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van Niel, Ed; Robertson, L.A.; Kuenen, J.G.

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PO Box 117
221 00 Lund
+46 46-222 00 00

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A mathematical description of the behaviour of mixed chemostat cultures of an autotrophic nitrifier and a heterotrophic nitrifier/aerobic denitrifier; a comparison with experimental data

E.W.J. van Niel, L.A. Robertson and J.G. Kuenen

Kluyver Laboratory for Biotechnology, Delft University of Technology, Delft, The Netherlands

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1. SUMMARY

A general, unstructured mathematical model has been used to describe the behaviour of nutrient-limited growth of two bacteria in a continuous co-culture. The experimental system consisted of a two-membered mixed culture of the heterotrophic nitrifier/aerobic denitrifier, *Thiosphaera pantotropha*, and the autotrophic nitrifier, *Nitrosomonas europaea*, competing for ammonia in chemostat culture. The outcome of competition was only dependent on the Monod constants and the growth yields of the two bacteria. The model shows that both bacteria will oxidize equal amounts of ammonia when the cell ratio of *T. pantotropha* / *N. europaea* is 260.

2. INTRODUCTION

Microbial interactions can be complex, especially in their natural or semi-natural environment. Waste water treatment systems can be a little simpler in that parameters such as temperature and pH are maintained within a defined range, but still involve a great number of prokaryotic and eukaryotic organisms. In some situations (e.g., reactor optimization) it is desirable to understand the interactions between desired physiological types and potential competitors. The most direct way of obtaining this information is to carry out controlled competition experiments with known bacteria, in the laboratory. From the data obtained, mathematical models can be derived and used to predict the behaviour of the bacterial populations when subjected to various environmental stresses.

It has been shown that several heterotrophic nitrifiers, including *Thiosphaera pantotropha*, are able to simultaneously nitrify heterotrophically

Correspondence to: E.W.J. van Niel, Department of Microbiology, Agricultural University, H. van Suchtelenweg 4, 6703 CT Wageningen, The Netherlands.

and denitrify aerobically [1]. Microorganisms which can nitrify, denitrify and remove organic carbon have obvious attractions for waste treatment, but before a reactor can be designed, it is necessary to understand which environmental factors will favour the heterotrophs rather than the autotrophs. The effect of environmental factors on autotrophic nitrification has been studied extensively [2–4], and dissolved oxygen has been identified as being of great importance [3–5]. Mathematical modelling of separate autotrophic nitrification and anaerobic denitrification with respect to different environmental factors has been reported by several authors [6–9]. Mathematical models describing the physiology of simultaneous nitrification and aerobic denitrification by suspended and immobilized axenic cultures of *T. pantotropha* was reported by Geraats et al. [10] and Hooijmans et al. [11].

When two or more organisms compete for one growth-limiting substrate in continuous culture, the outcome will depend on the maximum specific growth rates, the affinity of the organisms for the growth-limiting substrate and the dilution rate [12]. It has been demonstrated, both theoretically and experimentally, that, when all other relevant factors are held constant and are known, two or more species cannot coexist in continuous culture when their growth is limited by a single substrate [13–15]. However, stable coexistence is possible if growth is limited simultaneously by two or more substrates (e.g. [16–18]).

This paper presents a mathematical model based on co-culture experiments with an autotrophic nitrifier, *Nitrosomonas europaea*, and a heterotrophic nitrifier/aerobic denitrifier *Thiosphaera pantotropha* which were described by van Niel [19]. The two species were competing for ammonia and oxygen in chemostat cultures. Two of the variables used in the experimental work, the dissolved oxygen concentration and the *C/N* ratio, will be considered and a comparison made between the model and the experimental results.

3. MATERIALS AND METHODS

The experimental data used to test the model were derived from co-cultures of *Thiosphaera*

pantotropha LMD 82.5 and *Nitrosomonas europaea* (kindly provided by Dr. J.I. Prosser) in ammonia limited chemostat cultures. As described in the text, a variety of *C/N* ratios and dissolved oxygen concentrations were used at two dilution rates ($D = 0.04$ and 0.065 h^{-1}). The carbon and energy source for *T. pantotropha* was acetate, which was growth-limiting. *T. pantotropha* can use the nitrite, produced by *N. europaea*, as a nitrogen source, and was therefore not nitrogen-limited. Full descriptions of the media, analytical and cultural techniques and experimentally derived data have been published elsewhere [19].

