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Modelling of the Nitrifying compartment of MELiSSA

Review of models and Basis of a dynamic structured model of the Nitrifying compartment

- Review of biological kinetics and growth factors of nitrifying organisms
- Review of flows dynamics inside nitrifying bioreactors
- Basis of the modelling of the nitrifying bioreactor:
  flow model
  biological and physical kinetics

TECHNICAL NOTE 27.1

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T.N. 27.1: Modelling the nitrifying compartment
CRITICAL REVIEW OF MODELS

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INTRODUCTION

The aim of this Technical note is to complete the TN 23.2 which described the metabolism of *Nitrosomonas* and *Nitrobacter* by developing a dynamic model for their growth in a fixed bed column.

The model proposed will be as complete as possible, even if further asssumptions must be made to give a model more manageable for numerical treatment. As presented in TN 23.2, the model will be built from stoichiometries describing the metabolic behaviour of nitrifiers combined with biological kinetics, chemical relations and hydrodynamic equations of the column.

This TN will be divided into 4 sections.

In a first section, a bibliographic review of nitrification processes will be made. The review focus on the stoichiometric and kinetic representation of nitrification. That will provide a data base of kinetic parameters, usefull for the first numerical treatments of the model and for comparison with further results from UAB MELiSSA Laboratory on the nitrifying compartment.

The second section deals with hydrodynamics, and a special attention will be made on the hydrodynamic description of column and fixed bed reactors.

The third section presents the transfer limitations that could occur in the fixed bed and the relations developed to take these limitations into account in a dynamic model.

The last section proposes a model for the fixed bed nitrification column. The equations for hydrodynamics are associated to transfer and biological relations.

The continuation of the dynamic modelling of the nitrifying bed will be made in the next TN 27.2, involving numerical treatment, first simulations and parameter sensitivity.

I. Bibliographic review of the nitrification process.

The importance of nitrification has been recognised for more than one century. During waste treatment, the nitrification-denitrification systems have been developed for the nitrogen control preceding discharge of the treated waste. Then a lot of studies have been done on the effectiveness of biological nitrogen control processes and especially on the ability of nitrifying organisms to oxidise ammonia to nitrate.

In order to limit the field of the present study, the bibliographic reviewing will be focused on the overview of the different stoichiometric description of the nitrification by *Nitrosomonas* and *Nitrobacter* species, and on the models developed to study the growth kinetics and the growth factors of these nitrifying organisms.

1.1. - Stoichiometric representation of the autotrophic nitrification

❑ The most common representation of nitrification is the following set of reactions for the oxidation of ammonia and nitrite:

$$NH_4^+ + 1.5\,O_2 \rightarrow NO_2^- + 2\,H^+ + H_2O$$
$$NO_2^- + 0.5\,O_2 \rightarrow NO_3^-$$

In kinetics studies, such a representation is used to show the process and the main limiting substrates of the growth (O_2;NH_4^+;NO_2^-;NO_3^-) (Hunik et al., 1994) or to describe the pH limiting effect (Laudelout et al., 1976; Anthonisen et al.,1976).

Laudelout et al. used the stoichiometric yield of these equations to calculate the oxygen dynamic ($\frac{dC_{O2}}{dt}$) and to model growth kinetics in O_2 limiting conditions. In a similar way, Tanaka et al. (1981) defined the relationship between the molar reaction rate of nitrification and the oxygen consumption rate from these stoichiometries, as:

$$r_{O2} = \frac{3}{2} r_{NH4} + \frac{1}{2} r_{NO2}$$

Such a reduction of the elemental balance to this set of equations is allowed by the low growth rate of autotrophic nitrifiers. But it must be outlined that this representation is not sufficient to represent the complete elemental balance of the nitrification, and especially of the biomass synthesis associated to nitrification.

❑ A more complete representation of nitrification is usually proposed by introducing the biomass produced by ammonia and nitrite oxidation in the stoichiometric description.

Because of the low growth rate of nitrifiers, the biomass production rate is low and it is difficult to obtain enough biomass to analyse its composition. As a consequence, the chemical biomass composition usually found in literature is a mean biomass calculated for aerobic bacteria (Roels, 1983). Nevertheless, an elemental composition has been established by Forler (1994) for a nitrifying coculture cultived in fed-batch conditions.

The stoichiometries proposed by several authors are based upon the experimental determination of the mean ratio CO_2 consummed/N oxidised.

From Haug et al. (1972)

Nitrosomonas

$$55\ NH_3 + 5\ CO_2 + 76\ O_2 \longrightarrow C_5H_7O_2N + 54\ NO_2^- + 52\ H_2O + 54\ H^+$$

Nitrobacter

$$400\ NO_2^- + 5\ CO_2 + NH_3 + 195\ O_2 \longrightarrow C_5H_7O_2N + 400\ NO_3^- + 2\ H_2O$$

The nitrification was represented by the sum of these stoichiometries

$$9.7154\ NH_3 + 18.0308\ O_2 + CO_2 \longrightarrow CH_{1.4}O_{0.4}N_{0.2} + 9.5154\ NO_3^- + 9.2106\ H_2O + 9.5154\ H^+$$

A similar stoichiometry was proposed by Kowalski and Lewandowski (1981)

$$NH_4^+ + 1.83\ O_2 + 1.98\ HCO_3^- \longrightarrow 0.021\ C_5H_7O_2N + 1.04\ H_2O + 0.98\ NO_3^- + 1{,}88\ H_2CO_3$$

i.e.

$$9.5238\ NH_4^+ + 17.4286\ O_2 + 18.8571\ HCO_3^- \Downarrow CH_{1.4}O_{0.4}N_{0.2} + 9.9048\ H_2O + 9.3333\ NO_3^- + 17.9048\ H_2CO_3$$

Derived from these stoichiometries of nitrification, a simplified stoichiometric expression of the nitrification usually assumed to be (Ballay D., 1986):

$$10\ NH_3 + CO_2 + 18.2\ O_2 \longrightarrow CH_{1.4}O_{0.4}N_{0.2} + 9.8\ HNO_3 + 9.4\ H_2O$$

Stoichiometries for *Nitrosomonas* and *Nitrobacter*, mainly based upon a biomass biochemical composition extrapolated from *Rs. rubrum*, have been developed for the autotrophic nitrification in the previous studies of the nitrifying compartment (TN 23.2). These stoichiometries are metabolically structured, then such a description of the nitrification can be used to build a metabolically structured model of the phenomenum. However, it must be kept in mind that at the present time, the chemical biomass composition calculated ($CH_{1.6147}O_{0.3906}N_{0.1994}S_{0.0035}P_{0.0089}$) was different from the experimental one, determined by Forler (1994), ($CH_{1.9168}O_{0.4055}N_{0.1553}S_{0.0034}P_{0.0072}$). Moreover, the reserve metabolism of nitrifying organisms has not been taken into account, and it must be noted that Bock (1976) reported that *Nitrobacter agilis* growing on with organic matter stored large quantities of PHB, polyphosphate and possibly glycogen-like material. Laanbroek and Gerards (1993) reported that under no energy limiting condition, when another nutrient substrate became limiting, nitrifiers used the surplus energy of N-oxidation to synthesize reserve material (polyphosphate and/or PHB).

Nitrosomonas

$$CO_2 + 0.0089\ H_3PO_4 + 0.0035\ H_2SO_4 + 26.0166\ O_2 + 18.2606\ NH_3 \Downarrow CH_{1.6147}O_{0.3906}N_{0.1994}S_{0.0035}P_{0.0089} + 18.0612\ (NO_2^-,H^+) + 8.5391 H_2O$$

Nitrobacter

$$CO_2 + 0.0089\ H_3PO_4 + 0.0035\ H_2SO_4 + 38.8496\ O_2 + 79.8494\ (NO_2^-,H^+) + 0.1994\ NH_3 + 0.4914\ H_2O \Downarrow CH_{1.6147}O_{0.3906}N_{0.1994}S_{0.0035}P_{0.0089} + 79.8494\ (NO_3^-,H^+)$$

A nitrification which completely exhaust nitrite can be represented by adding these two previous equations. The following stoichiometry is the result of this addition:

$$CO_2 + 0.0089\ H_3PO_4 + 0.0035\ H_2SO_4 + 28.3838\ O_2 + 14.9289\ NH_3 \Downarrow CH_{1.6147}O_{0.3906}N_{0.1994}S_{0.0035}P_{0.0089} + 14.7295\ (NO_3^-,H^+) + 6.8733\ H_2O$$

The biomass defined by this stoichiometry represents the sum of the biomass of *Nitrosomonas* and *Nitrobacter* and is composed of 81% of *Nitrosomonas* and 19% of *Nitrobacter* (TN 23.2)

❑ An example of a metabolically structured model has been developed by Geraats et al. (1990) for the heterotrophic nitrification and denitrification by *Thiosphaera pantotropha*. This model involves a set of 6 metabolic reactions, associated to a metabolic rate r_i.

Metabolic reactions
- biosynthesis + maintenance
- catabolism
- oxydative phosphorylation
- ammonia oxidation
- nitrate reduction
- nitrite reduction

The reaction rates are further used to calculate the concentration profile of each compound from a relation of the form:

$$\frac{dC_{s_i}}{dt} = \text{Input} - \text{Output} + \sum_j r_j . \nu_{s_i,j}$$

Our aim is to build a similar model for the autotrophic nitrification using the metabolic description of nitrification developed in TN 23.2 and kinetic models proposed in the literature.

1.2 Growth yields, limiting factors and inhibitors of nitrification.

Growth parameters for ammonia and nitrite oxidisers have been measured in both batch and continuous flow systems involving pure cultures, and in mixed culture systems with enriched cultures, particulary for soil and sewage.

1.2.1 Growth rates and growth yields

Prosser (1989) has reviewed the growth rates and the growth yields of pure cultures of nitrifying bacteria. This section will summarise the main characteristics of the growth of nitrifying organisms that Prosser had reported.

The μ_{max} (maximum growth rate) values for both ammonia and nitrite oxidisers are low for pure and mixed culture systems under ideal conditions in comparison to those of heterotrophs. Values of μ_{max} lie usually in the range 0.014-0.064 h^{-1} (doubling time 11-50 h) (table 1), because μ_{max} is limited by the low energy gain from ammonia and nitrite oxidation (TN 23.3).

Table 1: Maximum specific growth rate (μ_{max}) values of pure cultures of nitrifying bacteria (* referenced by Prosser, 1989).

	μ_{max} (h^{-1})		
	Batch culture	Continuous culture	Reference
Ammonia oxidisers			
Nitrosomonas spp.			
Sewage isolate	0.016-0.058		Loveless and Painter (1968) *
Sewage isolate	0.012-0.043		
N. europea	0.088	0.063	Skinner et Walker (1961) *
N. europea	0.02-0.03		Drozd (1980) *
N. europea ATCC	0.052-0.066		Belser and Schmidt (1980) *
N. europea FH1	0.052-0.054		
N. europea	0.036	0.064	Helder and de Vries (1983) *
N. europea	0.017	0.039	Keen and Prosser (1987) *
N. marina	0.018		Glover (1985) *
Nitrosococcus oceanus	0.014		Glover (1985) *
Nitrosocistys oceanus	0.032		Belser and Schmidt (1980) *
Nitrosospira AV2	0.033-0.035		
Nitrosolobus AV3	0.043-0.044		
N. europea		0.057	Hunik et al. (1994)
Nitrite oxidisers			
Nitrobacter spp.			
N. sp	0.058		Gould and lees (1960) *
N. N. W.	0.039	0.033	Gay and Corman (1984) *
N. L.	0.025	0.018	
N. sp.	0.043	0.035	Keen and Prosser (1987) *
Nitrococcus mobilis	0.033		Glover (1985) *
N. agilis		0.036	Hunik et al. (1994)

It must be noted that the biomass activity does not depend solely on the rate of which cell mass may be doubled, *i.e.* the specific growth rate of biomass (μ):

- μ can therefore be increased by decreasing cell size for the same cell activity
- by the same way, cells with the same μ can have different cell activities, again due to cell size.

The determination of cell activity is then a complementary indicator for the estimation of the nitrification efficiency. That is the reason why the cell activity was calculated in batch culture (calculated per unit of cell) or in continuous culture (calculated per unit of biomass) (table 2).

Table 2: Cell and biomass activities for nitrifying bacteria (taken from Prosser, 1989).

Ammonia oxidisers		
Cell activity (fmol NO_2^- $cell^{-1}$ h^{-1})		
Nitrosomonas europea	20	Belser (1979)
Nitrosomonas europea ATCC	11	
Nitrosomonas sp.	23	
Nitrosomonas	0.9-5.1	Remacle and Deleval (1978)
Nitrosomonas FH1	23	Belser and Schmidt (1980)
Nitrosomonas marina	0.9-4.9	Glover (1985)
Nitrosomonas europea	1.0-7.0	Keen and Prosser (1987)
Nitrosococcus oceanus	13.7-31.3	Glover (1985)
Nitrosospira briensis	4	Belser (1979)
Nitrosolobus multiformis	23	
Biomass activity (nmol NO_2^- g biomass h^{-1})		
Nitrosomonas europea	4	Skinner and Walker (1961)
Nitrosomonas europea	30-200	Drozd (1980)
Nitrosomonas europea	7.5-16	Keen and Prosser (1987)
Nitrite oxidisers		
Cell activity (fmol NO_3^- cell h^{-1})		
Nitrobacter sp.	5.1-16.6	Remacle and Deleval (1978)
Nitrobacter spp.	9-42	Belser (1979)
Nitrobacter sp.	5.1-13.6	Keen and Prosser (1987)
Nitrococcus oceanus	6.7-11.4	Glover (1985)
Biomass activity (NO_3^- g biomass h^{-1})		
Nitrobacter sp.	15.7-25.2	Keen and Prosser (1987)

Both Keen and Prosser (1987) and Glover (1985) found cell activity to increase with specific growth rate in continuous culture.

Values for yield coefficients on ammonia and nitrite are given in table 3. Values are similar for both groups of nitrifiers and each cell must convert in the order of 10 times its own weight of ammonia (or nitrite) nitrogen to double mass. Experimental values of true growth yield and maintenance coefficients have been calculated by Keen and Prosser (1987). These values were obtained from a Pirt-Herbert growth model (see section 1.3). The maintenance was calculated from steady state continuous culture data, assuming the maintenance to be independent of the specific growth rate.

