PART IV

NITROGEN MONITORING AND CONTROL

CHAPTER 7

Monitoring and control of the nitrogen species concentration in the effluent of CIII

CHAPTER 7

MONITORING AND CONTROL OF THE NITROGEN SPECIES CONCENTRATION IN THE EFFLUENT OF CIII

Foreword

An on-line system for the continuous determination of ammonium, nitrite and nitrate in the reactor effluent will be implemented, allowing monitoring of the nitrifying efficiency under steady state conditions as well as during perturbations. Concentration data in the effluent are also required by the local nitrite control strategy, which was designed to minimise the concentration of nitrite in the effluent of CIII. The application of the mathematical model developed in previous chapters will also benefit from the availability of continuous concentration data eventually, allowing the simulation of different scenarios and the validation with experimental data.

7.1 INTRODUCTION

The nitrifying pilot reactor of the MELiSSA loop was designed to achieve total conversion of NH_4^+ into NO_3^-.The efficiency of the nitrifying reactor was tested under a wide range of operational conditions, and conversion rates close to 100% were achieved with incoming ammonium loads of up to 1.28 g $N\text{-}NH_4^+ \cdot L^{-1} \cdot d^{-1}$. However, under a number of unforeseen circumstances accumulation of nitrite or ammonium may occur in CIII. Some specific situations such as the decrease in the concentration of dissolved oxygen may, for different reasons, prevent the achievement of total nitrification, leading to the presence of nitrite or ammonium in the effluent of CIII.

Masot (2007) studied the the performance of CIVa (*Arthrospira platensis* compartment) using ammonium as a nitrogen source. It was concluded that concentrations above 79 mg $N\text{-}NH_4^+ \cdot L^{-1}$ resulted in inhibition of the growth of *Arthrospira*.

Nitrite is an unstable compound, which is toxic to humans at low concentrations. Its presence in drinking water has been regulated by European Community Directive 80/778/EEC, which states that the maximum admissible concentration is 0.03 mg $N\text{-}NO_2^- \cdot L^{-1}$. Nitrite has also been reported to be toxic for *Arthrospira* at high concentrations (Becker, 1994). However, experiments performed at the MPP with continuous connection of CIII and CIVa (Creus, 2000), showed that nitrite levels as high as those reported as inhibitory by Becker (1994) did not affect the correct performance of the *Arthrospira* compartment. It was concluded that, although nitrite can only be assimilated by *Arthrospira* up to a limited amount, higher concentrations are not toxic to it in the presence of other nitrogen sources (ammonia or nitrate). In these conditions, the excess of nitrite would simply be washed out of the reactor and could reach the crew compartment via the biomass used in food preparation and the recycled water.

The implementation of an on-line analysis system of the different nitrogen species in CIII, would allow monitoring the reactor efficiency under steady state and also during unforeseen perturbations. The toxic nature of nitrite requires the continuous monitoring of this nitrogen species in CIII to provide the control system with the required experimental data. Moreover, the absence of frequent, accurate experimental data of ammonium, nitrite and nitrate in the effluent, was an important limitation for the applicability of the model developed in Chapter 4 to the prediction

of the dynamic state. The potential of the mathematical model will be significantly improved with the availability of a continuous monitoring system.

7.1.1 NITROGEN SPECIES MONITORING STRATEGY

The different levels of ammonium, nitrite and nitrate occurring in CIII are presented in Table 7.1. The trade-off to select the most suitable analysers to monitor the concentration of ammonium, nitrite and nitrate in the effluent of CIII has to be based on several factors: (i) Accuracy: the capacity to provide concentration measurements, with a low deviation from the real value, both in the typical range, and during the transient state following a perturbation; (ii) Precision or repeatability: the capacity of a given analyser to produce the same measured value in two consecutive analyses of the same sample, usually expressed in terms of relative standard devition; (iii) frequency i.e. the duration of each analysis cycle; (iv) other factors such as the maintenance requirements or the necessity to implement an on-line dilution to achieve the required measure range were also taken into consideration.

Table 7.1 Concentration ranges of the different nitrogen species in compartment III.