A programme using a Runge-Kutta technique and written in the simulation language PSI/e [20] was used to solve the differential equations (1–5). The plots of the steady state equations (12–16) were plotted by means of a programme written in Atari ST Basic.

4. RESULTS AND DISCUSSION

4.1. The model

4.1.1. Assumptions

The model is based on the following assumptions and observations:

- the specific growth rate of the cultures is only dependent on the growth-limiting substrate concentrations in the chemostat (acetate for *T. pantotropha* and ammonia for *N. europaea*), and is equal to the dilution rate;
- growth can be described by Monod kinetics;
- ammonia assimilation by *N. europaea* can be neglected because it is less than 1% of the total ammonia in the feed;
- ammonia is used by *T. pantotropha* both as a nitrogen source and for heterotrophic nitrification;
- acetate is consumed exclusively by *T. pantotropha*;
- nitrogen assimilation is not limiting the growth of either *T. pantotropha* or *N. europaea*;
- nitrite is only used by *T. pantotropha* and exclusively for denitrification;
- nitrite produced by *T. pantotropha* does not

Table 1

Analytical data of conversion rates by *T. pantotropha* grown in mixed culture (data are from Ref. [19])

r_{sT1} (mmol· l ⁻¹ ·h ⁻¹)	r_{sT2} (mmol· l ⁻¹ ·h ⁻¹)	r_{sT3} (mmol· l ⁻¹ ·h ⁻¹)	C_{xT} (10 ¹¹ cells l ⁻¹)
0.38	0.032	0.052	17.9
0.40	0.055	0.046	19.0
0.40	0.036	0.043	20.3
0.40	0.047	0.054	18.0
0.41	0.092	0.010	19.0
0.46	0.092	0.034	21.0
0.48	0.109	0.032	22.8
0.49	0.092	0.020	22.5
0.50	0.108	0.032	25.3
0.58	0.086	0.050	29.8
0.62	0.069	0.049	37.0
0.62	0.123	0.001	31.5
0.70	0.107	0.042	35.8
0.75	0.128	0.012	36.0
1.24	0.189	0.026	67.5
1.40	0.213	0.016	80.0

r_{sT1} is the acetate consumption rate; r_{sT2} is the sum of ammonia assimilation rate and the heterotrophic nitrification rate; r_{sT3} is the aerobic denitrification rate and C_{xT} is the number of *T. pantotropha* cells.

in mixed cultures did not differ from that found for pure cultures ($(3.4 \pm 1.0) \cdot 10^{-3}$ mmol acetate · 10¹¹ cells⁻¹ · h⁻¹ [10]). With $\mu = 0.04$ h⁻¹, the other coefficients could be calculated. The results are listed in Table 2. The values of α_1 and α_2 are both comparable with the values found for *T. pantotropha* grown in pure culture. A value for α_3 for axenic *T. pantotropha* cultures has not yet been reported, and therefore cannot be compared. Figure 1 shows the measured and the calculated conversion rates of acetate by *T. pantotropha*. A linear relationship was observed between the calculated and measured acetate con-

Table 2

Comparison of the calculated stoichiometric coefficients of *T. pantotropha* in mixed culture with the stoichiometric coefficients of this bacterium grown in pure culture [10]

Coefficient	Mixed culture	Pure culture
	(10 ¹¹ cells · mmol ⁻¹)	
α_1	4.26 ± 0.61	4.06 ± 0.22
α_2	7.05 ± 1.58	7.31 ± 0.63
α_3	5.96 ± 1.40	-

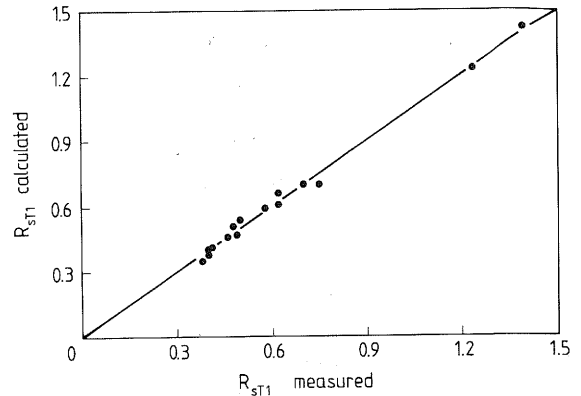


Fig. 1. Measured and calculated acetate utilization (mmol · l⁻¹ · h⁻¹) by *T. pantotropha* in mixed culture with *N. europaea*.

version rates. It is not relevant to apply the same procedure to *N. europaea* because all energy generated by substrate conversion is assumed to be used for biomass production and maintenance requirements.