The variability of the values, presented in tables 2 and 3, is mainly due to the different origins of the strains tested (waste water, soil...) and to the different culture conditions (pH, limitations...).

Table 3: Yield and maintenance coefficients for nitrifying bacteria (* referenced by Prosser, 1989)

Ammonia oxidisers		
Biomass and cell yield on ammonia		
Nitrosomonas sp.	0.42-1.40 g biomass mol^{-1}	Loveless and Painter (1968) *
Nitrisomonas europea	0.6-0.8 g biomass mol^{-1}	Drozd (1980) *
Nitrosomonas europea	1.26-1.72 g biomass mol^{-1}	Keen and Prosser (1987) *
Nitrosomonas ATCC	4.61-6.44 10^{12} cells mol^{-1}	Belser and Schmidt (1980) *
Nitrosomonas FH1	1.98-2.4410^{12} cells mol^{-1}	
Nitrospira sp.	8.04-10.6 10^{12} cells mol^{-1}	
Nitrosolobus sp.	1.85-2.24 10^{12} cells mol^{-1}	
Nitrisomonas europea	1.66 g biomass mol^{-1}	Hunik et al. (1994)
True growth yield	5.88 g biomass mol^{-1}	*
Maintenance coefficient	0.88 mol g $biomass^{-1}$ h^{-1}	*
maintenance *Nitrisomonas europea*	3.38 10^{-3}mol g $biomass^{-1}$ h^{-1}	Calculated from Hunik et al. (1994)
Carbon Yield on ammonia (ratio CO_2/NO_2^-)		
Nitrosomonas sp.	0.090	Wezernak and Gannon (1967) *
Nitrosomonas sp.	0.033	Helder and de Vries (1983) *
Nitrosomonas spp.	0.081-0.094	Belser (1984)*
Nitrosomonas marina	0.04-0.07	Glover (1985) *
Nitrosospira spp.	0.075-0.096	Belser (1984)*
Nitrosococcus oceanus	0.07-0.13	Gunderson (1966) *
Nitrosococcus oceanus	0.06-0.10	Carlucci and Strickland (1968) *
Nitrosococcus mobilis	0.014-0.031	Glover (1985) *
Nitrite oxidisers		
Biomass yield on nitrite		
Nitrobacter sp.	1.11-1.51 g biomass mol^{-1}	Keen and Prosser (1987) *
Nitrobacter agilis	0.58 g biomass mol^{-1}	Hunik et al. (1994)
True growth yield	9.8 g biomass mol^{-1}	*
Maintenance coefficient	0.78 mol g $biomass^{-1}$ h^{-1}	*
maintenance *Nitrobacter agilis*	7.92 10^{-3}mol g $biomass^{-1}$ h^{-1}	Calculated from Hunik et al. (1994)
Carbon Yield on nitrite (ratio CO_2/NO_3^-)		
Nitrobacter sp.	0.013-0.014	Schon (1965)*
Nitrobacter sp.	0.0125	Wezernak and Gannon (1967) *
Nitrobacter sp.	0.02	Helder and de Vries (1983) *
Nitrobacter spp.	0.02-0.03	Belser (1984)*
Nitrococcus mobilis	0.014-0.031	Glover (1985) *

1.2.2 Growth limiting factors

Two forms of limitations have been studied: substrates limitations and physical limitations introduced by the culture conditions.

❑ Substrates limitations

CO2: Bazin et al. (1982) have studied the potential causes for incomplete nitrification of ammonia supplied to a column reactor containing a coculture of *Nitrosomonas europea* and *Nitrobacter agilis*. They conclude that CO_2 was not limiting. Therefore, because of the rather high solubility of CO_2 and the low growth yield (table 3), it is usually assumed that CO_2 is not a growth limiting factor.

Considering that $K_La|_{O2} \approx K_La|_{CO2}$, the limiting gas CO_2 fraction can be calculated for oxygen limiting condition (TN 23.2).

From the stoichiometries (TN 23.2), it can be deduced that:

$$\frac{K_La|_{CO2}}{K_La|_{O2}} = \frac{Y_{CO2/X}}{Y_{O2/X}} \cdot \frac{C^*_{O2}}{C^*_{CO2}}$$

then

$$\frac{Y_{CO2/X}}{Y_{O2/X}} \cdot \frac{C^*_{O2}}{C^*_{CO2}} \approx 1 \qquad \text{or} \qquad C^*_{CO2} \approx \frac{Y_{CO2/X}}{Y_{O2/X}} \cdot C^*_{O2}$$

Using the values of the stoichiometric yield of equations reported in table 8 of TN 23.2, it comes for an aeration by air ($C^*_{O_2}$ =2.7218 10^{-4} mol/l):

$C^*_{CO_2}$ =9.5263 10^{-6} mol/l

which represents a molar fraction of 2.73 10^{-4} for CO_2 in the gas phase (calculated from relations of TN 17.1). In air, the mean CO_2 molar fraction is 3.5 10^{-4}. If instead of CO_2, the real form of carbon source is considered (HCO_3^-), the saturation concentration of CO_2, at which HCO_3^- becomes limiting, is:

$C^*_{CO_2}$ =2.205 10^{-7} mol/l

for a pH of 8. This is equivalent to a gas fraction of 6.32 10^{-6}.

This demonstrates that CO_2 limitation may occur for very low partial pressure of CO_2 after an O_2 limitation with air aeration (molar fraction of 0.2093).

O2: because ammonia and nitrite oxidisers have high oxygen requirement, the limiting effect of O_2 for nitrification has been fully studied, and is generally considered in the kinetics models as the main limiting substrate with the N-oxidised substrates.

Saturation constant (Ks) for pure culture of ammonia and nitrite oxidisers lies in the range of 0.25-2.5 mg dissolved O_2 l^{-1} (Painter, 1986). Similar values are reported for mixed culture activated sludges systems.

The Ks values of oxygen are higher than those for heterotrophs, and nitrifiers are therefore likely to be poor competitors for oxygen at low concentration. There is evidence that ammonia oxidisers have a greater saturation constant than nitrite oxidisers for oxygen. These differences can give rise to spatial separation of these two groups in attached biofilm limited by supply of oxygen by diffusion (Prosser, 1989).

NH_3,NO_2^-: They are respectively the energy sources for the growth of ammonia and nitrite oxidisers. As a consequence they are considered as the main limiting substrates. the saturation constant experimentally measured shows some degree of variability, but they are generally similar to, or greater than, concentration of ammonia or nitrite found in the environment from which the cells were originally isolated (table 4)

Table 4: Saturation constant for growth and activity of nitrifying bacteria.(* reported by Prosser, 1989)

Saturation constant for activity (Km)	Reference	Saturation constant for growth (K_{S_i})	Reference
Ammonia oxidiser			
Nitrosomonas sp. 14 mM NH_4^+ 3.6 mM O_2	Loveless and Painter (1968)*	*Nitrosomonas europea* 0.07 mM NH_4^+	Remacle and De Leval (1978)*
Nitrosomonas europea 0.4 mM NH_4^+	Suzuki (1974)*	*Nitrosomonas* sp. 0.055 mM NH_4^+	Helder and de Vries (1983)*
Nitrosomonas europea 0.12-10 mM (NH_4^++NH_3) 0.018-0.058 mM NH_3	Suzuki et al.(1974)*	*Nitrosomonas europea* 0.051 mM NH_4^+	Keen and Prosser (1987)*
Nitrosomonas sp. 1.1-3.8 mM NH_4^+	Laudelout et al. (1976)*	*Nitrosomonas europea* 1.25 mM NH_4^+ 5.05 10^{-3} mM O_2	Hunik et al. (1994) (model parameter values)
Nitrosomonas europea 0.5-0.7 mM NH_4^+	Drozd (1976)*		
Nitrite oxidisers			
Nitrobacter spp. 1.6-3.6 mM NO_2^-	Boon and Laudelout (1962)*	*Nitrobacter spp.* 0.178 mM NO_2^-	Gould and Less (1960)
Nitrobacter spp. 0.256 mM O_2 0.062 mM O_2	Peters et al. (1969)*	*Nitrobacter spp.* 0.045 mM NO_2^-	Remacle and De Leval (1978)*
		Nitrobacter spp. 0.267 mM NO_2^-	Helder and de Vries (1983)*
		Nitrobacter spp. 0.039 mM NO_2^- 0.015 mM NO_2^-	Gay and Corman (1984)*
		Nitrobacter agilis 0.36 mM NO_2^- 0.017 mM O_2	Hunik et al. (1994) (model parameter values)

Metal availability: Fe and Mg are important substrates for nitrifiers (Bazin et al., 1982). The influence of metal have been reviewed by Bazin et al. (1982) and the toxicity or inhibitory effect of some metals and other products has been reviewed by Zeghal (1992).

These problems will not be considered here.

❑ Physical limitations

pH: both ammonia and nitrite oxidation are considered to be optimal at neutral-alkaline pH values. Most of nitrifiers have an optimum pH in the range of 7.5-8 and growth within a pH range of approximately 2 pH units. The specific growth rate (μ) of both ammonia and nitrite oxidisers is affected by pH and is maximum at optimal pH value. The response of μ to pH reflects the pK value for the respective substrate:

- for NH_3:NH_4^+, the pK value is 9.25. Because of NH_3 is the substrate for ammonia oxidation, the decrease of pH decreases the concentration of ammonia by one order of magnitude for each pH unit. At low pH value, the maintenance required to maintain internal pH in addition to metabolic uptake may leave insufficient energy for growth. Above optimum pH value, the advantages of increased availability of free ammonia are counterbalanced by the need to maintain an internal value below that of the external medium ;

-for NO_2^-/HNO_2, the pK value is 3.15. At high pH value, the inhibition of growth result from a competitive inhibition by hydroxyl ion (OH^-) (Boon and Laudelout, 1962). At acid pH, limitation and inhibition occurs because of the increased production of HNO_2, and below pH 4, with formation of nitrogen oxide.

Moreover, the variation of pH value affects enzyme activities and HCO_3^- (carbon source) availability.

Temperature: as for all bacteria, the growth of nitrifying bacteria depends on temperature. An optimum value is 28°C. The effect of temperature on the saturation constant of oxygen (K_m^{O2}) was studied by Laudelout et al. (1976), Boon and Laudelout (1962) and Wijffels et al. (1995), using an Arrhenius relationship. As an example, the saturation constant of oxygen for immobilised *Nitrosomonas* was determined by Wijffels et al. (1995):

$$K_{O_2}^{Ns} = A\, e^{-\frac{B}{R.T}}$$

$A=9.04\ 10^{11}$ mol/m^3
B=72. 7 J/mol

It must be kept in mind that the temperature affects too the pK value of acid/base compound, the solubilities of compounds (TN 17.1) and dynamic parameters such as diffusion constants (Wijffels et al., 1995).

Transfer and diffusion limitations: transfer and diffusion phenomena must be considered in every processes. The effects of diffusion resistances of oxygen on nitrification kinetics was investigated by Beccari et al. (1992), who found an excellent agreement between experimental data and model prediction. Hoojmans et al. (1990) have introduced the diffusion limitation of O_2 in the metabolically structured model of growth of *Thiosphaera pantotropha* (Geraats et al., 1990) to model the oxygen profile in gel bead during the growth.

The dynamic model developed by Hunik et al. (1994) includes the mass transfer equation of several substrates (O_2, NO_2^-, NH_4^+, NO_3^-) in gel beads. the transfer and diffusion equations

are generally not easy to manage and to solve (the model developed by Hunik et al. takes about 5 h CPU time for 50 days of growth), but provides useful tools for the study and possible control of the process.

1.2.3 Growth inhibitory factors

❑ **Substrates inhibition**

At certain concentrations, the substrates or products of the nitrification act as inhibitors.

NH_3, NO_2^-, NO_3^-: The inhibition of nitrification by free ammonia and free nitrous acid (HNO_2) was studied by Anthonisen et al. (1976). They proposed a diagram combining pH and inhibitory effects of NH_3 and HNO_2 both on *Nitrosomonas* and *Nitrobacter*.

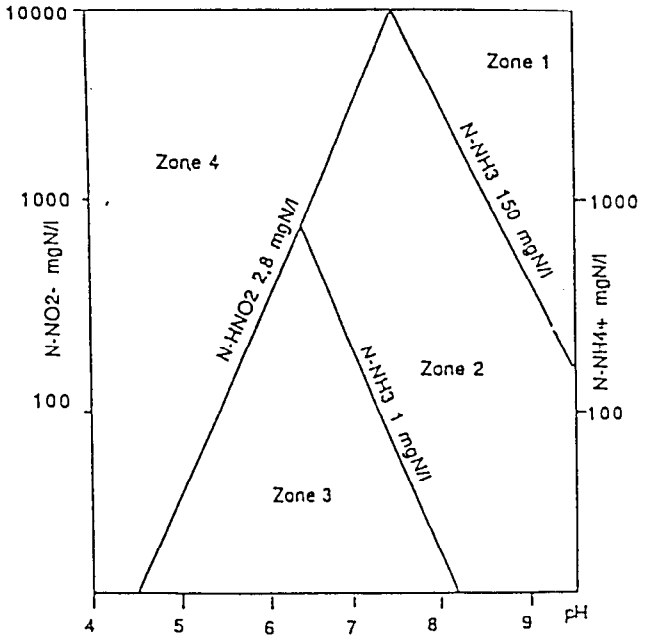


Figure 1: Anthonisen diagram. Zone 1: inhibition of *Nitrosomonas sp.* and *Nitrobacter Sp.* Zone 2: inhibition of *Nitrobacter Sp.* by NH_3. Zone 3: nitrification . Zone 4: inhibition of *Nitrobacter Sp.* by HNO_2.

A relation including both limiting and inhibitory effects of a substrate was used by Hunik et al. (1994) in the equation describing substrate consumption and biomass production in a nitrification process. Bazin et al. (1982) reviewed the ammonia, nitrite and nitrate inhibitory concentrations reported in literature for *Nitrosomonas* and *Nitrobacter* (table 5).

O_2: at high concentration, oxygen is inhibitory, and induces an increase in polyphosphate pools (Prosser, 1989). A free radical formation was suggested as the mechanism of inhibition. Nevertheless, oxygen inhibition seems more important in processes which are supplied with pure oxygen or high oxygen partial pressure than processes supplied with air.