Nitrogen compound	Normal operation (mg N·L^{-1})	Exceptional levels (mg N·L^{-1})
NH_4^+	Inlet: 300-600 Effluent: 0-10	Inlet: >700 Effluent: >100
NO_2^-	0-10	>50
NO_3^-	300-600	<200 >700

Ammonium and nitrate detection has been broadly studied and on-line analysis solutions based on different detection methods and analytical techniques have become increasingly common. However, nitrite detection techniques have only recently earned the necessary level of development required for their on-line implementation (Moorcroft et al., 2001). At the time the first decisions were made in the MELiSSA project regarding monitoring of the nitrogen species in CIII, the vast majority of the available analysers would not discern NO_2^- from NO_3^-, providing only a measurement of NO_x^- (NO_2^- + NO_3^-). Hence, the direct experimental measurement of the nitrite concentration was initially discarded, and a new working strategy was investigated, based on the indirect measurement of the nitrite concentration. The proposed strategy consists of experimentally measuring the ammonium and nitrate concentrations on-line and subsequently estimating the nitrite concentration. The proposed nitrite estimator is based on a simplified approach of a more complex

mathematical model developed by Blaise Pascal University (France) to describe the CIII pilot reactor operation (Poughon et al., 1999). The predictor requires the concentrations of all components of the liquid and gas phases entering the reactor, the flow rate and the state of the system at a given sampling time (t). The complete internal state at time (t) is then used to predict the behaviour of the nitrite concentration along a prediction horizon (H, typically 10h). The calculation of the nitrite concentration is directly affected by the sampling frequency, the accuracy and the precision of the ammonium and nitrate analysis equipment.

The performance of the ammonium and nitrate analysers will be tested under a number of experimental conditions to evaluate (i) the suitability of the analysers to monitor ammonium and nitrate in the reactor effluent and (ii) the precision of the obtained nitrite estimations and the feasibility of an indirect measure of the nitrite concentration.

7.1.2 NITRITE CONTROL IN COMPARTMENT III

One of the main objectives of the control strategy in CIII is to avoid nitrite accumulation. The actions of the nitrite control system will be based either on the experimental nitrite values provided by an on-line analyser or, in the case of the indirect measurement of nitrite, on the values estimated by the model.

The initial decision to estimate the nitrite concentration in CIII led to the development of a model-based predictive control strategy within the MELiSSA consortium (Sherpa Engineering, formerly ADERSA, France), as a key tool to achieve nitrite control in CIII. The main features of model based predictive control are (Richalet, 1993; Compas et al., 1994): (i) an internal model used for prediction, (ii) a reference trajectory that connects the estimated controlled variable (nitrite) at sampling time (t) to the future constraint, (iii) structured future manipulated variable and algorithmic solver and (iv) compensation of the model mismatch i.e. the error between the process and the model outputs. The predictive strategy designed for the nitrite control in CIII is based on the estimation of the nitrite concentration (controlled variable) and the subsequent actuation on the incoming flow rate (manipulated variable). The principle of the nitrite control strategy (Figure 7.1) is based on predicting the behaviour of the nitrite concentration on a horizon H (typically 10 h) following a sampling time. Input concentrations of all species and operational conditions at sampling time (t) are provided. The complete estimated state at time t is used by the predictor to estimate the nitrite concentration along a prediction horizon H (typically 10 h). The maximum value estimated between the

sampling time (t) and the other extreme of the prediction horizon (t+H) is compared to the constraint. The scenario is tested repeatedly with different values of the flow rate until the nitrite extreme matches the constraint.

The display of the user interface where the actuation on the liquid flow rate can be visualised is shown in Figure 7.2.