The extent of nitrification and denitrification changes with growth conditions. According to equation (8), the amount of acetate necessary for these processes also changes. As a consequence, the biomass yield of *T. pantotropha* will not be constant. The biomass yield is the quotient of the biomass production rate and the substrate consumption rate:

$$Y_{sT} = r_{xT}/r_{sT1} \quad (9)$$

4.1.4. Kinetic equations

The following step is to deal with the kinetics of growth and substrate consumption rates. The growth of the bacteria can be described by Monod growth kinetics:

$$\mu_i = (\mu_{\max,i} \cdot C_{sj}) / (K_{s,ij} + C_{sj}) \quad (10)$$

When growth is also oxygen limited, the interactive model for double substrate limitation [22] is used:

$$\mu_i = \mu_{\max,i} \cdot C_{sj} \cdot C_{s4} / (K_{s,ij} + C_{sj})(K_{s,i4} + C_{s4}) \quad (11)$$

The ammonium consumption rate by *N. europaea*, r_{sN2} , is expressed as follows:

$$r_{sN2} = \mu_N \cdot C_{xN} / Y_{sN} \quad (12)$$

accumulate in the culture, and will therefore be denitrified immediately [21];

- the only interactions between the two organisms are the competition for ammonia, and in some cases competition for oxygen;
- the behaviour of the organisms in co-culture is similar to that in pure cultures under the same physicochemical conditions.

4.1.2. Mass balance equations

The growth of *T. pantotropha* in continuous culture can be described by the following equation (for notation used, see list of symbols):

$$dC_{xT}/dt = (\mu_T - D) \cdot C_{xT} \quad (1)$$

and for *N. europaea*:

$$dC_{xN}/dt = (\mu_N - D) \cdot C_{xN} \quad (2)$$

The rate of acetate uptake by the heterotroph is given by the term r_{sT1} . The mass balance equation is then as follows:

$$dC_{s1}/dt = D \cdot (C_{s1,0} - C_{s1}) - r_{sT1} \quad (3)$$

Ammonia is assimilated and nitrified by both bacteria. The mass balance for ammonia includes an expression for the nitrification by the autotroph (r_{sN2}), as well as a composite term representing nitrification and assimilation by the heterotroph (r_{sT2}):

$$dC_{s2}/dt = D \cdot (C_{s2,0} - C_{s2}) - r_{sT2} - r_{sN2} \quad (4)$$

The nitrite produced by the autotroph accumulates (r_{sN3}), and is partly denitrified by the heterotroph (r_{sT3}). Nitrite assimilation by *T. pantotropha* is assumed, on the basis of experimental evidence, to be negligible. Thus the mass balance for nitrite can be written as:

$$dC_{s3}/dt = r_{sN3} - r_{sT3} - D \cdot C_{s3} \quad (5)$$

Using these five differential equations, the behaviour of the co-culture can be adequately described.

4.1.3. Linear relationship between observable conversion rates

For steady-state conditions in a chemostat, the conversion rate, r_I , is given by the product of the dilution rate and the concentration difference between the input and output from the chemostat:

$$r_I = D \cdot (C_{1,out} - C_{1,in}) = D \cdot \Delta C_I \quad (6)$$

where r_I is positive for production and negative for consumption of component i . In the case of *T. pantotropha*, r_{sT1} , r_{sT2} , r_{sT3} are negative and r_{xT} (is equal to $\mu_T \cdot C_{xT}$) is positive. The observable conversion rates are determined by the internal reaction rates. The internal reactions comprise acetate dissimilation and assimilation, ammonia assimilation, nitrification and denitrification. These reactions occur within the cell and cannot be observed directly. ΔC_{sT1} and ΔC_{xT} were found by measuring the amount of acetate consumed and the amount of biomass produced, respectively. The amount of ammonia assimilated and nitrified heterotrophically (ΔC_{sT2}) and the amount of nitrite denitrified aerobically (ΔC_{sT3}) were calculated indirectly from the nitrogen balances as described in Ref. [19]. The conversion rates are related by a linear equation with coefficients composed of stoichiometric coefficients of the internal reactions. The acetate conversion rate is a linear function of the biomass production rate, the ammonia conversion rate, the nitrite conversion rate and the maintenance energy requirements:

$$r_{sT1} = \frac{1}{\alpha_1} \cdot r_{xT} + \frac{\alpha_2}{\alpha_1} \cdot r_{sT2} + \frac{\alpha_3}{\alpha_1} \cdot r_{sT3} + m_T \cdot C_{xT} \quad (7)$$

