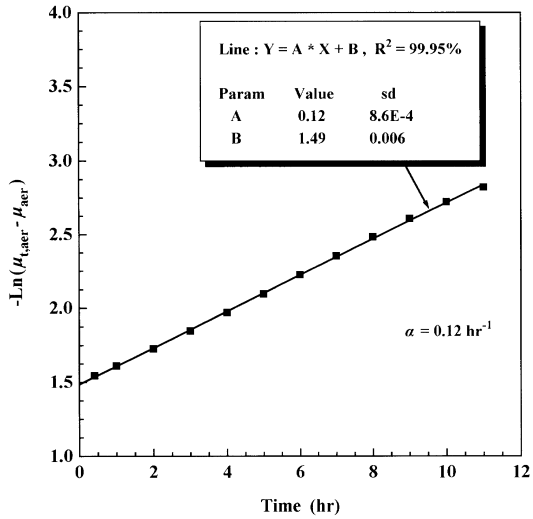

Fig. 6. $-\mathrm{Ln}(\mu_{t,aer}-\mu_{aer})$ vs time for the data obtained from the lag phase of the batch aerobic cultivation of *Ps. denitrificans* shown in Fig. 5. Evaluation of the adaptability parameter α from the slope of the line.

Cell growth:

$$\frac{dx}{dt} = \left[\mu_{aer} + \mu_{an1}(n_1, s_g, c_o) + \mu_{an2}(n_2, s_g, n_1, c_o)\right] \cdot x \tag{7}$$

Reduction of nitrate-nitrogen to nitrite-nitrogen:

$$\frac{dn_1}{dt} = -\frac{1}{Y_{n1}}\mu_{an1}(n_1, s_g, c_o) \cdot x \tag{8}$$

Generation of nitrite-nitrogen and reduction of nitrite-nitrogen to nitrogen gas:

$$\frac{dn_2}{dt} = \frac{1}{Y_{n1}}\mu_{an1}(n_1, s_g, c_o) \cdot x - \left[\frac{1}{Y_{n2}}\mu_{an2}(n_2, s_g, c_o) + v_{an}(n_2, s_g, c_o)\right] \cdot x \tag{9}$$

Organic carbon utilization:

$$\frac{ds_g}{dt} = -\frac{1}{Y_{x/s}}\mu_{aer} \cdot x - Y_{sn1}\left[\frac{1}{Y_{n1}}\mu_{an1}(n_1, s_g, c_o)\right] \cdot x - Y_{sn2}\left[\frac{1}{Y_{n2}}\mu_{an2}(n_2, s_g, c_o) + v_{an}(n_2, s_g, c_o)\right] \cdot x \tag{10}$$

Mass balance for oxygen:

$$\frac{dc_o}{dt} = k_L\alpha(c_o^* - c_o) - \frac{1}{Y_{x/o}}\mu_{aer} \cdot x \tag{11}$$

Aerobic specific growth rate:

$$\frac{d\mu_{aer}}{dt} = \alpha_{max\,2} \cdot \frac{K_{I3}}{K_{I3}+n_2}(\mu_{t,aer}-\mu_{aer}), \text{ if } \mu_{t,aer} > \mu_{aer} \tag{12}$$

or

$$\mu_{aer} = \mu_{t,aer}, \text{ if } \mu_{t,aer} \leq \mu_{aer} \tag{13}$$

where,

$$\mu_{t,aer}(s_g,c_o,n_1,n_2)=\mu_{max}\frac{s_g}{K_s+s_g}\frac{c_o}{K_o+c_o}\frac{K_{I1}}{K_{I1}+n_1}\frac{K_{I2}}{K_{I2}+n_2} \quad (14)$$

$$\mu_{an1}(n_1, s_g, c_o)=\mu_{m1}\frac{n_1}{K_{n1}+n_1}\frac{s_g}{K_{s1}+s_g}\frac{K_{Io1}}{K_{Io1}+c_o} \quad (15)$$