In the absence of dissolved oxygen, Bock et al. (1988) report the ability of *Nitrobacter* to grow using nitrate as electron donor and an organic substrate as C source. This study shows

the ability of *Nitrobacter* to reduce, in such growth conditions, nitrate to nitrite, to ammonia and to nitrogen gases (N_2O).

Hanaki et al. (1990) concluded that a low level of dissolved oxygen did not affect ammonia oxidation in pure suspended growth nitrifying reactor. They suggested that the doubling of the growth yield of ammonia oxidisers compensated the reduction of ammonia oxidation rate per unit biomass (activity). That can be linked with the previous observation on growth yield and cell activity.

Organic matter: In a suspended growth reactor, the inhibitory effect of organic loading on ammonia appears with low COD influent when an oxygen limitation occurs. But the interacting effect on nitrite oxidation has not been clearly determined (Hanaki et al., 1990)

On the basis of nitrogen balance calculation, Hanaki et al. concluded that there was occurrence of simultaneous nitrification and denitrification in a combined system: organic loading + oxygen limiting condition. That is consistant with the denitrifying behaviour of nitrifiers in absence of oxygen.

Nitrobacter agilis, growing chemoorganotrophically, generally conserves its nitrite oxidation ability, until the growth conditions induce the synthesis of cytochrome a_1 .

Table 5: Effects of inhibitory concentrations of nitrite and nitrate (taken from Bazin et al., 1982)

	Nitrogen concentration mg ml^{-1}	Effect	Reference
Nitrosomonas			
Nitrite	1400	36% inhibition of oxygen uptake	Meyerhof (1916)
	4200	100% inhibition of oxygen uptake	Meyerhof (1916)
	500	Prolong lag at all pH value but no inhibition at alkaline pH	Lewis (1959) Pokallus (1963)
	2500	100% inhibition-lag 50% inhibition-lag	Lewis (1959) Pokallus (1963)
Nitrate	-	No effect recorded	
Nitrobacter			
Nitrite	40	No lag, no inhibition	Aleem and Alexander (1960)
	130	Prolonged lag 2-3 days	Aleem and Alexander (1960)
	≤500	Added to exponentially growing culture - no effect	Aleem and Alexander (1960)
	1400	40% inhibition due to undissocied nitrous acid	Boon and laudelout (1962)
	159	Value of inhibitory constant	Hunik et al. (1992)
Nitrate	500	No effect	Aleem and Alexander (1960)
	1000-2000	Prolonged lag	Aleem and Alexander (1960)
	5000	No nitrification	Aleem and Alexander (1960)
	2000-5000	Added to active culture did not lead to significant depression NO3- accumulation does not interfere with NO2- oxidation	Aleem and Alexander (1960)
		Greater inhibition with increase aeration	Gould and lees (1960)
	188	Value of inhibitory constant	Hunik et al. (1992)

❑ Physical inhibition

Like substrates, physical parameters (dilution rate up to the washout conditions; nitrogen charge; hydraulic retention time...) and reactor design (reactor type; cells immobilized or not; type of support...) can affect the nitrification. The specificity of the process, fonctionning and studied at UAB Laboratory, will be discussed in a further section.

Light inhibition: light inhibition is significant in surface water and it was found 50% of inhibition of ammonia and nitrite oxidisers at light intensities approximately three order of magnitude less than the intensity of full sunlight. *Nitrobacter* appeared to be much more sensitive than *Nitrosomonas* to visible-blue and long-wavelength light which has suggested that the photooxidation of cytochrome c was the mechanism of inhibition by light (Prosser, 1989). For ammonia oxidisers, absence of oxygen i.e. high ammonia or hydroxylamine concentrations reduced the sensitivity to photo-inhibition.

1.3 Biological kinetics

1.3.1 Kinetics laws

❑ Specific growth rate - Definition and expressions -.

The growth of biomass (X), in general, has been observed to be described satisfactorily by:

$$r_x = \mu X$$

r_X is call growth rate, while μ, the specific growth rate, is defined as the change in biomass per unit of biomass.

Under condition wherein μ is observed to have a constant numerical value and in batch cultures, biomass is said to have an exponentially phase of growth:

$$X=X_0 e^{\mu t}$$

A decay rate, k_d, can be added to the previous expression of the growth rate:

$$r_x = \mu X - k_d X$$

Hurst (1992) reported that there is no evidence of the manifestation of decay during the exponential growth, or in declining growth phase in batch cultures. But there is much evidence consistent with concurrent growth and decay in slowly growing continuous culture systems.

μ as k_d, can vary depending on the environmental conditions, as well as the physiological state of the biomass. kd is assumed to be a system constant.

The most commonly used expressions of μ found in the literature are reported in table 6.

Table 6: Commonly expressions of the specific growth rate

μ	model type	Conditions of use	parameters
$\mu_m\left(1-\frac{X}{X_m}\right)$	Logistic	Growth limited by space (biomass limited)	μ_m maximum growth rate X_m maximum biomass population
$\mu_m.\frac{S_i}{K_{s_i}+S_i}$	Monod	Growth limited by substrate (S_i) consumption	μ_m maximum growth rate K_S half maximum growth rate saturation constant
$\begin{cases}\mu=\mu_m & S_i \geq 2.K_{s_i}\\ \mu=\mu_m\frac{S_i}{2.K_{s_i}} & S_i < 2.K_{s_i}\end{cases}$	Blackman	Growth limited by substrate (S_i) consumption	μ_m maximum growth rate K_{Si} half maximum growth rate saturation constant
$\mu_m.\frac{S_i}{(K_s+S_i)\left(1+\frac{S_i}{K_I\vert_{S_i}}\right)}$	Haldane	Growth limited by substrate (S_i) consumption at low concentration and by substrate inhibition at high concentration	μ_m maximum growth rate K_{Si} half maximum growth rate saturation constant K_I constant of inhibition

❑ Substrate and biomass kinetic relations (growth yield)

The rate (r_{Si}) of substrate Si utilisation can be related to the growth by:

$$r_{S_i}=\frac{-1}{Y_{X/S_i}}r_X$$

It must be noted that some authors prefer the notation q_S rather than r_S.

The numerical value of Y was found constant during the entire course of the growth curve (Hurst, 1992). In batch experiments, Y remained constant throughout the exponential and declining phases of growth. Nevertheless, Y may decrease due to cell decay. In that case, the yield has been termed the observed yield (Y_{obs}) while in other hands it is termed the true growth yield (Y_t), which, like k_d, is assumed to be a system constant.

As shown in table 6, the specific growth rate can depend on biomass or/and on one (or more) substrate. According to a Monod expression, the growth for a limiting substrate (S) can be represented by the set of equations:

$$\begin{cases}r_X=\frac{\mu_m.S_i}{K_{S_i}+S_i}X\\ r_{s_i}=\frac{-1}{Y_{X/S_i}}r_X\end{cases}$$

❑ Limiting or inhibitory substrate and growth kinetics (table 6)

The logistic law is based upon a biomass limiting growth (due to a lack of space), while the Monod expression is usually considered as the classical law for a substrate limiting reaction.

For systems wherein multiple interacting limitations occur, combination of Monod and logistic equations can be used, leading to an expression of the growth rate of the form:

$$r_X = \mu_m\left(1-\frac{X}{X_m}\right)\frac{S_1}{K_{S1}+S_1}\frac{S_2}{K_{S2}+S_2}.X$$

or, more generally of the form

$$r_X(X,S_1,S_2) = \mu_m\xi(X).\xi(S_1).\xi(S_2).X$$
$$\mu(X,S_1,S_2) = \mu_m\xi(X).\xi(S_1).\xi(S_2)$$

where ξ(i) is the normalised kinetic ($\xi = \frac{\mu}{\mu_m}$)associated to i.

For systems where there are non-interacting limitations, the expression of the growth rate takes a classical form :

$$r_X(X,S_1,S_2) = \mu_m.\min\{\xi(X).\xi(S_1).\xi(S_2)\}.X$$
$$\mu(X,S_1,S_2) = \mu_m.\min\{\xi(X).\xi(S_1).\xi(S_2)\}$$

Another class of models that describe deviations from classical Monod kinetics are models of microbial growth on inhibitory substrates. One model that has been used in many studies is a modification of the Monod equation, known as the Haldane equation:

$$\mu = \mu_m.\frac{S}{(K_S+S)\left(1+\frac{S}{K_i}\right)}$$

A special form the Haldane equation was introduced by d'Amado et al.(1984):

$$\mu = \mu_m.\frac{S}{\left(K_S+S+\frac{S^2}{K_i}\right)}$$

Bellgardt (1991) reported a general form of the specific growth rate with p alternative growth substrates, derived from the sum of the previous elementary kinetics:

$$\mu(S_1,....S_N) = \mu_1(S_1,....S_N)+\mu_2(S_1,....S_N)+....+\mu_P(S_1,....S_N)$$

As an example, a Monod-type kinetics for 2 substrates (S_1, S_2), where the first is preferred can be modelled as:

$$\mu(S_1,S_2) = \mu_1(S_1,S_2)+\mu_2(S_1,S_2)$$
$$\mu(S_1,S_2) = \mu_{m,1}.\frac{S_1}{K_{S1}+S_1}+\mu_{m,2}.\frac{S_2}{K_{S2}+S_2+\frac{S_1}{K_{I,1}}}$$

Tsao and Hanson (1975) proposed another form of the growth rate expression taking into account both combinations of essential and alternative substrates. Assuming m classes of essential substrates, the entire kinetics is given by:

$$\mu(S_1,....S_N) = (\mu_{S11}+.....\mu_{S1K}).(\mu_{S21}+.....\mu_{S2L}).....(\mu_{Sm1}+.....\mu_{SmN})$$

where K, L,...N, are the number of essential substrates in the i^{th} class.

❑ Growth and maintenance

Deviation from the prediction of the Monod equation have been noted for very low concentrations of limiting substrate (Pirt, 1975). At low substrate concentration, the endogenous metabolism of microorganisms can constitute a major drain of the energy needed to sustain growth. To account this drain, the Monod relation can be modified in the following way:

$$\mu = \mu_m.\frac{S}{K_s+S} - a$$

where a is the specific maintenance rate. The maintenance coefficient m, (Pirt, 1975), is related to a by:

$$m = \frac{a}{Y_t}$$

where Y_t is the growth yield.

According to Pirt, a relation can be established between Y_t, Y_{obs}, μ and m (for steady state conditions):

$$\frac{1}{Y_{obs}} = \frac{m}{\mu} + \frac{1}{Y_t}$$

It can be noted that the Monod -maintenance modified- relation, when the substrate is completely exhausted, is reduced to:

$$\mu = -a$$

which expresses the maintenance related decay of the population.

❑ Structured and unstructured models

The previously presented biological kinetic laws can be used for the building of structured or unstructured models.

In unstructured models, the biological reactions depends directly and solely on macroscopic variables that described the conditions in the bioreactor.

In contrast, structured models provide informations about the physiological states of the microorganisms (composition, adaptation to environmental changes). Moreover, Bellgardt

(1991), reported that processes with lag phase or diauxic growth cannot give a satisfactory simulation with unstructured models, while they could be simulated by structured models.

Nevertheless, because of the risk of the inability to verify experimental data and identify the coefficients of too complex models, the structured model should only include the most relevant intracellular processes. The study of these relevant processes was presented in TN 23.2.

1.3.2 Review of growth laws reported in nitrification studies and modelling

Prosser (1986) reviewed the experimental and theoretical models developed to describe the nitrification.

Several growth expression have been used by different author, depending on the growth conditions and limitations in which the process was performed.

Mc Laren (1971), and Prosser and Grey (1977) represented the growth of nitrifying bacteria by a logistic law. A continuous flow nitrification was described by Bazin and Sanders. (1973) by combining a Monod equation and a logistic law.

The Monod relations, involving single substrate limitation (Hanaki et al., 1990; Belser and Schmidt, 1980) or multiple substrates limitation (Laudelout et al., 1962; Beccari et al., 1992) are often used to model the biomass production and compounds consumption (or production). Good fits are usually obtained between experimental data and theoretical curves, and some of the parameters identified and calculated (μ_m, Y_S, K_S) are reported in tables 1, 2 and 4.

According to Pirt (1975), Keen and Prosser (1987) calculated a true growth yield (Yt) and a maintenance coefficient (m) for *Nitrosomonas europea* and *Nitrobacter sp.*. The values calculated in continuous cultures are reported in table 2.

Hunik et al. (1994) developed a model for the growth of *Nitrosomonas europea* and *Nitrobacter agilis* immobilized in k-carrageenan beads. Their growth model is based upon equations suggested by Beeftink et al. (1990). At low substrate concentrations, this model will perform as an Herbert model, while at high substrate concentration it acts as a Pirt model. The growth equations of a microorganism, as suggested by Hunik et al. (1994), are :

$$r_s = \frac{\mu.X}{Y} + m.X.\left[\frac{S}{(K_s + S).K_I}\right]$$

$$r_X = \mu.X - m.Y.X.\left[1 - \frac{S}{(K_s + S).K_I}\right]$$

$$\mu = \mu_m.\frac{S}{(K_s + S).K_I}$$

II Bioreactors and process dynamics

2.1 Design of the nitrifying fixed bed pilot reactor (Forler, 1994)

Using the results of all the previous experiments performed in the MELiSSA Laboratory, Forler (1994) defined the main characteristics of the nitrifying compartment: he adopted a fixed bed column packed with polystyrene beads (Biostyr).

The scheme of the bioreactor, containing the whole of equipment and the details of the column characteristics are reported from Forler (1994) in annex A.

A simplified scheme (sensors and actuators are not indicated) is proposed in figure 2, as the base for the building of the dynamic model of the compartment. It can be noted that the thermal control and energy balance (heating/cooling) is not yet included in this description.

The description of the 3 parts of the column (A, B, C) and of the flow patterns is summarised in table 7.

The column reactor can be assimilated to a series of two stirred tanks and of a column fixed bed reactor (table 7). Using hydrodynamics models for each of them, associated to the flow description of figure 2, will lead to the hydrodynamic model of the bioreactor (see section 3).

The description of the compartment involves flow rates, compositions parameters, and reactor design parameters (see section 2.3.1). These data will be used as variables in the model of the reactor.