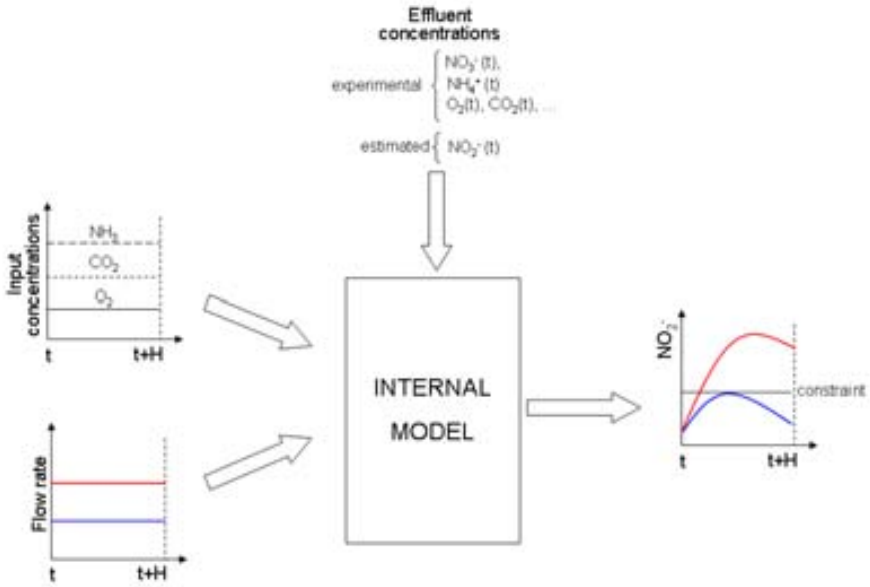


Figure 7.1 Principle of the nitrite control in theMELiSSA CIII (Adapted from Lobo and Lasseur, 2003).

Figure 7.2 Display of the liquid flow regulation user interface. The O_2, NH_3 and NO_3 measurements are present in the display, along with the NO_2^- concentration estimated by the nitrite predictor. The control action is applied to the input liquid flow rate whenever required by the system.

7.1.3 REVIEW OF ON-LINE ANALYSIS TECHNIQUES AND DETECTION SYSTEMS

Analysers developed for on-line measurements in flowing liquids are based on different principles. Among these techniques, Flow Injection Analysis (FIA) and Continuous Flow Analysis (CFA) are the most common techniques in laboratory analysis. Since the introduction of the FIA technique (Ruzicka et al, 1980) on-line techniques have been widely implemented in process analysers and the use of different on-line techniques on biotechnology processes is well documented (Ensafi and Kazemzadeh, 1999; Christensen et al., 1996; Pirsing et al., 1996).

In general, any analytical property related to the amount of analyte in a reproducible way is adequate to be implemented in these systems. The two analysis systems implemented in the analysers utilised in this thesis are potentiometry and spectrophotometry (the detection of the sample absorbance at a certain wavelength). These are properties that depend selectively on the measured amount of analyte in the sample, allowing determinations in which a previous separation step is, in principle, not required. The conversion of either absorbance or potential measurements performed by these analysers into concentration values is achieved by means of a calibration procedure.

In order to evaluate the performance of an analyser the following parameters, in addition to the previously mentioned accuracy and precision, have to be taken into consideration: (i) Sensitivity, given by the slope of the calibration curve. A high accuracy is obtained while measurements are performed in the linear range of the calibration curve. A decrease in the sensitivity during operation is an indication of a defective performance of the detection system, e.g. an ion selective electrode; (ii) low detection limit, which is the lower concentration at which the analyte can be distinguished from the interfering species present in the sample; (iii) response time, i.e. the time elapsed until a stable value is achieved; (iv) interference from other species present in the sample; (v) the optimal pH range of operation to avoid interference.

7.2 OBJECTIVES

The main aim of this chapter is the implementation of on-line instrumentation to monitor the concentration of ammonium, nitrite and nitrate in the effluent of CIII, thus providing continuous on-line data of the nitrifying efficiency in the reactor. In

order to achieve this final purpose, the following partial objectives have to be fulfilled:

- Characterisation of the performance of the ammonium and nitrate analysers and adaptation of their operation to the CIII sample matrix.
- On-line implementation and evaluation of the suitability of ammonium and nitrate analysers based on solid-state ion selective electrodes to monitor ammonium and nitrate in the reactor effluent.
- Evaluation of the feasibility of the estimation of the nitrite concentration using the data provided by the on-line ammonium and nitrate analysis instrumentation.

7.3 MATERIAL AND METHODS

7.3.1 OPERATIONAL CONDITIONS OF THE CIII PILOT REACTOR

The operational conditions in the reactor were kept constant during the analyser testing period, only altering the liquid flow rate and input ammonium concentration when required by the test specifications.