The results that are considered here were derived at only one dilution rate. Therefore, the maintenance energy requirements (m_T) cannot be calculated independently from the biomass yield on acetate (α_1). Equation (7) changes to:

$$r_{sT1} = \left(\frac{\mu}{\alpha_1} + m_T \right) \cdot C_{xT} + \frac{\alpha_2}{\alpha_1} \cdot r_{sT2} + \frac{\alpha_3}{\alpha_1} \cdot r_{sT3} \quad (8)$$

By using a linear regression procedure, it is possible to calculate the coefficients ($\mu/\alpha_1 + m_T$), α_2/α_1 , and α_3/α_1 . The conversion and production rates of the components during the steady states of the mixed cultures are listed in Table 1. Fitting of the coefficients gives $(\mu/\alpha_1 + m_T) = (1.28 \pm 0.09) \cdot 10^{-2}$; $\alpha_2/\alpha_1 = 1.65 \pm 0.29$ and $\alpha_3/\alpha_1 = 1.40 \pm 0.29$. To find values for α_1 , α_2 and α_3 , it was assumed that the value of the maintenance coefficient of *T. pantotropha* (m_T)

Because only one growth rate (0.04 h^{-1}) is considered here, the maintenance requirements of *N. europaea* are included in the yield.

The kinetics of ammonia assimilation by the heterotroph were not measured. It was assumed that zero order kinetics in the ammonia concentration were affective because of the very low affinity constant for this compound. The kinetics of heterotrophic nitrification can be calculated from the nitrogen balances of pure and mixed cultures. Even at relatively high ammonia concentrations ($> 1 \text{ mM}$), the rate of nitrification increased with the ammonia concentration. Considering the high K_m of ammonia monooxygenase (AMO), the first enzyme in the nitrification pathway, it was assumed that AMO was the rate-limiting step [19]. Nitrification can be described by Michaelis-Menten kinetics with a maximum specific activity for AMO, $Q_{\max,H}$ (h^{-1}). Total ammonia consumption rate by the heterotroph, r_{sT2} , is given in the next equation as the sum of the uptake rates for assimilation (r_{sTA}) and heterotrophic nitrification (r_{sTH}):

$$r_{sTA} = \alpha_n \cdot \mu_T \cdot C_{xT} \quad (13a)$$

$$r_{sTH} = \frac{Q_{\max,H} \cdot C_{s2}}{(K_{s,H2} + C_{s2})} \cdot C_{xT} \quad (13b)$$

Oxygen was assumed to not be limiting heterotrophic nitrification.

The rate of nitrite production, r_{sN3} , by *N. europaea* is equal to its ammonia consumption rate:

$$r_{sN3} = r_{sN2} \quad (14)$$

Denitrification activity of *T. pantotropha* is therefore the sum of the total reduction of nitrite formed by heterotrophic nitrification (equal to the heterotrophic nitrification rate (r_{sTH})) and of reduction of part of the nitrite formed by the autotroph. Denitrification was assumed to be zero order in respect of nitrite concentration because of the presence of nitrite concentrations (1–10 mM) sufficiently greater than the affinity constant for nitrite ($K_{sT3} \approx 10 \text{ } \mu\text{M}$ [23]). Furthermore, the amount of denitrification was assumed to be proportional to the amount of biomass. The rate of nitrite disappearance from the culture by denitrification, r_{sT3} , is therefore the difference

Table 3

Values of the parameters used in the model (for the units, see the Symbol list)

$\mu_{\max,N}$	0.076 ^a	$K_{sT,3}$	0.01 ^a	D	0.04
$\mu_{\max,T}$	0.35 ^a	$K_{s,H2}$	0.3 ^a		
$K_{sN,2}$	0.1 ^a	α_n	0.043 ^a		
$K_{sN,4}$	0.012 ^a	Y_{sN}	0.11 ^a		
$K_{sT,1}$	0.02 ^a	$Q_{\max,H}$	0.0045 ^b		
$K_{sT,4}$	0.002 ^a	Q_D	0.0020 ^b		

^a Derived from pure cultures [19,23].

^b Derived from co-culture experiments [19].

between total denitrification rate ($Q_D \cdot C_{xT}$) and heterotrophic nitrification rate (r_{sTH}):

$$r_{sT3} = Q_D \cdot C_{xT} - r_{sTH} \quad (15)$$

4.1.5. Values of the parameters

Some of the parameters were determined from experiments with pure cultures, and others were derived from the co-culture experiments [19]. The values of the parameters are listed in Table 3.