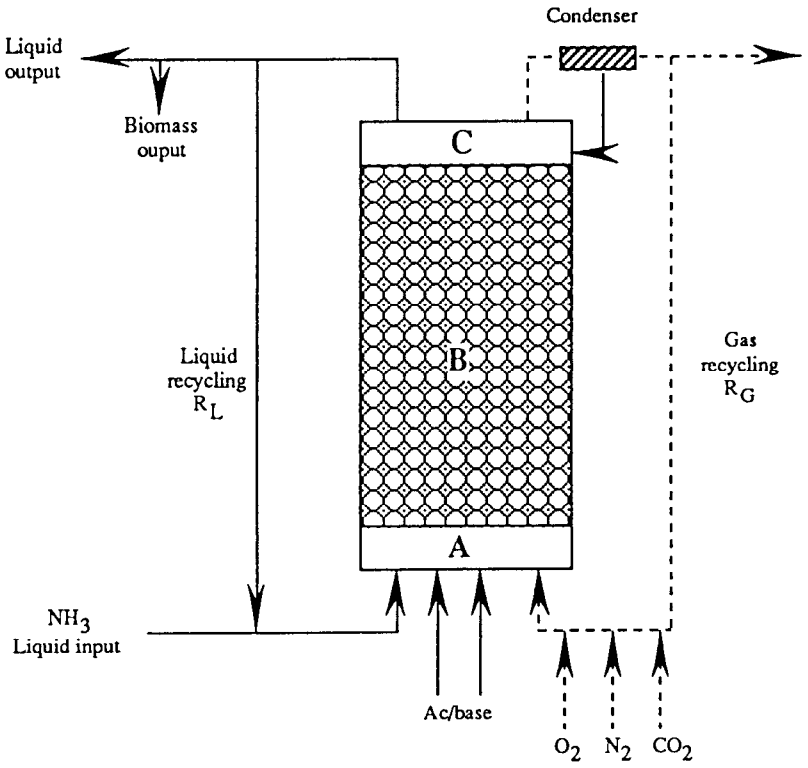


Figure 2: Schematic representation of the nitrifying reactor. Dasheds line are for gas flow.

Table 7: Design of the nitrifying column.

Column reactor	Total height: 0,760 m Total volume: 8,5 l Diameter: 112 - 120 mm	
Part A	Under magnetic agitation Inputs of gas and liquid on the column	Can be assumed to be a stirred tank. No active biomass is considered in this part of the reactor.
Part B	Void fraction: 0.52	This part of the column corresponds to the fixed bed where occurs the biological reactions
Part C	Outputs of gas and liquid on the column	Can be assumed to be a stirred tank. No active biomass is considered in this part of the reactor. Gas liquid interface at the top of the column
Input flows	Liquid (including NH3 loading) Acid/base under pH control Gases (O_2+CO_2)under P_{O2} and gas flow rate control	
Output flows	Liquid (NO_3^-) Gas	
Recycling flows	Liquid (R_L)	$R_L = \frac{\text{Liquid flow rate entering the column}}{\text{input flow rate}}$
	Gas (R_G)	$R_G = \frac{\text{Gas flow rate entering the column}}{\text{input flow rate}}$

2.2 Hydrodynamic behaviour of a reactor

For continuous processes, two extreme hydrodynamic behaviours are generally considered, between which the globe of more realistic reactors models is stretched (Lapidus and Amundson, 1977). These two ideal behaviours are:

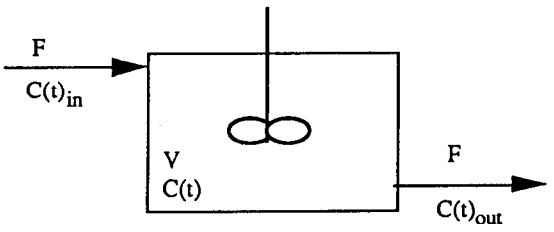
- the stirred tank reactor (or back mixed reactor)
- the plug flow tubular reactor

The stirred tank reactors can be working in continuous or batch conditions.Three ideal reactors are then usually considered: continuous stirred tanks reactor, batch stirred tank reactor and plug flow reactors.

2.2.1 Ideal reactors: characteristics and dynamics

❑ Stirred tank

The feed entering the reactor is supposed to be immediately diluted into the reactor volume, because of perfect mixing. Accordingly, the exit stream would have the same composition as that within the reactor.



The dynamic of the reactor is defined by the relation

$$V\frac{dC}{dt} = F\left(C(t)_{in} - C(t)_{out}\right) + V.r(C)$$

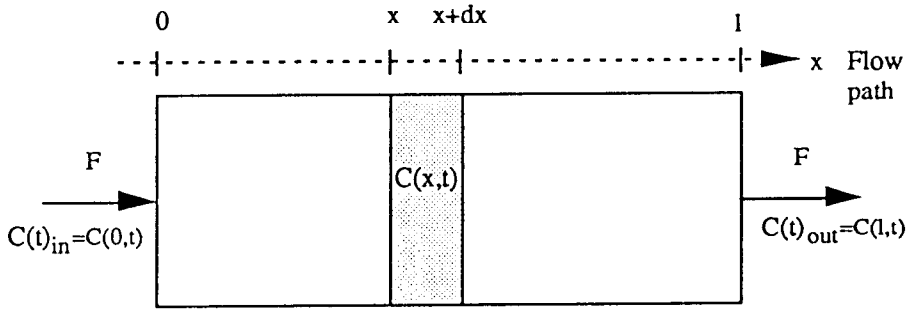
and the initial condition

$$C(0) = C_{in}$$

where
C() is the concentration of the compound C
F is the volumetric flow rate
r(C) is the reaction rate of C

❑ Plug flow

The plug flow reactor assumes complete mixing in the radial direction, but allows for no diffusion in the flow (especially no back mixing). As a result, the velocity, temperature and composition profile are flat over any cross sectional area perpendicular to the flow, but the composition varies along the flow path.



The dynamic of the reactor is defined by the system:

$$\begin{cases} \dfrac{\partial C}{\partial t} = -\bar{v}\dfrac{\partial C}{\partial x} + r(C) \\ \dfrac{\partial C}{\partial x} = 0 \qquad \text{for} \quad x = 0 \\ C(x,0) = C_{in}(x) \end{cases}$$

where

$\bar{v}$ is the average velocity ($\bar{v} = \dfrac{F}{\text{area of the tube section}}$)

❑ Batch reactor

This represents an unsteady-state process in regard to the composition of the reaction mixture. An assumption of complete mixing implies a homogenous composition at any time.

2.2.2 Non ideal flow

Deviations from ideality, assumed in developing the basic reactor design equations, are present in practical reactors. Important deviations from the continuous ideal flows can be outlined (Perry and Green, 1984):

❑ Deviation from plug flow

Sources of deviation from plug flow are:
- channelling of the reacting fluids through the catalyst packing and presence of stagnant fluid pockets;
- presence of velocity and temperature gradients in the radial direction;
- diffusion in the direction of flow and back mixing as a consequence of fluid turbulence, thermal convective transport and molecular diffusion.

These two last effects have led to introduce diffusion-dispersion terms in the ideal plug flow model, and to build dispersion models. The equation of plug flow can be rewritten for isothermal, incompressible flow of fluid under a constant flow rate in a tube as:

$$\frac{\partial C}{\partial t} = E_x \frac{\partial^2 C}{\partial x^2} + E_r \cdot \left(\frac{\partial^2 C}{\partial r^2} + \frac{1}{r} \frac{\partial C}{\partial r} \right) - \bar{v} \frac{\partial C}{\partial x} + R(C)$$

E_X: axial dispersion term
E_r: radial dispersion term

❑ Deviation from stirred tank

Sources of deviation from perfectly mixed tank are:
- short circuiting and by-passing of the reacting fluid (i.e. certain portion of fluid may proceed directly from the inlet to the product discharge without mixing with the content of the reactor);
- internal recycling of fluid;
- presence of stagnant fluid pockets.

❑ Residence time distribution and hydrodynamic behaviour of a reactor

The residence time distribution (RTD) data resulting from the flow of a tracer through a reactor can be used to predict the performance and the behaviour of a reactor. The 3 typical techniques of introducing a tracer into the reactor are the step input, the pulse input and the cyclic input, which leads to a characteristic shape of tracer signal-response curves at the exit (figure 3).

For a convenient data treatment, a dimensionless time τ is used, defined as:

$$\tau = \frac{\theta}{\bar{\theta}}$$

where $\theta = t - t_0$ (t_0 is the time of tracer input)

$\bar{\theta}$ is the mean residence time

The dimensionless parameter $\varepsilon(\tau)$ can be used as a measure of RTD of the tracer at the exit of the reactor. $\varepsilon(\tau)$ is defined as:

$$\begin{cases} \varepsilon(\tau) = \dfrac{dF(\tau)}{d\tau} \\ F(\tau_0) = \int_0^{\tau_0} \varepsilon(\tau) d\tau \end{cases} \quad \text{and} \quad \int_0^{\infty} \varepsilon(\tau) d\tau = 1$$

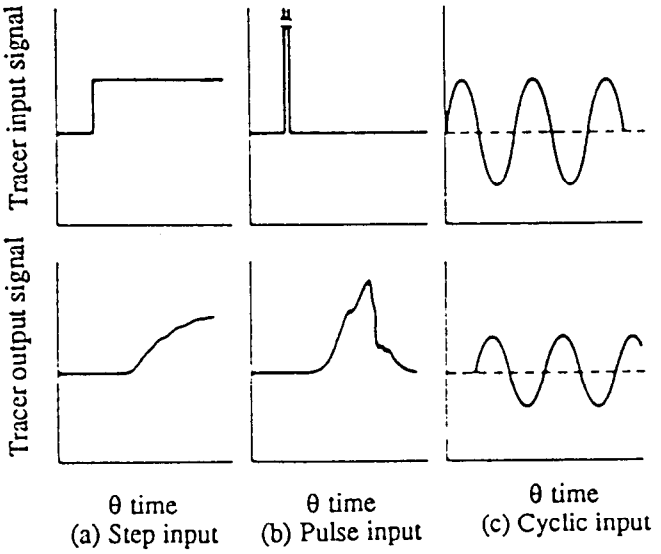


Figure 3: tracer input and characteristic signal-response curves.

$\varepsilon(\tau)$ can be considered as the fraction of the tracer at the exit of the reactor, the dimensionless residence time t of which is between t and t+dt. For a step impulse of C_0, $\varepsilon(\tau) = \dfrac{C(\tau)}{C_0}$.

$F(\tau_0)$ is the fraction of tracer which dimensionless residence time is lower than τ_0.

Typical E and F curves for ideal and non ideal behaviour are reported in annex B (Wen and Fan, 1974).

2.3 Hydrodynamic of a fixed bed.

2.3.1 Characterization of a fixed bed column.

❑ Characteristics of the bed and of the support

The choice and the characteristics of the bed and of the support for the nitrifying compartment were studied and defined by Zeghal (1992) and Forler (1992, 1994). The support chosen for the bacteria fixation is constituted by polystyrene beads named BIOSTYR. In order

to calculate the dynamic behaviour of the fixed bed, Forler (1994) determined the physical parameters of the support. These parameters are reported in table 8.

Table 8: Characteristics of the support

Variable	Values
Mean bead diameter (d_b)	4.1 mm ± 0.5
Shape factor of the sphere	1
Bead polystyrene density (ρ_p)	18.66 kg.m^{-3}
Bead density (ρ_b)	17.60 kg.m^{-3}
Fixed bed density (ρ_{fb})	9.60 kg.m^{-3}
Void fraction (ε)	0.4852
Mean hydraulic diameter (d_h)	2.56 mm

Some other parameters can be interesting in order to calculate or to determine the behaviour of the bed, such as the liquid (ε_L) and the gas (ε_G) void fractions defined respectively as the volume of the bed occupied by liquid and by gas ($\varepsilon=\varepsilon_L+\varepsilon_G$). Theses values can be calculated by using the relation reported by Forler (1992):

$$\frac{\mu|_G . v_0|_G}{\mu|_L . v_0|_L}.(1-\varepsilon_G)^2.(\varepsilon-\varepsilon_G)^3-\varepsilon^3.(1-\varepsilon+\varepsilon^2)=0$$

Applied to a fixed bed of a total void fraction of 0.52, and feeded by 3l/min of air and 20.8 ml/min of liquid (input of 2.8 ml/min and recirculation of 18 ml/min), in a column of 120 mm diameter, the relation gives :

- ε_L= 0.236
- ε_G=0.285

This calculated values are quite different from the experimental ones determined at UAB Laboratory (ε_L= 0.47 ; ε_G= 0.05).

❑ Characterisation of the flow through the bed

The behaviour of a fluid can be defined by using several dimensionless number.

The Reynolds number, related to a fluid, characterises the type of flow

$$Re=\frac{d\ .v.\rho}{\mu}$$

where d=characteristic dimension of the flow channel
v=linear velocity
ρ= density of the fluid
μ=viscosity of the fluid

For the flow through the fixed bed, Forler (1992) used the hydraulic Reynolds number for the gas and the liquid:

Gases $$Re_h|_G=\frac{2}{3}.\frac{d_p.v_o|_G.\rho|_G}{(1-\varepsilon_G).\mu|_G}$$

Liquids $$Re_h|_L = \frac{2}{3}.\frac{d_p.v_o|_L.\rho|_L}{(1-\varepsilon_L).\mu|_L}$$

Three types of flow behaviour are considered:
Re<1: laminar flow
1<Re<700 : transient flow
Re>700 : turbulent flow.

Using the experimental condition described for the void fractions calculation, and taking the bead radius as the characteristic dimension (dp), the hydraulic Reynolds numbers calculated are:

$Re_h|_G$=1.19 with the calculated ε_G and $Re_h|_G$=0.897 with the mesured ε_G
$Re_h|_L$=0.109 with the calculated ε_G and $Re_h|_L$=0.16 with the mesured ε_L

The Schmidt number is used when diffusion processes occur :

$$N_{sc} = \frac{D_M.\rho}{\mu}$$

where D_M is the molecular diffusion coefficient.