The aeration in the reactor was maintained at around 1.5 $L \cdot min^{-1}$, which ensured that the oxygen was not limiting at any stage during the tests. With this flow rate, an average dissolved oxygen concentration of approximately 50% of saturation was attained in the packed bed, with some fluctuations during the transient states following a load step. The temperature was 28ºC during all the experiments. The pH value was controlled (7.8-8.2) using $NaHCO_3$ as base and CO_2 as acid. The composition of the reactor feeding medium is presented in Table 7.2.

Table 7.2 Composition of the culture medium used in CIII. pH is set to 8.1-8.2 with Na_2CO_3; $MgSO_4 \cdot 7H_2O$ and $CaCl_2 \cdot 2H_2O$ are sterilised separately and subsequently added to the main solution.

Compound	Concentration ($g \cdot L^{-1}$)
$(NH_4)_2SO_4$	1.32
$FeSO_4 \cdot 7H_2O$	0.0025
$CuSO_4 \cdot 5H_2O$	$4 \cdot 10^{-6}$
Na_2HPO_4	0.71
KH_2PO_4	0.68
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.177
$ZnSO_4 \cdot 7H_2O$	$4.3 \cdot 10^{-6}$
$MgSO_4 \cdot 7H_2O$	0.052
$CaCl_2 \cdot 2H_2O$	$7.4 \cdot 10^{-4}$
$NaHCO_3$	0.8

7.3.2 DESCRIPTION OF THE NITRATE ANALYSER (AQUATEC S.A., AQUANITRA®)

7.3.2.1 Detection system

The selected nitrate analyser (AQUATEC S.A., Aquanitra®) uses a solid state Ion Selective Electrode (ISE) as a detection system. Its on-line operation is based on the Flow Injection Analysis (FIA) technique. An ISE is mainly composed of a membrane containing elements that are selective to the analyte, coupled with an internal reference that provides both sides of the membrane with a constant potential.

AQUANITRA® uses a solid-state ISE, which only differs from a conventional ISE in the absence of a liquid internal reference. In solid-state ISEs the liquid internal reference is replaced with a conducting material, an epoxy resin, which is deposited over the surface of the selective membrane providing a constant potential. The composition of the membrane of the solid state ISE used by the AQUANITRA® analyser (Beltran et al., 2002) can be found in Table 7.3.

Table 7.3 Composition of the selective membrane of the ISE of the AQUANITRA® nitrate analyser (Beltran et al., 2002).

Compound	Composition (%)
Ionophore (Tridodecyl-methyl-ammonium nitrate)	6.5%
Plastifying agent (2-nitrophenyl octyl ether)	61%
PVC (high molecular weight)	32.5%

The relation between the concentration of the analyte and the potential (E) detected by electrodes based on selective membranes is described by the Nickolskii-Eisenman equation (Equation 7.2). This expression is based on the Nernst equation (Equation 7.1) with an added term that allows the quantification of the interference of the different ions present in the sample matrix. E^0 is the cell constant; R is the universal gas constant (R=8.314 $J \cdot K^{-1} \cdot mol^{-1}$), T is the absolute temperature (K), z is the number of electrons transferred in the cell reaction or half-reaction and F is the Faraday constant (F=9.648 $C \cdot mol^{-1}$) ; $K_{x,y}^{pot}$ is the potentiometric selectivity coefficient and can be described as the ratio analyte/interference below which 100% of the response of the ISE is due to interference; a_x and a_y are the activities of the analyte and the interferent ion; z_x and z_y are the charges of the analyte and the interfering ion.

$$E = E^0 \pm \frac{RT}{zF} \cdot \log(a_x) \quad \textbf{7.1}$$

$$E = E^0 \pm \frac{RT}{zF} \cdot \log\left(a_x + \sum_y K_{x,y}^{pot} \cdot a_y^{z_x/z_y}\right) \quad \textbf{7.2}$$

Activity (a_x, a_y) can be directly related to the ion concentration by means of the activity coefficient (f_i) through the Debye-Hückel equation (Equation 7.3), where z_i is the charge of each ion and I is the ionic strength, which can be calculated by means of Equation 7.4, and c_i is the ion concentration.

$$\log f_i = \frac{0.51 \cdot z_i^2 \cdot \sqrt{I}}{1 + \sqrt{I}} \quad \textbf{7.3}$$

$$I = 0.5 \cdot \sum c_i \cdot z_i^2 \quad \textbf{7.4}$$

The electrical signal generated by the nitrate ions in the selective electrode is compared to that of a reference electrode (AgCl/Ag) giving a potential that is then converted into a concentration value using the calibration curve obtained from calibration with three nitrate standard solutions. The electrical potential due to the presence of nitrate is compared to the base line potential given by the carrier and the reagent modifier.