4.2. Simulation with the model

Development of the co-culture from start-up to steady-state was simulated by solving the five differential equations (1–5) numerically. The model was then tested for the behaviour of the bacterial community under steady state conditions.

4.2.1. Development of the co-culture

The simulation involved the behaviour of a community made up by a pure culture of *N. europaea*, grown to steady-state under ammonia limitation and under the conditions to be tested, to which *T. pantotropha* and acetate had been added [19]. From Fig. 2, it can be seen that *T. pantotropha* is expected to grow exponentially until the acetate becomes limiting. During the exponential phase, the heterotroph will consume ammonia in increasing amounts. As a consequence, the autotrophic population, and thus the nitrite concentration, will decrease. Figure 2A shows the simulated development of a co-culture with a dissolved oxygen concentration of 50% air saturation, a dilution rate of 0.04 h^{-1} and a C/N ratio of 2. A steady state should be reached after

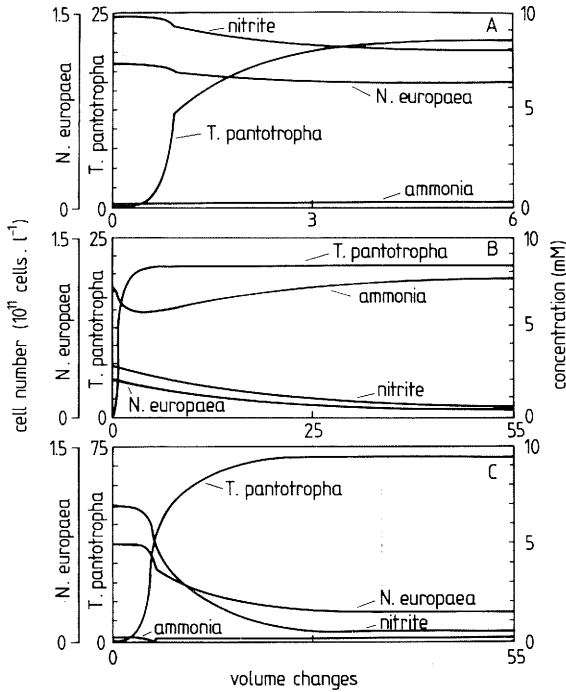


Fig. 2. Graphic representation of the numerical solutions of the differential equations describing the development of a mixed culture of the heterotroph *Thiosphaera pantotropha* and the autotroph *Nitrosomonas europaea* and the concentrations of ammonia and nitrite for three different sets of C/N ratios and air saturations at a dilution rate of 0.04 h^{-1} : (A) $118 \mu\text{M}$ oxygen, C/N ratio 2, 10 mM acetate, 10 mM ammonia; (B) $10 \mu\text{M}$ oxygen, C/N ratio 2, 10 mM acetate, 10 mM ammonia; and (C) $118 \mu\text{M}$ oxygen, C/N ratio 9.6, 32.5 mM acetate, 6.8 mM ammonia.

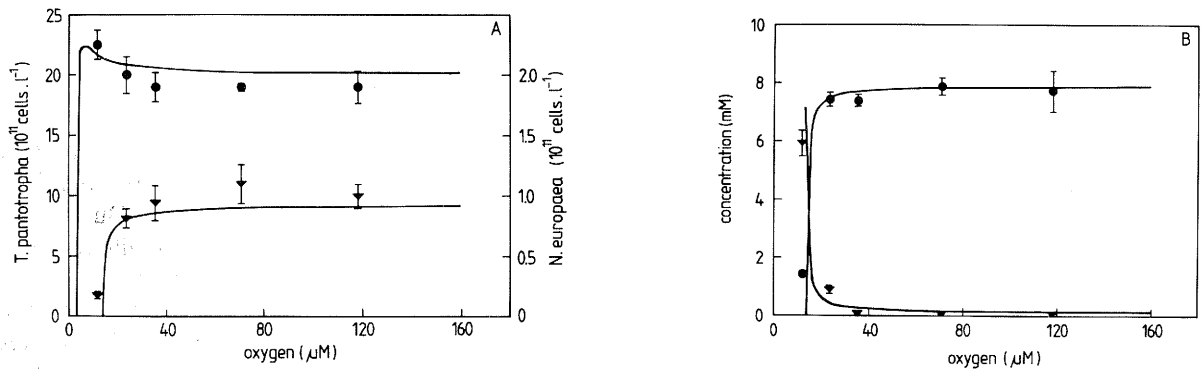


Fig. 3. Graphic representations of steady-state cell concentrations of a two membered culture of *Thiosphaera pantotropha* and *Nitrosomonas europaea* at various dissolved oxygen concentrations. The experimental results are plotted as points: (A) (●) *T. pantotropha*; (▼) *N. europaea*; (B) (●) nitrite; (▼) ammonia. The lines represent the solution of the steady-state equations. The error in the measurements is indicated by vertical bars.