❑ **Pressure drop**

An equation for evaluating the pressure drop through a packed bed was developed by Ergun, both for laminar and turbulent flows:

$$\frac{\Delta P}{L} = \left(\frac{170.(1-\varepsilon).\mu_{visc}}{dp.v.\rho} + 1.75\right).\frac{1-\varepsilon}{\varepsilon^3}.\frac{v^2.\rho}{dp}$$

where
v=superficial mass flow rate
L=depth of the bed
ε=voidage of the bed
ρ=density of the reacting fluid
μ_{visc}=viscosity of the reacting fluid
d_p=mean bead diameter

This relation is available for the complete range of Reynolds values (laminar and turbulent flow). Restrictions in the use of the equation are that the particle must not derivate too much from the sphere shape and that the diameter of the particles must be considerably smaller than the diameter of the bed (<1/20). Moreover, the Ergun relation, as well as the hydraulic Reynolds used above, was established for two-phases systems, and there is no evidence that it can be applied to a three-phase system such as our nitrifying column.

❑ Behaviour of a nitrifying bed

The dynamic and the behaviour of nitrification in a glass bead column has been studied by Bazin et al. (1982).They made interesting experiments by measuring the transient and the steady state behaviour of the column (measuring the profile of the effluent nitrite and nitrate concentration) as a function of the changes in flow rate and in the nutrients inputs. They concluded that one of the most characteristic properties of nitrification columns is the short term asymmetric responses in effluent nitrite and nitrate concentrations which occur after change in flow rate. The reason for this asymmetry is still unclear. Nevertheless, comparing models of different authors, they supposed that some aspects of the response are a function of the physical structure of the system rather than being derived from any biological property.

Cox et al. (1980) studying bacteria distribution in a continuous flow nitrification column observed that for unlimited conditions (incomplete nitrification ?), the bacteria were found only in the nutrient input region of the column. After 7 month growth, they were observed most commonly in monolayer, less commonly in layers of about 20 cells, and rarely in pile of about 100 cells. Further in the column was found a layer of slime covering the glass beads, and no bacteria were found. Cox et al. (1980) suggested that the growth was neither limited by diffusion of metabolites through a microbial film nor limited by competition between bacteria for space on the surface of the bead.

2.3.2 Homogenous fluids through fixed-fluidized bed

The flow pattern in a fixed bed reactor can not be represented by ideal models or mixed model, but N-compartment-in-series models (see section 3.4) or dispersion models (see below) can generally fit experimental data (Wen and Fan,1974). These authors represented a packed bed reactor by a dispersion plug flow associated to stirred tanks (figure 4).

Wen and Fan (1974) reviewed the works about dispersion of fluids through fixed and fluidized beds. They deduced from several experimental data the correlations for axial (Ex) and radial (Er) dispersion for gases and liquid Newtonian fluid through packed beds. But it must be kept in mind that these models are for homogenous systems.

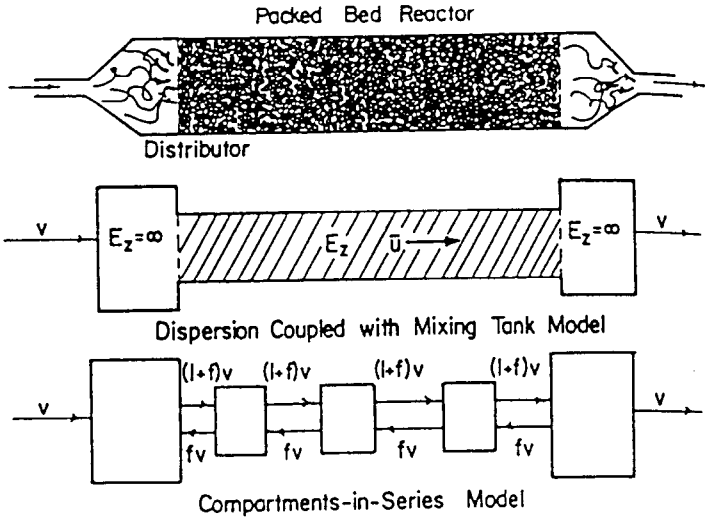


Figure 4: Model representation of a packed bed

❑ Axial dispersion of liquid through fixed and fluidized bed

The classical axial dispersed plug flow model is:

$$\frac{\partial C_{s_i}}{\partial t} = E_r . \frac{\partial^2 C_{s_i}}{\partial x^2} - \bar{v} . \frac{\partial C_{s_i}}{\partial x} + r_{s_i}$$

This model can be used for fixed bed, by taking $\bar{v}$ as the interstitial velocity, *i.e.*:

$$\bar{v} = \frac{v_0}{\varepsilon}$$

v_0: the superficial velocity

ε: voidage of the bed

E_X is called the axial dispersion coefficient based on interstitial velocity. Wen and Fan (1974) proposed a correlation equation for both fixed and fluidized bed, based on the scattering of 482 available experimental data points (standard deviation of 46%):

$$\frac{\varepsilon . N_{Pe.a}}{[X]} = 0.20 + 0.011 . Re^{0.48}$$

$N_{Pe.a}$: Peclet number for axial dispersion $= \frac{\bar{v} . d_h}{E_x}$

d_h: hydraulic diameter of particle

Re: Reynolds particle number $= \frac{d_h . v_0 . \rho}{\mu}$

[X]=1 for fixed bed

$[X] = \frac{Re|_{mf}}{Re}$ where $Re|_{mf}$ is the Reynolds at minimum fluidization.

The relation is applicable in the Reynolds range from 10^{-3} up to 10^3. From analysis of this relation compared to the Schmidt number ($\frac{D_M . \rho}{\mu}$), for the liquid system, they concluded that even at low Reynolds number, the molecular diffusion is not important.

❑ Axial dispersion of gases in a packed bed

Unlike liquids, dispersion of gases in fixed or packed beds is affected by the molecular diffusion. Wen and Fan reported that the resulting correlation for axial dispersion of gases (assuming additivity of molecular diffusion effect and turbulent mixing) flowing through a fixed bed can be expressed as:

$$\frac{1}{N_{Pe.a}} = \frac{0.3}{N_{SC} . Re} + \frac{1}{2} . \underbrace{\frac{1}{1 + 3.8 (Re . N_{SC})^{-1}}}_{\text{Corrective factor of Edwards and Richardson}}$$

❑ **Radial dispersion of gases and liquid in packed bed**

As it was for axial dispersion, empirical correlation were determined by Wen and Fan (1974) from several results of experimental investigations.

Assuming $\frac{r}{x^2}.\frac{E_x}{E_r} <<< 1$, for gaseous dispersion:

$$\frac{1}{N_{Pe.r}} = \frac{0.4}{(R_e.N_{SC})^{0.8}} + \frac{0.09}{1+\frac{10}{R_e+N_{SC}}}$$

$0.4<Re<500$
$0.77<N_{SC}<1.2$

and for liquid dispersion:

$$N_{Pe.r} = \frac{17.5}{Re^{0.75}} + 11.4$$

2.3.3 Heterogeneous systems

The nitrifying bioreactor is a three phases reactor (solid-liquid-gas), and then it is defined as a heterogeneous system. In reality, all the previous hydrodynamic models are essentially homogeneous . In order to use the results concerning the homogeneous models for heterogeneous reactors, we have to consider, that the reactor behaves as aperfectly mixed reactor, which is an important simplification.

For a complex heterogeneous reactor (as fine particle fluidized bed used for chemical processes), a number of investigations was conducted to elucidate the gas flow behaviour, because of the fail of ideal and homogenous dispersion models. As a result of these efforts, two kinds of distribution models, the residence time distribution models and bubbling bed models were proposed (Wen and Fan, 1975), the schematic description of which is reported in figure 5.

2.4 The N tanks in series model

As previously mentioned, the N tanks model can be used to describe the flow pattern of a homogenous fluid through a fixed bed. The interest of such a model is its ability to represent both the plug-flow, the axial dispersion flow and the stirred tank models.

The most general representation of the N stirred tank model is the N-series-back-mix stirred tank model (figure 4).

For special coefficients values, the model can work as one of the previous ideal model:
Conditions for a stirred tank
N=1

Conditions for a plug flow

N -> ∞

f=0

Conditions for an axially dispersed model

N -> ∞

and the axial dispersion term can be approximated by (Wen and Fan, 1974)

$$E_x = \frac{f}{N}.\frac{v.L^2}{V} \qquad \text{or} \qquad E_x = \frac{1}{2.N}.\frac{v.L^2}{V} \quad (\text{if } f = 0)$$

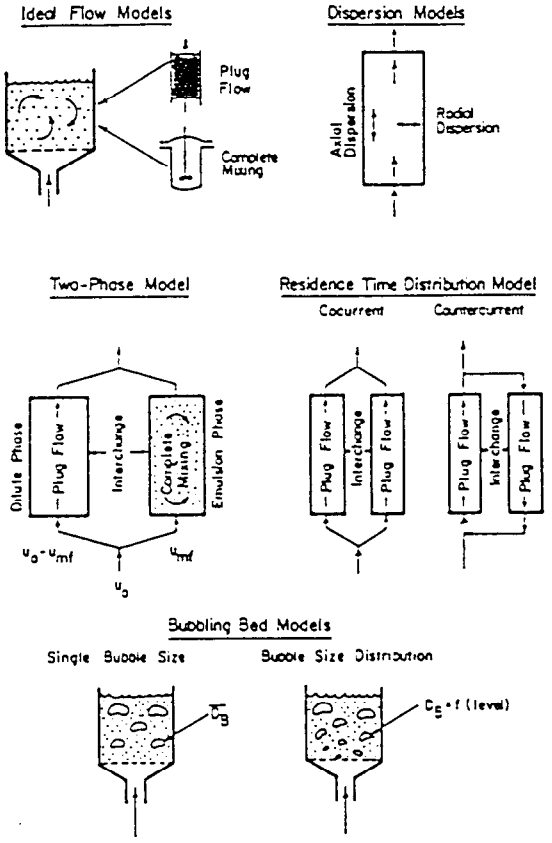


Figure 5: Schematic representation of models for heterogeneous reactors.

2.5 Influence of recirculation

The design of the MELiSSA nitrifying compartment involves recirculation loops for gas and liquid. A recirculation loop on a plug flow model increases the mixing of the compounds in the fluid, and then a plug flow with a recirculation tend towards the behaviour of a stirred tank.

The behaviour of such a system composed by a column with a recirculation loops is then attempted to lay between plug flow and stirred tank, depending in fact of the recirculating factor (recirculating flow rate/input flow rate) and of the volume of the reactor.

III Transfer limitations

The transfer phenomena and limitations are the third aspect to consider in the building of a dynamic model for a bioreactor. We will limit here the study to mass transfer and focus on classical gas-liquid transfer limitations and biofilm transfer limitation. The importance of transfer and diffusion limitations have been reviewed in section 1.2.2.

3.1 Gas-liquid

The transport of a compound from the gas to the liquid phase (or from the liquid to the gas phase) is an important aspect which must be considered for the design of a bioreactor. In the case of aerobic processes, the mechanism of oxygen transfer from the bulk of the gas phase into the bulk of the liquid suspension of single cell organisms is controlled by the liquid phase mass transfer resistance. This process is schematically illustrated in figure 6.

At the gas-liquid interface, there is a thermodynamic equilibrium between gas and liquid phases. In the bulk of the liquid, the concentrations of the compounds which are exchanged between gas and liquid are different. In the case of a transfer from the gas phase to the liquid phase (case of oxygen for aerobic processes) the concentration in the bulk liquid is lower than the equilibrium concentration, inducing a concentration gradient which is the driving force for diffusion from the gas to the liquid (figure 6). In the opposite case, *i.e.* production of a volatile compound inside the liquid phase and transfer to the gas phase (case of CO_2 and more generally of the volatile metabolites), the concentrations in the liquid are greater than the equilibrium concentrations.

Generally speaking, the microorganisms are sensitive to the bulk concentrations which can reach limiting values in the case of consumption or inhibiting values in the case of the production of volatile metabolites. These bulk concentrations depend on the equilibrium concentrations and on the intensity of the transfer *i.e.* the gas-liquid transfer rates.

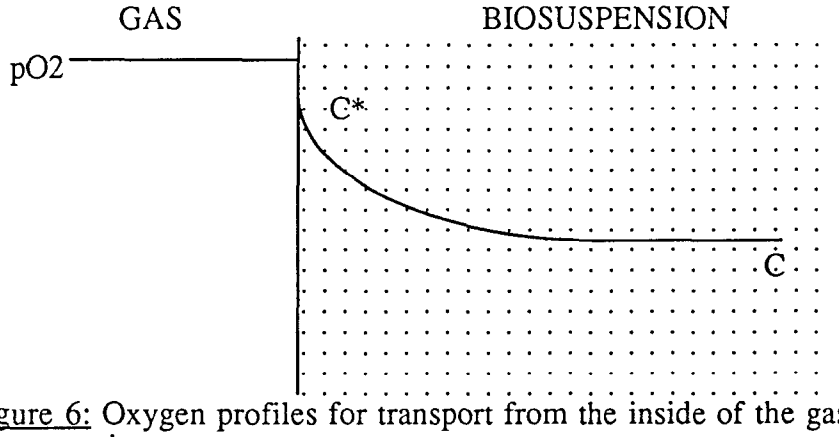


Figure 6: Oxygen profiles for transport from the inside of the gas phase into the bulk of a biosuspension

The intensity of transfer depends on the driving forces *i.e.* the difference between the equilibrium concentrations and the bulk concentrations, and on the mass transfer coefficients which are under control of physical parameters such as diffusion coefficients and interfacial area. Mathematically, the exchange phenomenum between the gas and liquid phases is

represented by the non-steady state balance of compound i in the liquid phase. For a perfectly mixed situation of the liquid, the balance takes the following form:

$$\frac{dC_i}{dt} = K_L a|_i \ (C_i^* - C_i) + r_i$$

where C_i is the concentration of the dissolved gas i

C_i^* is the concentration of compound i at equilibrium with the gas composition

r_i is the production rate of i by a microorganism ($r_i > 0$ is a production, $r_i < 0$ is a consumption)

$K_L a|_i$ is the volumetric transfer coefficient of compound i in liquid phase.

Generally, r_i is a function of the concentration in the bulk liquid C_i and the form of this function is determined by the study of the microbial kinetics.

It must be noted that $K_L a|_i$ is a key parameter for characterising the transfer of the compound i. It is the product of the gas transfer coefficient (K_L) and of the interfacial area per unit of dispersion (a). These two parameters depend on the reactor design and working conditions.