7.3.2.2 Interference

The use of ISEs makes it necessary to study the possible interferences derived from the use of a potentiometric method. Although the selectivity of these sensors is high, they are not specific, and thus the use of complex culture media may have a negative effect on the performance of the ISE.

Interference from the different ions present in the culture medium fed to CIII (Table 7.2) was studied for each of the analysers. Interference is usually due to ions that have the same charge as the analyte (Sandblom et al., 1967; Egorov et al., 1997). In addition to the compounds in Table 7.2, NO_2^- from partial oxidation is also expected to interfere with the detection of NO_3^- and interference from HCO_3^- is very likely to be present due to the further addition of Na_2CO_3 by the pH control system. The interference of some of these species was studied during the characterisation process of the nitrate analyser and will be addressed in further sections of this chapter.

7.3.2.3 Reagents

The use of a potentiometric method and more so, the use of a solid state electrode, reduce the maintenance requirements of the analyser considerably. The main reagents necessary for the operation of the on-line nitrate analyser AQUANITRA® are listed below and were prepared following the indications of the manufacturer's instructions (AQUATEC S.A., 2002).

De-ionised water: used as the carrier continuously flowing through the system during an analysis cycle.

Modifier reagent (Na_2SO_4): The modifier reagent is a solution with a high buffer capacity as well as a high ionic strength. The use of a highly concentrated Na_2SO_4 increases the ionic strength of the sample and makes it possible to establish a linear dependence of the measured electric potential on the nitrate concentration. The concentration and pH of this solution are two of the main parameters that need to be set in order to achieve a good performance of the on-line nitrate analyser. The concentration and pH of this solution were used to minimise the effect of possible interferences such as HCO_3^-, one of the main interferences encountered in the studied system. The reagent is prepared by dissolving 14.25 g of Na_2SO_4 in 1 L of MilliQ water. The pH value must be adjusted to 2.3 by means of H_2SO_4. The final solution should contain in addition 1 mg $NO_3^-{\cdot}L^{-1}$

Nitrate standard solution: three standard solutions are required, whose concentrations are selected according to the required range of analysis.

7.3.2.4 Sample analysis and calibration

Before the start of normal operation with the nitrate on-line analyser a calibration cycle needs to be carried out. This procedure takes place automatically and consists of a three-point standard calibration performed with a frequency that can be adjusted in the main display of the analyser. The standard solutions are selected in such a way that the expected range of sample concentrations is contained within this range.

An analysis cycle starts with the sample being injected into the system in order to replace the sample remaining from previous analyses. Periods of time within 0 and 600 seconds can be selected for this purpose, and a value for this parameter should be selected according to the set-up of the sampling loop.

A detailed scheme of the analyser and its main components is presented in Figure 7.3. During operation of the analyser a continuous stream of de-ionised water flows through the system while the standards and the sample are switched to the main carrier flow by means of different electro valves. The carrier flow (de-ionised water) containing the injected sample is subsequently pumped to a mixing chamber, where a flow of modifier reagent (Na_2SO_4) is incorporated. The concentration and pH of this solution are selected in such a way that the buffer capacity is sufficient to avoid the effect of interfering species. The flow then goes through a de-bubbling chamber, designed to remove any gas from the liquid flow that might lead to incorrect measurements when the sample reaches the detection system.

The analysis cycle starts with the main flow, which contains only the carrier (de-ionised water) and the modifier reagent (Na_2SO_4), going through the nitrate ISE and then through the reference electrode. Under these conditions the potential between the two electrodes is measured and sent to the microprocessor, which stores it as the base line potential. After this elapse of time, known as stabilisation time, the sample is switched into the system by means of a valve, after which it reaches the mixing chamber and subsequently becomes part of the main flow. The homogenised flow finally flows through a de-bubbling chamber, where CO_3^{2-} and HCO_3^- present in the sample are removed as CO_2.