$$\mu_{an2}(n_2,s_g, n_1,c_o)=\mu_{m2}\frac{n_2}{K_{n2}+n_2}\frac{s_g}{K_{s2}+s_g}\frac{K_{In1}}{K_{In1}+n_1}\frac{K_{Io2}}{K_{Io2}+c_o} \quad (16)$$

$$v_{an}(n_2, s_g, c_o)=v_{n2}\frac{n_2}{K_{n2}+n_2}\frac{s_g}{K_{s2}+s_g}\frac{K_{Io3}}{K_{Io3}+c_o} \quad (17)$$

and

$$Y_{x/s}=Y^{max}_{x/s}-b_1\cdot n_1-\frac{b_2\cdot n_2}{b_3+n_2} \quad (18)$$

$$Y_{x/o}=\frac{Y_{x/s}}{Y^{max}_{o/s}} \quad (19)$$

The oxygen inhibition coefficients are:

K_{Io1}: describes the inhibition of dissolved oxygen on the nitrate reduction to nitrite
K_{Io2}: describes the inhibition of dissolved oxygen on the nitrite reduction through the energy producing pathway, and
K_{Io3}: describes the inhibition of dissolved oxygen on the nitrate reduction through the non energy producing pathway.

As can be easily seen, this model reduces to the previously developed ones under strictly anoxic and strictly aerobic conditions respectively. The kinetic parameters for each one of the separate models are given in Tables 1 and 2.

Up to this point, the evaluation of all the other parameters of the kinetic model has been made by manipulating the model's equations in such a way that the parameters could be determined via linear plots. In the case of the K_{Ioi} inhibition coefficients such a manipulation was not possible due to the fact that the inhibitory effects of dissolved oxygen on denitrification became evident when its concentration in the culture medium fell below 0.1 mg/l. Measurements of dissolved oxygen concentrations in the region and less than 0.1 mg/l were not reliable in our monitoring system, since its minimum sensitivity was 1% of the oxygen saturation value. Therefore, the best values of the K_{Ioi} coefficients were determined using least-square calculations (minimization of the sum of the squares of the differences between predicted and experimental values). The Levenberg-Marquardt method of optimization was used for varying the values of the parameters and for determination of their best fitting values. K_{Io1} was found to be 0.087 and K_{Io2}, K_{Io3} were 0.0015 and 0.058 mg/l respectively. These values of the oxygen inhibition coefficients clearly show that each one of the denitrification steps in equation 1 is inhibited by dissolved oxygen to a different degree, with the step of nitrate reduction to nitrite being the less sensitive and the nitrite reduction through the energy associated pathway being almost completely inhibited. Hochstein *et al.* (1984), Kawakami *et al.* (1985) and Bonin *et al.* (1989) have reported similar results in *Paracoccus halodenitrificans*, *Paracoccus denitrificans* and *Pseudomonas nautica* respectively in their studies of the inhibitory effect of oxygen on the activity of denitrifying enzymes. In the continuous cultures of *Pa. halodenitrificans*, when the oxygen supply was in excess, N_2OR (N_2O reductase) was blocked. In the presence of about 0.1 mg/l dissolved oxygen, nitrite was the major product of intermediate, and when the oxygen concentration was greater than 0.1 mg/l, nitrite reduction decreased, and nitrate accumulated in the medium. From the modelling standpoint, Batchelor (1982) used a single dissolved oxygen inhibition coefficient (K_o) of 0.25 mg/l vs 0.20 mg/l that was used in the ASM2 of IAWQ (1995) for the description of oxygen inhibition on the denitrification process.