3.2 Biofilm

Because of the design of the bioreactor, a biofilm transfer limitation must be considered. However, it could be noted that, as previously mentioned in section 2.3.1, the cell layers observed on glass beads by Cox et al.(1980) are monolayers, less commonly layers of about 20 cells, and rarely pile of about 100 cells. Based on calculation of the quantity of cells per glass bead, Cox et al.(1980) suggested that, in the region where bacteria are mainly distributed, the mean thickness of the biofilm is 7.7 μm (equivalent to among 8 layers of cells of 1μm thick). Assuming that O_2 becomes limiting for the third of the 26.4 μm of the maximum calculated thickness of the microbial biofilm, Cox et al. (1980) deduced that the O_2 biofilm transfer is not a limiting factor.

The substrate concentration in the biofilm, as a function of time and position in the biofilm (assuming a spherical geometry), is given by:

$$\frac{\partial S_i|_B}{\partial t} = D_{S_i}|_B \left(\frac{\partial^2 S_i|_B}{\partial b^2|_B} + \frac{2}{b|_B} \frac{\partial S_i|_B}{\partial b|_B} \right) - r_{S_i}$$

with boundary conditions

for $b|_B = R_0$ $\qquad \frac{\partial S_i|_B}{\partial b|_B} = 0$

for $b|_B = h_b$ $\qquad S_i|_B = S_i|_{liq}$

and where

D_{S_i} is the diffusion coefficient of the compound S_i in the biofilm

r_{Si} is the reaction rate of the compound S_i

To model the biofilm limitation profile in steady state condtions, a combined diffusion-reaction equation for spherical geometry, can be used, as de Gooijer et al. (1991) do it:

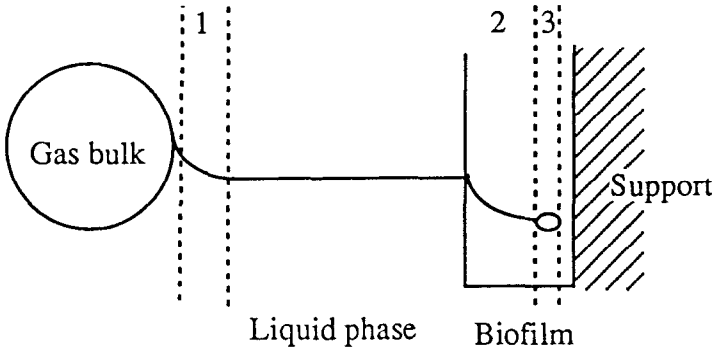
$$\frac{d^2 S_i|_B}{db|_B^2} = \frac{1}{D_{S_i}|_B} \cdot r_{S_i} - \frac{2}{b|_B} \cdot \frac{dS_i|_B}{db|_B}$$

and using as boundary conditions for a biofilm of a thickness h_b:

$S_i|_B = S_i|_{liquid\ medium}$ at $b|_B = R_0 + h_b$

$\frac{\partial S_i|_B}{\partial b|_B} = 0$ at $b|_B = R_0$

The regime analysis for a compound transfer between the different phases of a bioprocess can be performed in order to determine the limiting effect of a transfer versus the other limiting diffusion effects of the process. For a fixed biomass column reactor, 3 steps can be considered (figure 7):



1- transfer from gas bulk to liquid phase (see section 3.1)

2- diffusion through the biofilm

3- transfer into the cell to the enzyme complex.

Figure 7: Different transfer steps for fixed biomass

The regime analysis can be made by comparing the time constant of the different transfer rates (table 9). It can be noted that for oxygen, de Gooijer et al. (1991) calculated that with a characteristic time constant for the transfer into the cell of 10^{-4} s^{-1}, the third step is 100000 timesfaster than the other and can then be neglected.

Table 9: Calculation of the time constant for the regime analysis of a compound transfer rate.

Step	Calculation of time constant for a compound i
1	$T_{S_i} = \frac{1}{k_l a\|_{S_i}}$
2	$T_{S_i} = 0.1 \frac{h_b^2}{D_{S_i}\|_B}$
3	$T_{S_i} = 0.1 \frac{h_{cell}^2}{D_{S_i}\|_B}$

IV Proposed model of autotrophic nitrification for compartment III reactor design

4.1 Main assumptions

1 - The column is assumed to be troncated into 3 parts: A, B (fixed bed) and C
2 - The fixed bed can be assimilated to a N in series stirred tanks reactor with back-flow for gas and liquid
3 - Even if it exits free biomass in the liquid medium, no biological reactions are considered in the parts A and C of the column. Only the fixed biomass is active, while the free biomass (in liquid) activity is neglected.
4 - A steady-state behaviour is considered for the transfer rates.

4.2 The column model design

The model chosen to represent the hydrodynamic behaviour of the column is the N in series stirred tanks reactor model (see section 2.4). From the schematic description of the nitrifying reactor (see section 2.1), a flow diagram can be established assuming that the fixed bed (part B of the column) is truncated into N stirred tanks.

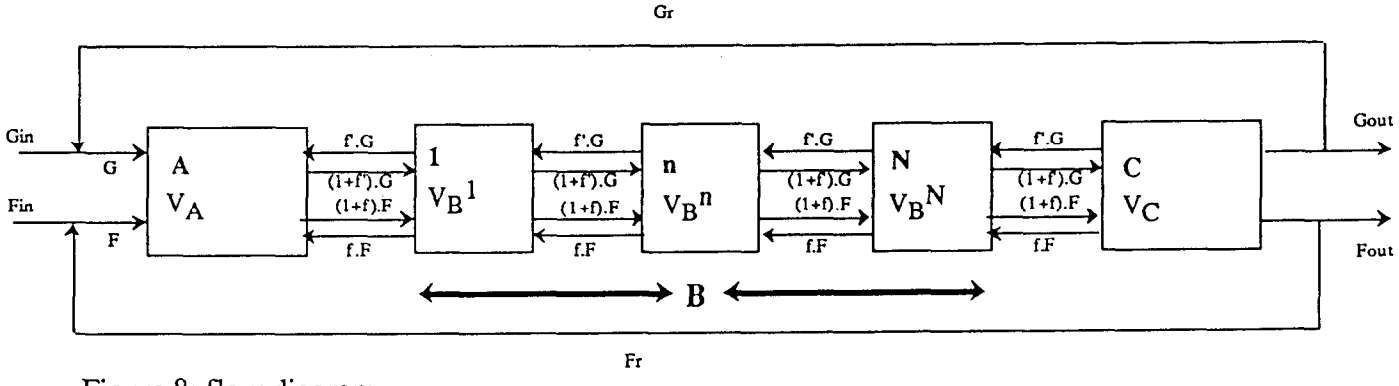


Figure 8: flow diagram

The column design can be defined by its set of relations for volumetric flow rates, volumes and void fraction:

- volumetric flow rates and volumetric flow rates ratio

$$F = F_{in} + F_r = F_{in}(1 + R_L)$$

$$G = G_{in} + G_r = G_{in}(1 + R_L)$$

$$R_L = \frac{F_r}{F_{in}}$$

$$R_G = \frac{G_r}{G_{in}}$$

If it is assumed that there is a variation of volume both in gas and liquid phases, it can be written that:

$$G_{out} = G_{in}$$
$$F_{out} = F_{in}$$

- volumes

$$V_B^n = \frac{V_B}{N}$$

where V_B^n is the total volume of tank n of part B of the column, and V_B is the total volume of the part B of the column. With N=1, the column is assumed to be a stirred tank, while for N>10 the column is not far from a plug-flow behaviour.

-void fraction

$$\varepsilon = \varepsilon_L + \varepsilon_G$$

where ε is the void fraction of the fixed bed and ε_L and ε_G are respectively the volume fraction occupied by liquid and gas in the column. ε_L and ε_G can be calculated using the relation derived from the pressure drop Ergun relation proposed by Forler (1992).

4.3 Hydrodynamic model

4.3.1 Liquid flow description

❑ **Part A (input mixing part of the column)**

$$\frac{\varepsilon_L}{\varepsilon} V_A \frac{dC_{Si}^A|_L}{dt} = F_{in}.C_{Si}^{in}|_L + F_r.C_{Si}^{out}|_L - (f+1).F.C_{Si}^A|_L + f.F.C_{Si}^1|_L + \frac{\varepsilon_L}{\varepsilon} V_A.\phi_{Si}^A|_{GL}$$

with: $\phi_{Si}^A|_{GL}$ the Gas-Liquid transfer term (mol/unit volume. unit time)

❑ **Part B (fixed bed)**

For N stirred tanks

$$\varepsilon_L.V_B^n.\frac{dC_{Si}^n|_L}{dt} = (1+f).F.C_{Si}^{n-1}|_L + f.F.C_{Si}^{n+1}|_L - (f+1).F.C_{Si}^n|_L - f.F.C_{Si}^n|_L + \varepsilon_L.V_B^n.\phi_{Si}^n|_{GL} + \varepsilon_L.V_B^n.\phi_{Si}^n|_{LB}$$

with: n the indice of the tank, 1<n<N

$\phi_{Si}^n|_{GL}$ the Gas-Liquid transfer term (mol/unit volume. unit time)

$\phi_{Si}^n|_{LB}$ the Liquid-Biofilm transfer term (mol/unit volume. unit time)

the boundary conditions $C_{Si}^0|_L = C_{Si}^A|_L$

$C_{Si}^{N+1}|_L = C_{Si}^C|_L$

❑ **Part C (output mixing part of the column)**

$$\frac{\varepsilon_L}{\varepsilon} V_C \frac{dC_{Si}^C|_L}{dt} = (f+1).F.C_{Si}^N|_L - f.F.C_{Si}^C|_L - F_r.C_{Si}^C|_L - F_{out}.C_{Si}^C|_L + \frac{\varepsilon_L}{\varepsilon} V_C.\phi_{Si}^C|_{GL}$$

with: $C_{Si}^{out}|_L = C_{Si}^C|_L$

$\phi^{c}_{Si}|_{GL}$ the Gas-Liquid transfer term (mol/unit volume. unit time)

4.3.2 Gas flow description

The description of the gas flow can be made in a similar way of the liquid flow

❑ **Part A (input mixing part of the column)**

$$\frac{\varepsilon_G}{\varepsilon} V_A \frac{dC^{A}_{Si}|_G}{dt} = G_{in}.C^{in}_{Si}|_G + G_r.C^{out}_{Si}|_G - (f'+1).G.C^{A}_{Si}|_G + f'.G.C^{1}_{Si}|_G - \frac{\varepsilon_L}{\varepsilon} V_A.\phi^{A}_{Si}|_{GL}$$

with: $\phi^{A}_{Si}|_{GL}$ the Gas-Liquid transfer term (mol/unit of volume. unit of time)

❑ **Part B (fixed bed)**

For N mixed tanks

$$\varepsilon_G.V^{n}_{B}.\frac{dC^{n}_{Si}|_G}{dt} = (1+f').G.C^{n-1}_{Si}|_G + f'.G.C^{n+1}_{Si}|_G - (f'+1).G.C^{n}_{Si}|_G - f'.G.C^{n}_{Si}|_G - \varepsilon_L.V^{n}_{B}.\phi^{n}_{Si}|_{GL}$$

with: n the indice of the tank, 1<n<N

$\phi^{n}_{Si}|_{GL}$ the Gas-Liquid transfer term (mol/unit of volume. unit of time)

$\phi^{n}_{Si}|_{LB}$ the Liquid-Biofilm transfer term (mol/unit of volume. unit of time)

the boundary conditions $C^{0}_{Si}|_G = C^{A}_{Si}|_G$

$C^{N+1}_{Si}|_G = C^{C}_{Si}|_G$

❑ **Part C (output mixing part of the column)**

$$\frac{\varepsilon_G}{\varepsilon} V_C \frac{dC^{C}_{Si}|_G}{dt} = (f'+1).G.C^{N}_{Si}|_G - f'.G.C^{C}_{Si}|_G - G_r.C^{C}_{Si}|_G - G_{out}.C^{C}_{Si}|_G - \frac{\varepsilon_G}{\varepsilon} V_C.\phi^{C}_{Si}|_{GL}$$

with: $C^{out}_{Si}|_L = C^{C}_{Si}|_L$

$\phi^{c}_{Si}|_{GL}$ the Gas-Liquid transfer term (mol/unit of volume. unit of time)

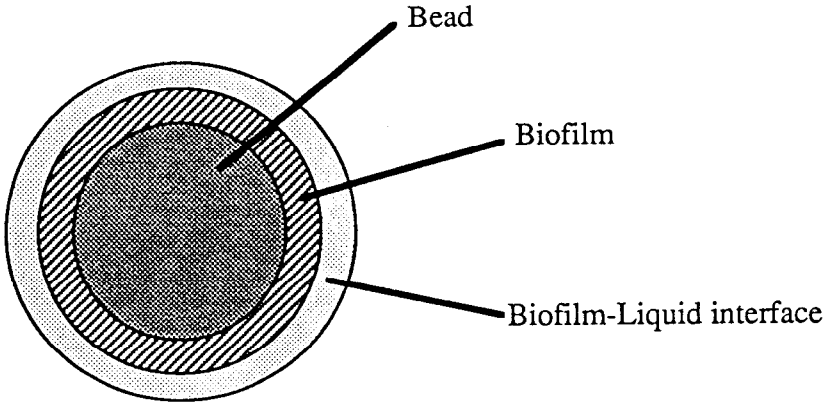
4.4 Transfer and reactional terms

❑ **Gas-Liquid mass transfer**

$$\phi^{\alpha}_{Si}|_{GL} = K_L a|_{Si}.(C^{*}_{Si}|_L - C^{\alpha}_{Si}|_L)$$

❑ Liquid-biofilm mass transfer and biological reaction rate

3 levels of complexity can be considered, assuming different behaviours



1 - Assuming a transfer resistance between the biofilm and the liquid bulk, and a steady-stade transfer limitation in the biofilm

$$\phi_{Si}^{\alpha}\big|_{LB} = K_L a\big|_{Si}^{Biofilm} . (C_{Si}^{\alpha}\big|_{L} - C_{Si}^{\alpha}\big|_{B=hb+Ro})$$

where $C_{Si}^{\alpha}\big|_{B=hb+Ro}$ is the concentration of S_i at the surface of the film. This relation is associated to the reactions involved in the biofilm and represented by the sytem:

$$\frac{d^2 C_{Si}^{\alpha}\big|_B}{db\big|_B^{\,2}} = -\frac{1}{D_{Si}\big|_B} . r_{Si} - \frac{2}{b\big|_B} . \frac{dC_{Si}^{\alpha}\big|_B}{db\big|_B}$$

with the boundary conditions

$$\frac{\partial C_{Si}^{\alpha}\big|_B}{\partial b\big|_B} = 0 \qquad \text{at} \qquad b\big|_B = R_0$$

$$\frac{\partial C_{Si}^{\alpha}\big|_B}{\partial b\big|_B} = -\frac{1}{D_{Si}\big|_B} . \phi_{Si}^{\alpha}\big|_{LB} \qquad \text{at} \qquad b\big|_B = h_b^n + R_0$$

and r_{Si}, is the biological reaction rate (>0 for production and <0 for consumption)