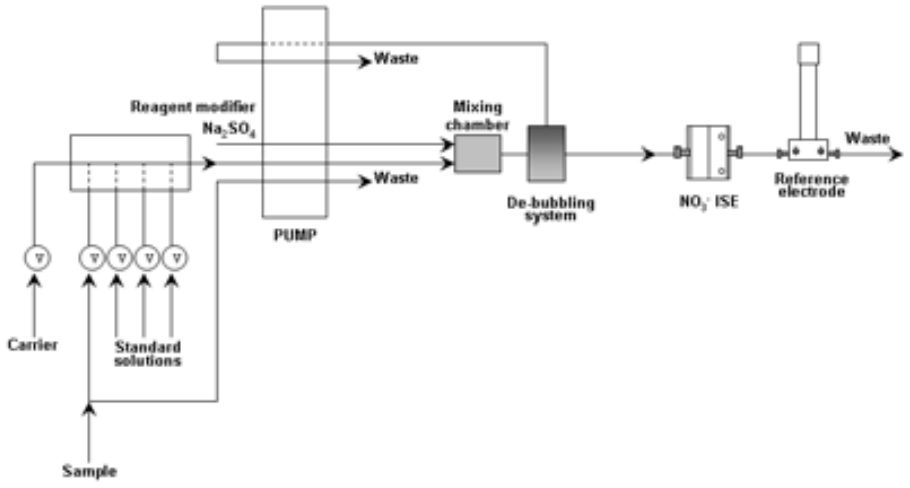


Figure 7.3 Flow diagram of the on-line nitrate analyser (Aquanitra®). Description of the main components.

The concentration of the samples is obtained as a function of the measured potential peaks by means of Equation 7.5 where: V is the peak height in mV, K_1 is the y-axis incercept in mV and K_2 is the slope of the calibration curve in $mV \cdot dec^{-1}$.

The concentration value thus obtained is expressed in $mg \cdot L^{-1}$. The conversion between measured potentials and concentration is automatically performed by the analyser with the information obtained from the calibration cycle.

$$C = 10^{\frac{V - K_1}{K_2}} \qquad \textbf{7.5}$$

7.3.3 DESCRIPTION OF THE AMMONIUM ANALYSER (AQUATEC S.A., AQUAMONIA®)

7.3.3.1 Detection system

The ammonium analyser is based on the same detection system as the nitrate analyser i.e. the measurement of the electric potential generated by the ions present in the sample is related to the analyte concentration through the Nickolskii-Eisenman equation (Equation 7.2). The composition of the membrane of the solid state ISE used by the AQUAMONIA® ammonium analyser (Alegret et al., 1989) is described in Table 7.4.

Table 7.4 Composition of the selective membrane of the ISE of the AQUAMONIA® ammonium analyser (Alegret et al., 1989).

Compound	Composition (%)
Ionophore	1%
Plastifying agent	67%
PVC (high molecular weight)	32%

7.3.3.2 Interference

The main species known to interfere with the NH_4^+ ISE are ions such as K^+ and Na^+, which have the same charge as the analyte. However, the configuration of the AQUAMONIA® on-line analyser was designed to avoid any interference. The sample is treated with a highly alkaline solution that causes the conversion of NH_4^+ into NH_3, and the consequent separation from the dissolved single charged positive ions. The gaseous NH_3 then flows through a selective membrane, after which the pH is decreased again and the analyte is subsequently detected by the ISE as NH_4^+.

7.3.3.3 Reagents

The main reagents necessary for the operation of the on-line nitrate analyser AQUAMONIA® are:

De-ionised water: the sample is injected onto a de-ionised water flow that acts as a carrier.

NaOH: A 1 N solution of NaOH was prepared by dissolving 40 g of NaOH in 1 L of MilliQ water. The role of this reagent is to increase the pH of the solution so that NH_4^+ is transformed into NH_3 and can flow through the gas selective membrane, leaving interference behind.

Tris-hydroximethyl-amino-methane: A 0.01M solution of Tris at pH 7.4-7.5 was prepared by dissolving 1.21 g of Tris in 900 mL of MilliQ water and subsequently setting the pH at a value between 7.4 and 7.5 with a solution of 50% HCl. The Tris solution is used to decrease the pH again once the alkaline solution containing the gaseous NH_3 has crossed the membrane, so that the NH_4^+ present in the sample can be detected by the ammonium ISE.