Transition from aerobic to anoxic conditions

In order to study the response of a culture of *Ps. denitrificans* grown aerobically to a change to anoxic conditions, another batch experiment was carried out (Fig. 7). Initial concentrations of nitrate and nitrite were 374 and 0.62 mg N/l respectively, while 40 mM glutamate were used as initial carbon concentration. At the mid-exponential phase the gas

Table 1. Description and values of the kinetic parameters for the anoxic model

Parameter	Symbol	Value	Units	Process
Maximum specific growth rate	μ_{m1}	0.10	h^{-1}	$NO_3^-\rightarrow NO_2^-$
Nitrate half saturation constant	K_{n1}	0.77	mg NO_3^--N/l	$NO_3^-\rightarrow NO_2^-$
Growth yield coefficient	Y_{n1}	1.02	$\frac{\text{mg dry cell mass}}{\text{mg } NO_3^- - N}$	$NO_3^-\rightarrow NO_2^-$
Nitrate inhibition constant	K_{In1}	8.75	mg NO_3^--N/l	$NO_2^-\rightarrow N_2$
Maximum specific growth rate	μ_{m2}	0.10	h^{-1}	$NO_2^-\rightarrow N_2$
Maximum specific nitrite nongrowth-associated utilization rate	v_{n2}	0.019	h^{-1}	$NO_2^-\rightarrow N_2$
Nitrite half saturation constant	K_{n2}	0.28	mg NO_2^--N/l	$NO_2^-\rightarrow N_2$
Growth yield coefficient	Y_{n2}	1.41	$\frac{\text{mg dry cell mass}}{\text{mg } NO_2^- - N}$	$NO_2^-\rightarrow N_2$
Half saturation constant	K_{s1}	0.050	mM glutamate	$NO_3^-\rightarrow NO_2^-$
Yield coefficient	Y_{sn1}	0.015	$\frac{\text{mmol glutamate}}{\text{mg } NO_3^- - N}$	$NO_3^-\rightarrow NO_2^-$
Half saturation constant	K_{s2}	0.037	mM glutamate	$NO_2^-\rightarrow N_2$
Yield coefficient	Y_{sn2}	0.017	$\frac{\text{mmol glutamate}}{\text{mg } NO_2^- - N}$	$NO_2^-\rightarrow N_2$

Table 2. Description and values of the kinetic parameters for the aerobic model

Parameter	Symbol	Value	Units
Maximum specific growth rate	μ_{max}	0.41	h^{-1}
Glutamate half saturation constant	K_s	0.025	mM glutamate
Oxygen half saturation constant	K_o	0.065	mg/l
Nitrate inhibition constant	K_{I1}	992.75	mg NO_3^--N/l
Nitrite inhibition constant	K_{I2}	273.82	mg NO_2^--N/l
Volumetric mass-transfer coefficient	$K_L\alpha$	33.4	h^{-1}
Maximum growth yield coefficient based on glutamate	$Y_{x/s}^{max}$	96	$\frac{\text{mg dry cell mass}}{\text{mmol glutamate}}$
Maximum growth yield coefficient based on oxygen	$Y_{x/o}^{max}$	1.36	$\frac{\text{mg dry cell mass}}{\text{mg oxygen}}$
Maximum oxygen utilization coefficient based on glutamate	$Y_{o/s}^{max}$	70.4	$\frac{\text{mg oxygen}}{\text{mmol glutamate}}$
Nitrate inhibition coefficient on the growth yield	b_1	0.057	$\frac{\text{mg dry cell mass·l}}{\text{mmol glutamate·mg } NO_3^- \text{–N}}$
Maximum nitrite inhibition coefficient on the growth yield	b_2	60.24	$\frac{\text{mg dry cell mass}}{\text{mmol glutamate}}$
Half saturation constant of nitrite inhibition on the growth yield	b_3	96.97	mg NO_2^--N/l

supply to the fermentor was changed from air to high purity argon. As expected, no change of the nitrate concentration was observed during the initial aerobic phase, but immediately after the switch to anoxic conditions the nitrate and nitrite reduction was initiated, indicating again the existence of previously synthesized denitrifying enzymes. After 6.5 h of anoxic growth, the gas supply was changed again to air with flowrate 1.4 l/min, and the culture remained under aerobic conditions for about 2.5 h. The cell growth rate did not seem to increase significantly (aerobic lag phase), while nitrate and nitrite reducing activity ceased immediately after the exposure to high dissolved oxygen concentrations. Nitrate and nitrite concentrations remained almost constant throughout the aerobic phase. After this short aerobiosis period, a return to anoxic conditions resulted in a immediate recovery of the nitrate and nitrite reduction activity (which was inhibited under the previous aerobic conditions). The cell growth also continued without any lag phase. During both the above anoxic phases nitrite accumulation was observed, but the nitrite concentrations were very close to those predicted by the strictly "anoxic" model.