Assuming that the biofilm thickness is small enough compared to the bead radius, the spherical geometry from which the previous relation has been established can be neglected and the profile in the biofilm can then be represented by:

$$\frac{d^2 C_{Si}^{\alpha}\big|_B}{db\big|_B^{\,2}} = -\frac{1}{D_{Si}\big|_B} . r_{Si}$$

with the same boundary conditions

2 - Assuming a no transfer resistance between the biofilm and the liquid bulk, and a steady-stade transfer limitation in the biofilm:

$$\phi^{\alpha}_{Si}\big|_{LB} = -D_{Si}\big|_{B} \left.\frac{\partial C^{\alpha}_{Si}\big|_{B}}{\partial b\big|_{B}}\right|_{B=hb+Ro}$$

This relation is associated to the reactions involved in the biofilm and represented by the sytems:

$$\frac{d^2 C^{\alpha}_{Si}\big|_{B}}{db\big|_{B}^{\,2}} = -\frac{1}{D_{Si}\big|_{B}}.r_{Si} - \frac{2}{b\big|_{B}}.\frac{dC^{\alpha}_{Si}\big|_{B}}{db\big|_{B}} \quad \text{or for plane geometry} \quad \frac{d^2 C^{\alpha}_{Si}\big|_{B}}{db\big|_{B}^{\,2}} = -\frac{1}{D_{Si}\big|_{B}}.r_{Si}$$

with the boundary conditions

$$\frac{\partial C^{\alpha}_{Si}\big|_{B}}{\partial b\big|_{B}} = 0 \qquad \text{at} \qquad b\big|_{B} = R_0$$

$$C^{\alpha}_{Si}\big|_{B} = C^{\alpha}_{Si}\big|_{L} \qquad \text{at} \qquad b\big|_{B} = R_0 + h^{n}_{b}$$

3 - The third level is based on the hypothesis that transfer resistance and transfer diffusion in the biofilm can be neglected. The Liquid-biofilm term is then reduced to the biological reactional rate:

$$\phi^{\alpha}_{Si}\big|_{LB} = r_{Si}$$

❑ Increase of the biofilm thickness with growth of the bacteria

Assuming a mean geometry of a cell of 1 x 1 x 1 µm and that 1mg of dry biomass is equivalent to an average of $3.7.10^9$ cells (Hunik et al., 1994) (Cox et al., 1980, proposed a value of $5\ 10^9$), a relation can be established giving an average thickness of the biofilm as a function of the quantity of bacteria fixed:

$$h^n(m) = C^{n}_{X}\big|_{B}\ 3.7\ 10^{-3}\ \frac{\varepsilon_L}{(1-\varepsilon)}\ \frac{R_0}{3}$$

with $C^{n}_{X}\big|_{B}$ the concentration of biomass fixed (g/l)
Ro the mean radius of the beads (m)

It can be noted that a volume of 10^{-18} m^3 and a volumetric density of 901 kg/m^3 for wet biomass (70% water), calculated from the previous assumption are in the range of the commonly observed values for microorganisms.

Cox et al.(1980) determined that the O_2 transfer rate in the nitrifying biofilm becomes limiting at a thickness of 8.8 µm. Using the previous relation, this is equivalent to a concentration of 1.8 g biomass/l.

4.5 Physical reactions (acid-base equilibrium)

The problem of acid-base equilibria, linked to the Gas-Liquid equilibria, has been studied in TN 17.1 and TN 23.1 (see these TN for much details about acid-base phenomena).

❑ Ammonia

$$NH_3 + H_2O \rightleftharpoons NH_4^+ + OH^-$$

$$K_{NH3} = \frac{C^{\alpha}_{NH4+}|_L \cdot C^{\alpha}_{OH-}|_L}{C^{\alpha}_{NH3}|_L}$$

$$\ln K_{NH_3}(T) = \frac{A_1}{T} + A_2 \ln T + A_3 T + A_4$$

The parameters proposed by Edwards (1978) (TN 23.1) are:
A_1= -3 335.7
A_2= 1.4971
A_3=-0.0370566
A_4= 2.76
Temperature range: 273.15 - 498.15 K

❑ Phosphoric acid

$$H_3PO_4 \rightleftarrows H_2PO_4^- + H^+$$

$$H_2PO_4^- \rightleftarrows HPO_4^{2-} + H^+$$

$$HPO_4^{2-} \rightleftarrows PO_4^{3-} + H^+$$

$$K_{H3PO4} = \frac{C^{\alpha}_{H2PO4-}|_L \cdot C^{\alpha}_{H+}|_L}{C^{\alpha}_{H3PO4}|_L} \quad \text{and,} \quad pK_{H3PO4}\ (25°C)=2.16$$

$$K_{H2PO4} = \frac{C^{\alpha}_{HPO4}|_L \cdot C^{\alpha}_{H+}|_L}{C^{\alpha}_{H2PO4}|_L} \quad \text{and,} \quad pK_{H2PO4}\ (25°C)=7.21$$

$$K_{HPO4} = \frac{C^{\alpha}_{PO4}|_L \cdot C^{\alpha}_{H+}|_L}{C^{\alpha}_{HPO4}|_L} \quad \text{and,} \quad pK_{HPO4}\ (25°C)=12.32$$

❑ Sulfuric acid

$$H_2SO_4 \xrightarrow{\text{immediate}} HSO_4^- + H^+$$

$$HSO_4^- \rightleftarrows SO_4^{2-} + H^+ \qquad \text{and } pK_{HSO4}(25°C)=1.98$$

❑ **Carbon dioxide and its ionic forms**

$$CO_2 + H_2O \underset{k'_{-1}}{\overset{k'_1}{\rightleftharpoons}} HCO_3^- + H^+$$

$$HCO_3^- \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} CO_3^{2-} + H^+$$

$$K_1 = \frac{C^{\alpha}_{HCO3-}|_L \cdot C^{\alpha}_{H+}|_L}{C^{\alpha}_{CO2}|_L}$$

$$K_3 = \frac{C^{\alpha}_{CO3--}|_L \cdot C^{\alpha}_{H+}|_L}{C^{\alpha}_{HCO3-}|_L}$$

$$\ln K_1(T) = \frac{-12092.1}{T} - 36.7816 \ln T + 235.482$$

$$\ln K_3(T) = \frac{-12431.7}{T} - 35.4819 \ln T + 220.067$$

4.6 Biological reactions

The building of the stoichiometries has been explained in TN 23.2

❑ **Active biomass biosynthesis (anabolism)**

For the two organisms:

$$CO_2 + 0.0089\ H_3PO_4 + 0.0035\ H_2SO_4 + 2.9468\ NTP + 1.2879\ H_2O + 0.1994\ NH_3 + 2.1502\ CofH_2$$

$$\Downarrow\ r_1^{Ns} \text{ or } r_1^{Nb}$$

$$CH_{1.6147}O_{0.3906}N_{0.1994}S_{0.0035}P_{0.0089} + 2.9468\ (NDP + Pi) + 2.1502\ Cof$$

❑ **Energetic metabolism**

For *Nitrosomonas*:

$$NH_3 + \frac{5}{4}O_2 + 4H^+_{in} + \frac{1}{2}UQ \longrightarrow 4H^+_{out} + (NO_2^-, H^+) + \frac{1}{2}UQH_2 + \frac{1}{2}H_2O \qquad r^{Ns}2a$$

$$UQH_2 + 6H^+_{in} + \frac{1}{2}O_2 \longrightarrow UQ + 6H^+_{out} + H_2O \qquad r^{Ns}2b$$

$$NAD^+ + UQH_2 + 4H^+_{out} \rightleftarrows NADH, H^+ + UQ + 4H^+_{in} \qquad r^{Ns}2c$$

$$3H^+_{out} + ADP + Pi \rightleftarrows 3H^+_{in} + ATP + H_2O \qquad r^{Ns}2d$$

For *Nitrobacter*:

$$(NO_2^-,H^+) + H_2O + \text{Cyt } c_{ox} \longrightarrow (NO_3^-,H^+) + \text{Cyt } c_{red} + 2H^+ \qquad r^{Nb}_{2a}$$

$$\text{Cyt } c_{red} + 8H^+_{out} + NAD \longrightarrow \text{Cyt } c_{ox} + NADH,H + 6H^+_{in} + H_2O \qquad r^{Nb}_{2b}$$

$$\text{Cyt } c_{red} + \frac{1}{2}O_2 + 4H^+_{in} \rightleftarrows \text{Cyt } c_{ox} + H_2O + 2H^+_{out} \qquad r^{Nb}_{2c}$$

$$3H^+_{out} + ADP + Pi \rightleftarrows 3H^+_{in} + ATP + H_2O \qquad r^{Nb}_{2d}$$

❑ Maintenance

For the two organisms:

$$ATP + H_2O \longrightarrow ADP + Pi \qquad r^{Ns}_3 \text{ or } r^{Nb}_3$$

❑ Other reactions

Some other reactions, such as the reserve metabolism or the catabolism can be introduced. The catabolism can be of special interest if remaining organic compounds are considered in the reactor. At the present time these other reactions are not considered.

Assuming steady state conditions for NTP (i.e. ATP) and reduced Cofactor (i.e. NADH,H+), the system of equations can be be reduced for each organism to 2 equations:

Nitrosomonas

$$CO_2 + 0.0089\ H_3PO_4 + 0.0035\ H_2SO_4 + 5.4269\ O_2 + 4.5341\ NH_3 \Downarrow CH_{1.6147}O_{0.3906}N_{0.1994}S_{0.0035}P_{0.0089} + 4.3347\ (NO_2^-,H^+) + 3.8433\ H_2O \qquad (r^{Ns}_{gr})$$

$$NH_3 + 1.5\ O_2 \rightarrow (NO_2^-,H^+) + H_2O \qquad (r^{Ns}_m)$$

Nitrobacter

$$CO_2 + 0.0089\ H_3PO_4 + 0.0035\ H_2SO_4 + 6.5106\ O_2 + 15.1714\ (NO_2^-,H^+) + 0.1994\ NH_3 + 0.4914\ H_2O \Downarrow CH_{1.6147}O_{0.3906}N_{0.1994}S_{0.0035}P_{0.0089} + 15.1714\ (NO_3^-,H^+) \qquad (r^{Nb}_{gr})$$

$$(NO_2^-,H^+) + 0.5\ O_2 \rightarrow (NO_3^-,H^+) \qquad (r^{Nb}_m)$$

❑ Biological kinetics

The maintenance is proportionnal to the active biomass concentration:

$$r_m^{Ns} = m^{Ns}.C_{X-Ns}\big|_B$$

$$r_m^{Nb} = m^{Nb}.C_{X-Nb}\big|_B$$

The definition of the maintenance coefficient m is given in section 1.3.1. The maintenance rates are then defined as concentrations of substrate for maintenance (respectively NH_3 and HNO_2 for *Nitrosomonas* and *Nitrobacter*) by time unit.

The growth, represented by the first set of equations, takes the form:

$$r_{gr}^{Ns} = \mu^{Ns}.C_{X-Ns}\big|_B$$

$$r_{gr}^{Nb} = \mu^{Nb}.C_{X-Nb}\big|_B$$

μ^{Ns} and μ^{Nb} are respectively the specific growth rate of *Nitrosomonas* and *Nitrobacter*. The specific growth rate can be expressed as a function of limiting substrate concentration. A relation extrapolated from Hunik et al. (1994) can be used:

$$\mu = \mu_{max}.\prod_{\substack{\text{Limiting}\\ \text{substrate j}}} \frac{C_j\big|_B}{(Ks_{sj} + C_j\big|_B).Ki}$$

$$\text{where } Ki = \prod_{\substack{\text{Inibitory}\\ \text{Substrate k}}} \left(1 + \frac{C_k\big|_B}{I_k}\right)$$

Considering that the decay rate resulting of the maintenance (see section 1.3.1), depends on the growth performances, the growth rate can be written as:

$$r_X^{Ns} = r_{gr}^{Ns} + \left(\frac{\mu^{Ns}}{\mu_{max}^{Ns}} - 1\right).Y_{X/Si}^{Ns}.r_m^{Ns}$$

$$r_X^{Nb} = r_{gr}^{Nb} + \left(\frac{\mu^{Nb}}{\mu_{max}^{Nb}} - 1\right).Y_{X/Si}^{Nb}.r_m^{Nb}$$

i.e.

$$r_X^{Ns} = \mu^{Ns}.C_{X-Ns}\big|_B + Y_{X/Si}^{Ns}.m^{Ns}.\left(\frac{\mu^{Ns}}{\mu_{max}^{Ns}} - 1\right).C_{X-Ns}\big|_B$$

$$r_X^{Nb} = \mu^{Nb}.C_{X-Nb}\big|_B + Y_{X/Si}^{Nb}.m^{Nb}.\left(\frac{\mu^{Nb}}{\mu_{max}^{Nb}} - 1\right).C_{X-Nb}\big|_B$$

The substrate consumption and production rates can be expressed as:

$$r_{Si}^{Ns} = \frac{1}{Y_{X/Si}^{Ns}}.r_X^{Ns} + \frac{1}{Y_{Smt/Si}^{Ns}}r_m^{Ns}$$

These expressions of growth rate and of substrate consumption are similar to those proposed by Hunik et al. (1994).

❑ Free biomass and fixed biomass

It can be assumed that a fraction of the biomass produced is washed out in the liquid phase. The production rate of free biomass can be considered as a function of the growth rate, and described by the relation

$$r_X^{Ns-free} = K_{wo}.r_X^{Ns}$$
$$r_X^{Nb-free} = K_{wo}.r_X^{Nb}$$

where K_{WO} is the fraction of the biomass produced that is washed out in the liquid phase.