about five volume changes. Under conditions less favourable to *N. europaea*, it will take longer to reach a steady state. Thus, at an oxygen concentration of 5% of air saturation, this will take more than 40 volume changes (Fig. 2B). A similar effect was also observed at higher C/N ratios (Fig. 2C).

4.2.2. Steady-state situations

At steady state ($dC_{xT}/dt = dC_{xn}/dt = dC_{s1}/dt = dC_{s2}/dt = dC_{s3}/dt = 0$), none of the concentrations will change. This means that the differential equations change into algebraic equations, and that the biomass and substrate concentrations can be written as functions of the dissolved oxygen concentration and the dilution rate. Solving equation (1) for steady-state situations, combined with equation (10) gives, for acetate:

$$C_{s1} = \frac{D \cdot K_{s,T1}}{\mu_{\max,T} - D} \quad (16)$$

Solving equation (3) combined with equations (8) and (11) leads to the steady-state equation for the biomass of *T. pantotropha*:

$$C_{xT} = (C_{s,10} - C_{s1}) \cdot (K_{s,T4}/C_{s4} + 1) \cdot Y_{sT} \quad (17)$$

Solving equation (2) combined with equation (11) gives the steady-state equation for the ammonia concentration:

$$C_{s2} = \frac{D \cdot (K_{s,N4} + C_{s4}) \cdot K_{s,N2}}{(\mu_{\max,N} - D) \cdot C_{s4} - D \cdot K_{s,N4}} \quad (18)$$

Solving equation (4) combined with equation (12) gives the steady state equation for the biomass of *N. europaea*:

$$C_{xN} = \left\{ (D \cdot (C_{s20} - C_{s2}) - r_{sT2}) \cdot (K_{s,N2} + C_{s2}) \cdot (K_{s,N4} + C_{s4}) \cdot Y_{sN} \right\} \cdot \left\{ \mu_{\max,N} \cdot C_{s2} \cdot C_{s4} \right\}^{-1} \quad (19)$$

Finally, solving equation (5) combined with equation (15) provides an expression for the steady state equation for nitrite:

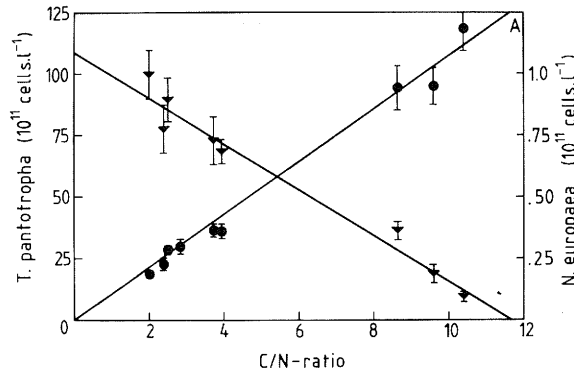
$$C_{s3} = C_{xN}/Y_{sN} - (Q_D \cdot C_{xT} - r_{sTH})/D \quad (20)$$

4.2.3. Oxygen as a variable

In Fig. 3A and B, the solutions for the equations (17–21) are plotted as lines, with the dissolved oxygen concentration as the variable. The symbols represent experimentally-derived results. At low dissolved oxygen concentrations, *N. europaea* was more affected than *T. pantotropha*, because of its lower affinity for oxygen. This can be seen from the simulation.

4.2.4. C/N ratio as a variable

The solutions for the equations (17–21) with the C/N ratio as the variable, calculated as $2 \cdot C_{s10}/C_{s20}$ are shown in Fig. 4A and B. The simulation predicts that *N. europaea* will wash out totally at a C/N ratio higher than 11.6. At this critical C/N ratio, the heterotroph will switch from energy- and carbon limitation to nitrogen limitation. It can be seen that there is a good correlation between the model and the experimental data.