All the kinetic parameters obtained from the batch cultivations of *Ps. denitrificans* under transient conditions of growth are summarized in Table 3. For the prediction of the theoretical performance of each one of the batch systems that were run, equations (7)–(19) were integrated using the kinetic parameters shown in Tables 1–3. Figures 1, 4, 5 and 7 show the time courses of cell growth, and nitrate, nitrite and dissolved oxygen utilization, as well as the theoretically predicted profiles for each one of these variables. It can be easily seen that the

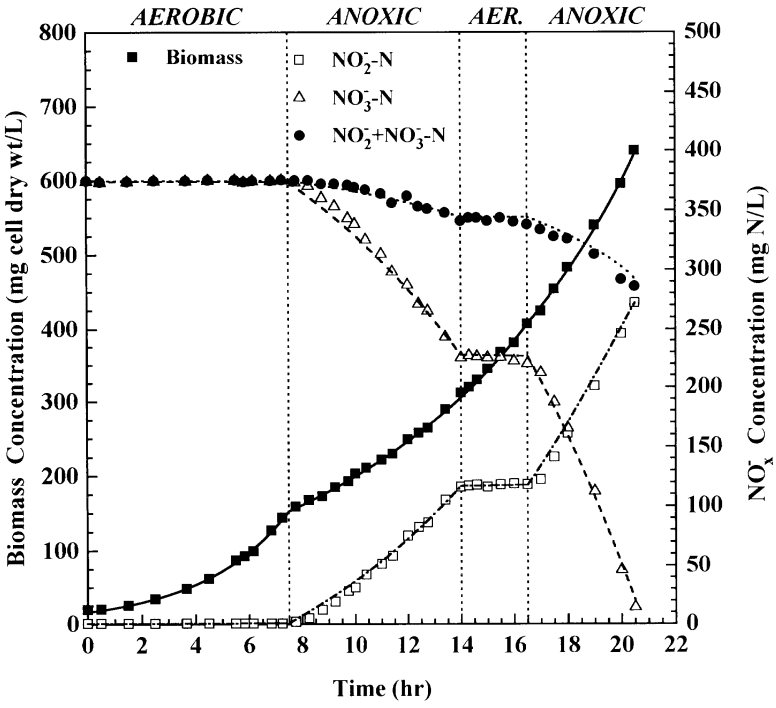

Fig. 7. Response of a batch culture in aerobic conditions to a change to anoxic conditions for 6.5 h, return to aerobic conditions for 2.5 h and then shift back to anoxic conditions. Experimental and theoretical profiles of biomass, nitrate-, nitrite- and total nitrogen concentrations for each growth phase.

Table 3. Description and values of the kinetic parameters obtained from transient batch experiments with *Ps. denitrificans* from aerobic to anoxic conditions of growth and vice-versa

Parameter	Symbol	Value	Units
Maximum adaptability parameter for aerobic to aerobic transition	α_{max1}	1.30	h^{-1}
Maximum adaptability parameter for anoxic to aerobic transition	α_{max2}	0.13	h^{-1}
Nitrite inhibition constant	K_{I3}	57.2	mg NO_2^--N/l
Oxygen inhibition coefficient on the nitrate reduction	K_{Io1}	0.087	mg/l
Oxygen inhibition coefficient on the nitrite reduction through an energy producing pathway	K_{Io2}	0.0015	mg/l
Oxygen inhibition coefficient on the nitrite reduction through a non energy producing pathway	K_{Io3}	0.058	mg/l

agreement between experimental data and model-predicted concentration profiles in all these cases is excellent.