The evolution of the free biomass (in the liquid phase) can then be calculated from the relation:

$$\varepsilon_L.V_B^n.\frac{dC_{Si}^n|_L}{dt} = (1+f).F.C_{Si}^{n-1}|_L + f.F.C_{Si}^{n+1}|_L - (f+1).F.C_{Si}^n|_L - f.C_{Si}^n|_L + \varepsilon_L.V_B^n.r_X^{biomass-free}$$

❑ Production of slime by the nitrifiers

The production of slime by the nitrifiers has been noted by Cox et al. (1980) (see section 3.2). It seems that the slime prevents from the fixing of new bacteria. This phenomenum is not well-known, thus for simplicity it can be supposed that no free bacteria (even free nitrifiers) can grow on the beads after the innoculation of the column and the beginning of nitrification. As a consequence, if all nitrifying bacteria fixed on a bead died, no more bacteria can be fixed on this bead.

4.7 List of parameters involved.

A lot of coefficients are involved in the proposed model. These coefficients are listed here. Some values for these parameters can be found in the literature (see section 1) or from experiments performed at ESTEC laboratory (Forler, 1994) and UAB Laboratory. The numerical treatment of the model and the addition of further assumptions will be performed in TN 27.2.

Hydrodynamic parameters

Liquid flow :	F	Volumetric flow rate in the column
	Fin	Volumetric flow rate of the input flow to the column
	RL	Recycling factor
	f	back-mix factor
Gas flow :	G	Volumetric flow rate in the column
	Gin	Volumetric flow rate of the input flow to the column
	RG	Recycling factor

	f'	back-mix factor
Column design :	VA	Volume of the part A of the column
	VB	Volume of the part B of the column
	VC	Volume of the part C of the column
	N	Number of equivalent stirred tanks
	ε_L	volumetric fraction occupied by liquid
	ε_g	volumetric fraction occupied by gas
	ε	voidage of the bed

ε_L and ε_g can be calculated using the relation developed by Forler (1992) and extrapolated from the Ergun relation.

Constants associated to the compounds

Dissociation constants : K_A or pK_A for a defined temperature, or parameters for the calculation of K_A by a relation (see TN 17.1)

Gas-Liquid transfer : K_La (or K_L and a)
C*, the saturation concentration at a defined temperature, or parameters for the calculation of C* (see TN 17.1)

Liquid-biofilm transfer : $D|_s$ the diffusion coefficient in the biofilm

$K_La|^{Bio}$ (or $K_L|^{Bio}$ and $a|^{Bio}$) the transfer coefficients from the liquid bulk to the biofilm

Kinetic parameters for each microorganism :

K_S	saturation constant
I_S	inhibitory constant
μ_m	maximum specific growth rate
m	maintenance coefficient

Conclusion

This bibliographic review of the nitrification process provides many published values that can further be used to perform the numerical integration of the model proposed. The nitrification was mainly studied by measuring the conversion yields and the limiting and inhibitory substrates, but rarely by using a structured approach of the stoichiometries.

The proposed model has been established for autotrophic conditions. Nevertheless, the stoichiometries can be easily completed to take into account an heterotrophic behaviour.

The model proposed involves many coefficients. But, by adding further assumptions, the number of coefficients can be reduced to a form more manageable (this is in particular the case with the 3rd step of complexity for the liquid-biofilm description)

The numerical treatment of the model, using literature data(including the works performed at ESTEC laboratory) and, when possible UAB MELiSSA Laboratory data, will be further performed in TN 27.2.

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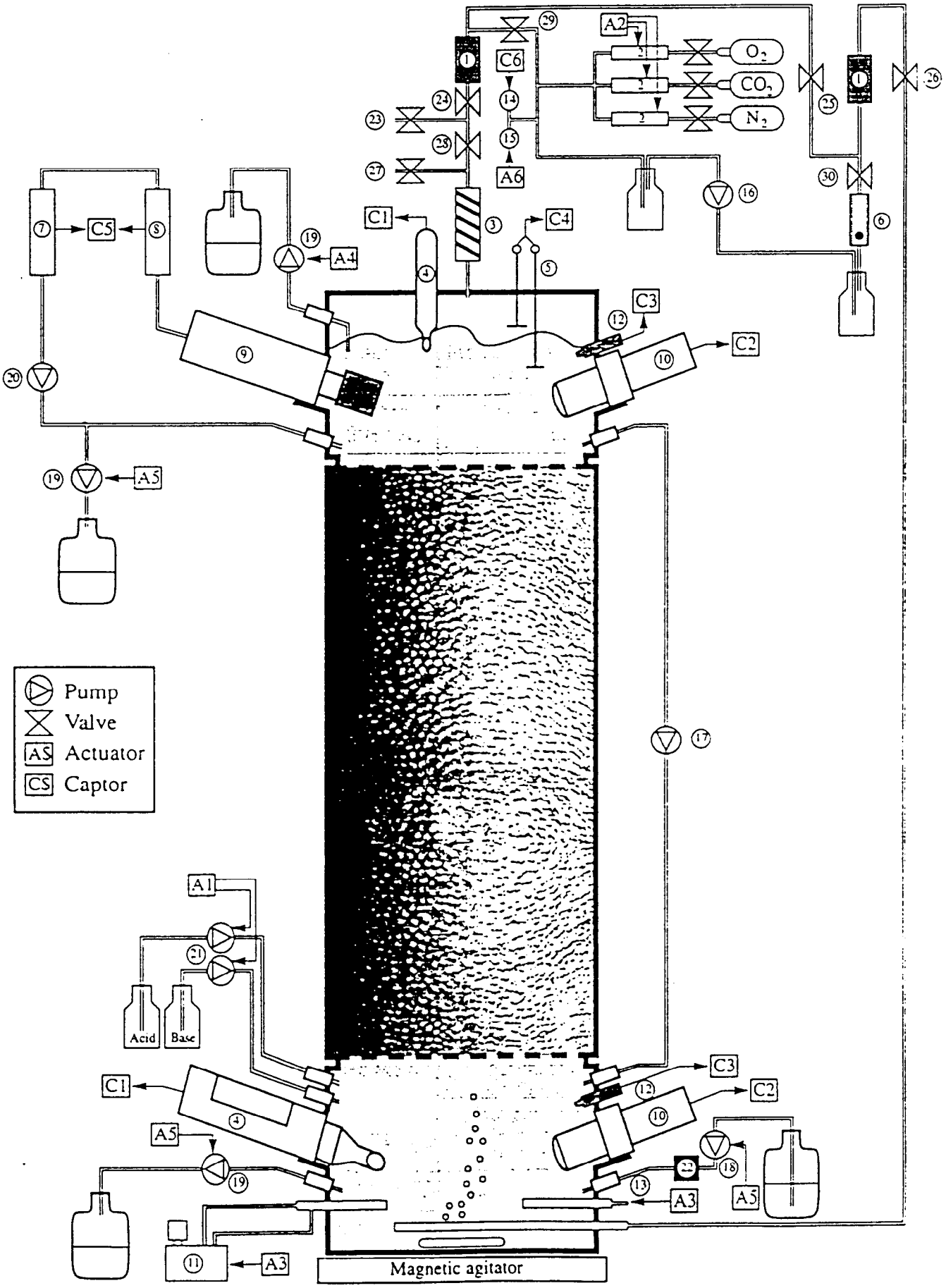
Annex A

Design of the nitrifying column (Forler, 1994)

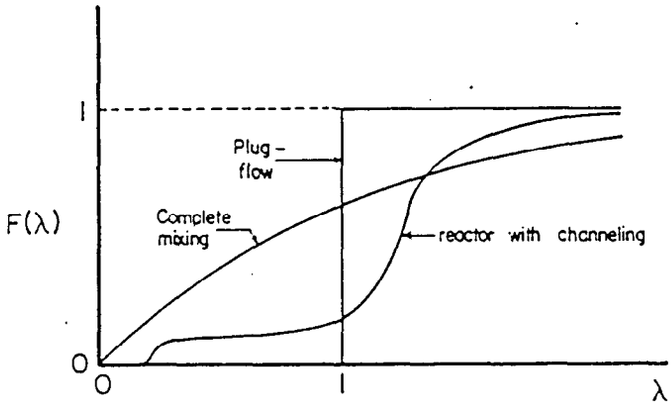


Ref. N°	Material	Trademark	Action	Input	Output
1	Air Filter (x2)	Bioengineering	Keep the gas loop sterile		
2	Mass Flow Controller (x3)	MKS 2259-BX	Control of O_2, CO_2, N_2 flow	3	2
3	Condenser	Bioengineering	Keep the gas loop safe from humidity		
4	pH Probe (x2)	Ingold Infit 764-50 Mark I	Measure of pH on top and bottom of the column		2
5	Level Probe (x2)	Heuser Lindeman	Indication of the liquid level on the top of the column		3
6	Air Flow Meter	KDG Mobrey 1100 3033/JC	Measure of the air pump flow		
7	NH_4^+ Analyser	AMTAX Dr. Lange LYX720	On line measurement of $N\text{-}NH_4^+$ liquid concentration	3	4
8	NO_3^- Analyser	NITRAX Dr. Lange LPG234	On line measurement of $N\text{-}NO_3^-$ liquid concentration	3	4
9	Filtration probe	PERSEP Tech-sep (RP)	Continuous filtration of the analysis liquid medium	3	2
10	PO_2 Probe (x2)	Ingold Infit 322 7568	Measure of pO_2 on top and bottom of the column		2
11	Thermostatic Bath	Haake 3568/7983	Cooling of the column, the condensor and the PERSEP	3	2
12	Temperature Probe (x2)	PT 100	Measure of temperature on top and bottom of the column		2
13	Heater	Heuser Lindeman	Heating of the column	3	
14	Pressure Sensor	Phoenix contact 276/8858	Measure of the pressure in the gas loop		2
15	Pressure Valve	ASCO 58729017	Regulation of the pressure in the gas loop	2	
16	Air Pump	KNF Verder	Maintain the flow and the pressure in the gas loop	3	
17	Mechanical Pump	Ismatec MSZ	Maintain the liquid recirculation between top and bottom of the reactor	3	
18	Peristaltic Pump	Ismatec MCMS3	Liquid medium input	5	
19	Peristaltic Pump	Ismatec MCMS3	Liquid medium output	5	
20	Peristaltic Pump	Ismatec MCMS3	Liquid analysis loop	5	
21	Peristaltic Pump (x2)	Heuser Lindeman	Acid/Base injection	3	
22	Filter 0.22 µm	Millipore Millipac 200	Filtration of medium input		

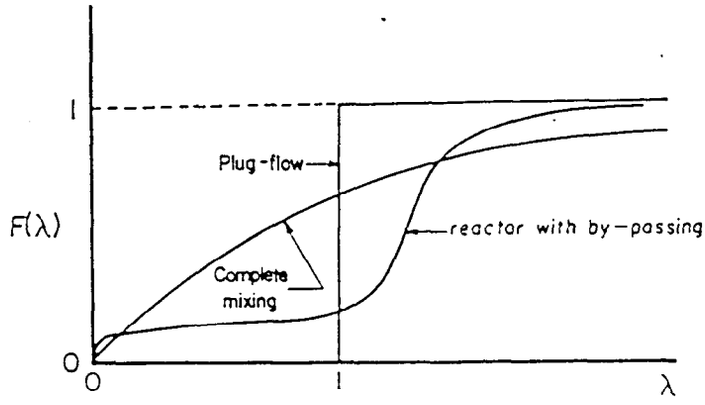
Annex B

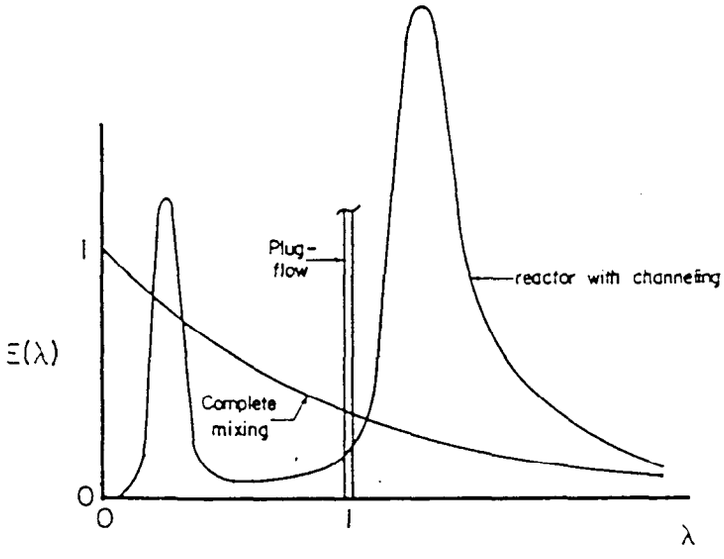
RTD curves (Wen and Fan, 1974)

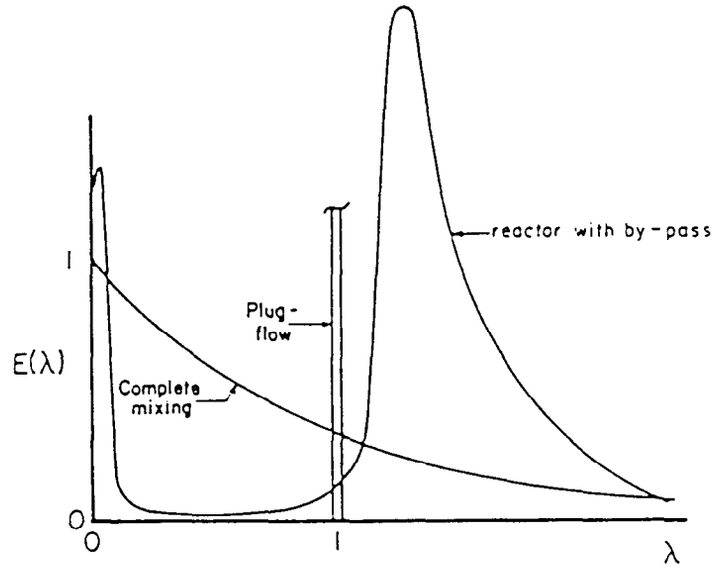
CHANNELING



BY-PASSING







DEAD SPACE