4.3. Competition for ammonia between the heterotroph and the autotroph

The amount of ammonia used by *T. pantotropha* is dependent on the acetate concentration in the feed. The steady-state ammonia concentration in the culture is, in most cases, defined by *N. europaea*. The utilization rates of ammonia by the heterotroph and the autotroph can be related to the difference between the incoming and outgoing ammonia flow rates of the continuous coculture by means of a linear equation:

$$r_{sTA} + r_{sTH} + r_{sN2} = (C_{s2,0} - C_{s2}) \cdot D \quad (21)$$

Under the conditions studied here ($D = 0.04 \text{ h}^{-1}$; 50% air saturation; 10 and 0.13 mM ammonia in the feed and culture, respectively), equation (21) can be changed into:

$$1.72 \cdot 10^{-3} \cdot C_{xT} + 1.36 \cdot 10^{-3} \cdot C_{xT} + 3.54 \cdot 10^{-1} \cdot C_{xN} = 3.95 \cdot 10^{-1} \quad (22a)$$

or:

$$3.08 \cdot 10^{-3} \cdot C_{xT} + 3.54 \cdot 10^{-1} \cdot C_{xN} = 3.95 \cdot 10^{-1} \quad (22b)$$

From equation (22a), a cell ratio $C_{xT}/C_{xN} = 260$ is derived. At this ratio the amount of ammonia nitrified by the heterotroph equals the amount of ammonia nitrified by the autotroph. This is similar to the results found experimentally [19]. At a cell ratio $C_{xT}/C_{xN} = 115$, *T. pantotropha* used, ammonia assimilation included, as much ammonia as *N. europaea*. Equation (22b) represents a

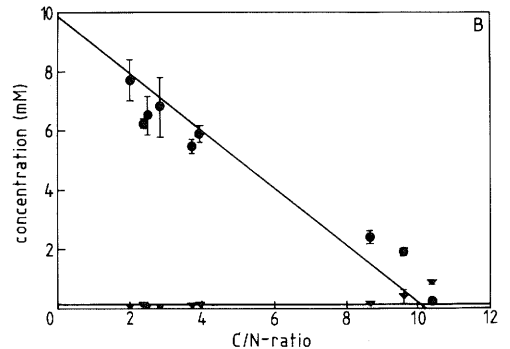


Fig. 4. Graphic representations of steady-state cell concentration of a two membered culture of *Thiosphaera pantotropha* and *Nitrosomonas europaea* at various C/N ratios. The same notation is used as in Fig. 3.

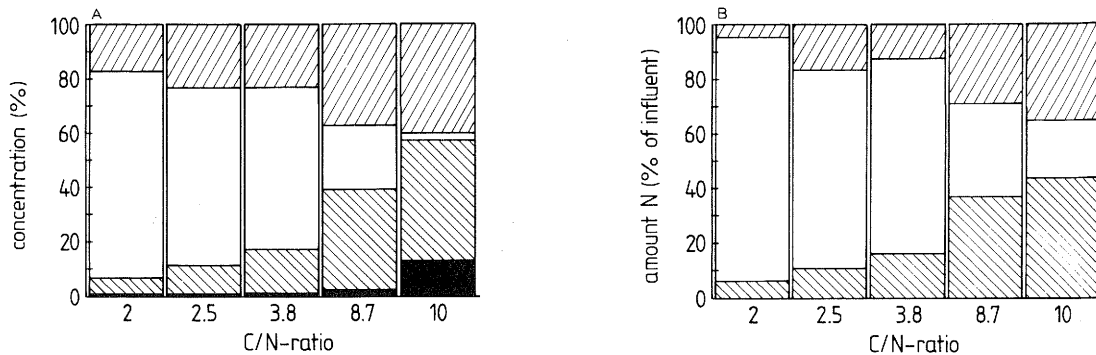


Fig. 5. (A) Conversion of ammonia (as a percentage of the total ammonia in the feed) by a mixed culture of *Thiosphaera pantotropha* and *Nitrosomonas europaea* at several C/N ratios into biomass (▨), for nitrite accumulation (□) and to nitrogen gas (▧); (■) ammonia in the effluent. (B) Conversion of ammonia (as a percentage of total converted ammonia) by assimilation into biomass (▨), by autotrophic nitrification (□) and by heterotrophic nitrification (▧).

linear equation describing combinations of *T. pantotropha* and *N. europaea* cell numbers under the conditions described above. The values of the coordinates are entirely dependent on the acetate concentration. A *N. europaea* population cannot establish itself under these conditions ($C_{xN} = 0$, eqn. 22b) when there are $128 \cdot 10^{11}$ *T. pantotropha* cells present, as the heterotroph has become nitrogen rather than carbon limited.