CONCLUSIONS

The growth of a facultative aerobic and denitrifying bacterium, *Ps. denitrificans*, has been previously studied under strictly anoxic (Kornaros *et al.*, 1996) and strictly aerobic conditions (Kornaros and Lyberatos, 1997) and accurate kinetic models describing the observed growth characteristics have been developed. In this work, the transient responses of cultures with cells of *Ps. denitrificans* to changes from anoxic to aerobic conditions and vice-versa were investigated and an appropriate mathematical model was developed. The model reduces to the previous ones for aerobic and anoxic conditions respectively. Dissolved oxygen seemed to act as an inhibitor on the activity of the denitrifying reductases rather than as a repressor of their synthesis. Its inhibitory effect on each one of the steps of the denitrification pathway was modeled by incorporating into the "anoxic" model three inhibition coefficients. The estimated values of these coefficients clearly indicate that the enzymes associated with denitrification (NaR, NiR, NOR and/or N_2OR) present a different sensitivity toward dissolved oxygen concentration. Nitrite reduction to nitrogen gas via a non energy forming pathway was inhibited by about 30% more than nitrate to nitrite reduction, while nitrite reduction to nitrogen gas through an energy producing pathway was almost completely inhibited.

The difference of the magnitude of oxygen inhibition on each one of the denitrification steps resulted in large amounts of nitrite accumulated in the culture medium and probably in incomplete denitrification if NO and/or N_2O was also accumulated. If this type of behavior (toward dissolved oxygen concentrations) is followed by other denitrifying bacteria as well under transient conditions of growth in an actual denitrifying WWTP, then great care has to be taken for the conditions of its operation, due to the toxicity of the produced nitrogenous compounds that are either remaining in the liquid phase (NO_2^-) or escaping to the atmosphere (NO, N_2O).

On the change from aerobic to anoxic growth conditions, cells of *Ps. denitrificans* presented a very rapid adaptability to the anoxic environment, exhibiting full denitrifying activity and no lag phases, while on the reverse change, from anoxic to aerobic conditions, very long lag phases of adaptation were observed.

The overall mathematical model that was developed in the present work is able to accurately describe the behavior of a representative denitrifying bacterium, *Ps. denitrificans*, under strictly aerobic, strictly anoxic and transient conditions of growth. Simple kinetic and stoichiometric functionalities were used to describe the aerobic and anoxic processes mainly based on Monod kinetics for all components that can influence the reaction rates. It is, of course, realized that in a real wastewater treatment process extraneous factors such as formation of activation sludge flocs, mixed cultures, complex substrates etc. may affect the system performance. However, a kinetic model based on a pure culture and a well-defined medium allows assessment of various operating parameters (dissolved oxygen concentration, carbon:nitrate or carbon:nitrite ratio) directly on the denitrification process. Therefore, it may prove useful as a guide for the development or improvement of general activated sludge model frameworks of the type developed by the IAWQ Task Group (IAWQ, 1995). An extended version of the ASM2 could include formation and reduction of nitrite nitrogen by the heterotrophic biomass as well as substrate utilization for these processes. Such an upgrading of the ASM2 would help in the assessment of the operating conditions of a WWTP under which nitrite accumulation could be expected and subsequently, in the manipulation of appropriate operating parameters in order to decrease the nitrite concentrations in the treated effluent. Introduction of appropriate oxygen inhibition coefficients on the denitrification steps in the ASM2, as were used in equations (7)–(19), instead of one global saturation/inhibition oxygen coefficient would be a second step of upgrading which would markedly increase the accuracy and predictive abilities of such model frameworks. Although such revisions would increase the complexity of ASM2, the availability of such a reliable model will help the engineer/researcher/teacher to explore through dynamic simulation, alternative system configurations, to troubleshoot at full-scale WWTPs, to optimize individual processes

and also develop more economic and environmentally friendly design guidelines.

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