Ammonium standard solutions: two ammonium standards carefully selected according to the expected concentration of the sample are necessary to perform the calibration of the analyser.

7.3.3.4 Sample analysis and calibration

Calibration of the Aquamonia® ammonium analyser is carried out by means of two standard solutions of different concentrations. During calibration the standard solution with the lowest concentration is first injected into the system and the peak of potential is recorded by the microprocessor, as well as the maximum potential given by the sample and the second standard solution. The concentration of the samples can then be computed from the parameters obtained by this calibration procedure.

When a sample analysis is programmed to start, the analyser automatically opens the valve that supplies the carrier flow to the system and switches on the peristaltic pump so that deionised water, the reagent modifier (0.01M Tris-hydroximethyl-amino-methane with a pH=7.48) and 1M NaOH are pumped into the system. A flow diagram of the analyser is shown in Figure 7.4.

After the sample is injected into the carrier, the flow is mixed with the sodium hydroxide and it circulates counter-current with the Tris solution through a diffusion chamber containing a gas selective membrane. During this first stage of the analysis the potential between the ion selective electrode and the reference electrode is measured and will constitute the baseline potential to which the signal given by ammonium is compared when the sample flows through the system.

After the stabilising time (set on the analyser display), the valve switches to allow the sample flow into the system. As the sample is mixed with the sodium hydroxide, the pH increases all the dissolved ammonium turning into ammonia. Part of this gas crosses the gas permeable membrane and, as it flows through the diffusion chamber, it is dissolved in the Tris solution (pH=7.48) and recovers its ionic form, which can be detected by the electrode. The voltage between the reference electrode and the ion selective electrode increases as the sample containing the analyte (ammonium) reaches the detection system. The microprocessor records the maximum achieved value of voltage between the two electrodes, which can be visualised as a peak of voltage in the analyser screen. After the injection time the valve switches back to the de-ionised water position so that the baseline is recovered.

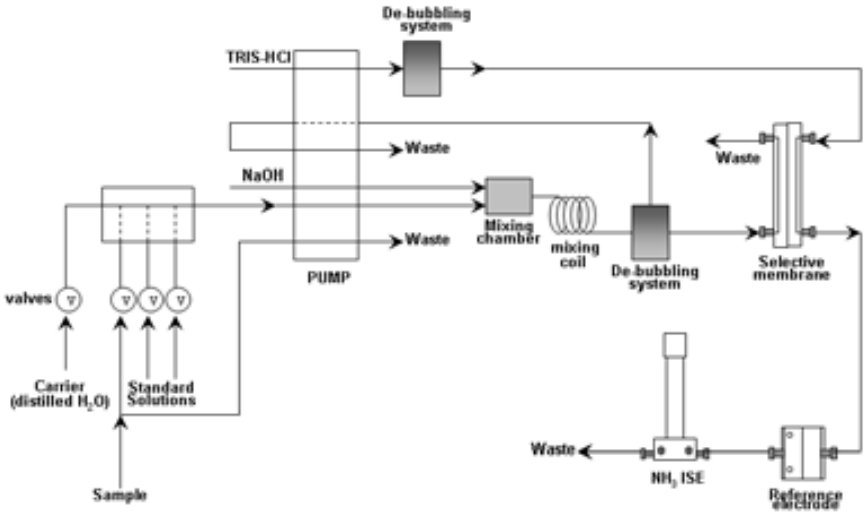


Figure 7.4 Flow diagram of the on-line nitrate analyser (Aquamonia®). Description of the main components.