4.4. Conversion of ammonia by the mixed culture

The incoming ammonia is partly converted by the co-culture to nitrite and nitrogen gas, and partly assimilated into the biomass. Nitrogen gas is entirely formed by *T. pantotropha*. This organism denitrifies all of the nitrite produced by its heterotrophic nitrification system, and part of the nitrite produced by *N. europaea*. More *T. pantotropha* biomass is produced at higher C/N ratios. As a result, more ammonia is assimilated or nitrified heterotrophically and denitrified to nitrogen gas (Fig. 5A and B). From Fig. 5, it can be seen that at every C/N ratio, the amount of ammonia nitrified and denitrified by *T. pantotropha* approximately equals the amount of ammonia assimilated. At C/N ratios higher than 11.5, only *T. pantotropha* will remain in the (ammonia-limited) culture. About 50% of the incoming ammonia will then leave the system in the form of biomass and 50% in the form of nitrogen gas.

4.5. Conclusions

- The model describes the experimental results adequately.
- In ammonia limited co-cultures, under the specified conditions, at cell ratios of *T. pantotropha* / *N. europaea* of 260 and higher, more ammonia is nitrified by the heterotroph than the autotroph.
- At C/N ratios above 11.5, *N. europaea* will disappear completely from the culture, under these conditions. At these high C/N ratios, *T. pantotropha* was nitrogen- rather than carbon-limited.
- Heterotrophic nitrification by *T. pantotropha* has a lower activity and a lower affinity for ammonia than autotrophic nitrification [19]. In spite of that, the heterotrophic population is able to oxidize more ammonia than the autotroph because of higher cell numbers. It appears that the affinity for substrate is not decisive in this form of competition, but only affects the extent to which the heterotroph must be in excess to successfully compete with the autotroph.

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SYMBOL LIST

C_{sj}	concentration substrate j	$\text{mmol} \cdot \text{l}^{-1}$
$C_{sj,0}$	concentration substrate j in feed	$\text{mmol} \cdot \text{l}^{-1}$
C_{xi}	concentration biomass organism i	$10^{11} \text{ cells} \cdot \text{l}^{-1}$
D	dilution rate	h^{-1}
$K_{s,i}$	affinity constant of organism i for substrate j	$\text{mmol} \cdot \text{l}^{-1}$
$K_{s,p}$	affinity constant for process p for substrate j	$\text{mmol} \cdot \text{l}^{-1}$
m_i	maintenance energy requirements of organism i	$\text{mmol} \cdot 10^{11} \cdot \text{cells}^{-1} \cdot \text{h}^{-1}$
q_i	nitrification quotient of organism i	$\text{mmol N} \cdot \text{h}^{-1} \cdot 10^{11} \text{ cells}^{-1}$
Q_D	specific denitrification activity	$\text{mmol N} \cdot \text{h}^{-1} \cdot 10^{11} \text{ cells}^{-1}$
$Q_{\max,p}$	maximum specific activity of process p	$\text{mmol} \cdot \text{h}^{-1} \cdot 10^{11} \text{ cells}^{-1}$
$r_{s,i}$	substrate consumption rate of organism i on substrate j	$\text{mmol} \cdot \text{l}^{-1} \cdot \text{h}^{-1}$
r_{xi}	biomass production rate of organism i	$10^{11} \text{ cells} \cdot \text{h}^{-1}$
t	time	h
$Y_{\max,si}$	maximum growth yield of organism i	$10^{11} \text{ cells} \cdot \text{mmol}^{-1}$
Y_{si}	growth yield of organism i	$10^{11} \text{ cells} \cdot \text{mmol}^{-1}$
Greek symbols		
α_j	stoichiometric coefficient substrate j	$10^{11} \text{ cells} \cdot \text{mmol}^{-1}$
α_n	stoichiometric coefficient	$\text{mmol NH}_3 \cdot 10^{11} \text{ cells}^{-1}$
$\mu_{\max,i}$	maximum specific growth rate organism i	h^{-1}
μ_i	specific growth rate organism i	h^{-1}
Δ	difference sign for incoming and outgoing concentration subscripts	
Subscripts		
1	acetate	
2	ammonia	
3	nitrite	
4	oxygen	
A	assimilation	
D	denitrification	
H	heterotrophic nitrification	
N	<i>N. europaea</i>	
T	<i>T. pantotropha</i>	

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