In order to calculate the concentration, the microprocessor requires a calibration procedure, which in this analyser is carried out by a two-point standard calibration. The standards used for this calibration will be selected in such a way that the interval of concentrations contains the expected concentration range of the samples. The concentration of the samples can be calculated by means of Equation 7.6, where C stands for the NH_4^+ concentration in $mg \cdot L^{-1}$; V represents the peak height in mV; K_1 is the y-intercept in mV; K_2 is the slope of the calibration curve in $mV \cdot decade^{-1}$. As in the nitrate on-line analyser these units indicate that, by using this modified constant, the concentration is directly obtained in $mg \cdot L^{-1}$, instead of obtaining the logarithm of the concentration and subsequently calculating the concentration value from it.

$$C = 10^{\left(\frac{V-K_1}{K_2}\right)} - f \qquad \textbf{7.6}$$

Finally, the parameter f ($mg \cdot L^{-1}$) describes the loss of lineality of the ISE response at low concentrations (Equation 7.7). When the analyser was developed, the possibility to fit this parameter from experimental data was considered (Barquero, 2001). However, it would have been necessary to use a third standard and thus increase the calibration time of the analyser to estimate this parameter. Eventually it was concluded that this parameter could be estimated as follows by taking into account the properties of a FIA system concerning relative measurements.

$$f = 10^{-\frac{K_1}{K_2}} \qquad \textbf{7.7}$$

7.3.4 MAINTENANCE REQUIREMENTS OF THE ION SELECTIVE ELECTRODES

As previously discussed, optimal performance of an ion selective electrode is defined by several parameters, the most important being: (i) low detection limit, (ii) response time and (iii) precision. The evolution of these parameters over the lifetime of the electrode can be used to define a protocol for the replacement of the electrodes. Due to the operation of the ammonium and nitrate analysers being based on the interpolation of potential measurements (mV) in a calibration curve, the calibration procedure has a very important role in the correct performance of the analyser.

As established by the Nickolskii-Eisemann equation (Equation 7.2), the slope of the calibration curve has a constant value that gradually decreases because of continuous operation of the analysers, eventually leading to a loss of sensitivity in the concentration measurements. The adaptation of this equation to the specific operation of the analysers AQUANITRA® and AQUAMONIA® is expressed by Equations 7.5 and 7.6 respectively (AQUATEC S.A., 2001).

Electrodes should be replaced when the slope of the calibration curve reaches a certain value that is established according to the accuracy requirements of the application they are used for and to the recommendations given by the supplier. In the case of CIII of the MPP, the analysers are used for control purposes, and thus a high sensitivity is essential.

The protocol defined for the replacement of the ion selective electrodes was thus based on two constraints: (i) the slope of the calibration curve that establishes

the sensitivity of the concentration measurements and (ii) the maximum effective operation time of the electrode. The values for these parameters were based on the recommendations given by the analyser supplier and were modified during the characterisation process in order to fulfil the requirements of operation in the MPP (i.e. more conservative values were adopted to ensure accuracy of the results). Following this protocol, the constraints were set for the correct performance of the ammonium and nitrate ISEs (Table 7.5). The ISEs should be replaced as soon as either the slope of the calibration curve or the maximum time established for each electrode in Table 7.5 is exceeded.

The ammonium analyser has lower maintenance requirements concerning the ion selective electrode than the nitrate analyser due to the use of a membrane that prevents damaging the electrode. However, the membrane should be replaced once every week to secure optimal performance of the equipment and the electrode.

Table 7.5 .Range of the calibration curve slope and electrode lifetime to guarantee the correct performance of the ammonium and the nitrate on-line analysers.

Analyser	Slope calibration curve (K_2)	Electrode lifetime
Ammonium	$40<K_2<80$	6 months
Nitrate	$45<K_2<65$	2 months

7.3.5 NITRITE ANALYSER (APPLIKON® ANALYTICAL, ADI 2019 HD)

7.3.5.1 Detection method

All the commercial analysers evaluated during the trade-off that led to the final selection of an on-line nitrite analyser were based on the implementation of the same analytical method used for the detection of low nitrite concentrations i.e. the azo-dye standard method (Clesceri et al., 1998).The main difference between the evaluated analysers was the analytical technique used for on line implementation of the chemical detection method, rather than the detection system they use.

The azo-dye method determines NO_2^- through the formation of a reddish purple azo-dye, produced under acidic conditions (pH 2.0 to 2.5), by coupling diazotised sulphanilamide with N-(1-naphtyl)-ethylenediamine dihydrochloride (NEDD) according to the reaction depicted in Figure 7.5.

This method allows the detection of nitrite concentrations that range from 10 to 1000 µg $N\text{-}NO_2^- \cdot L^{-1}$.

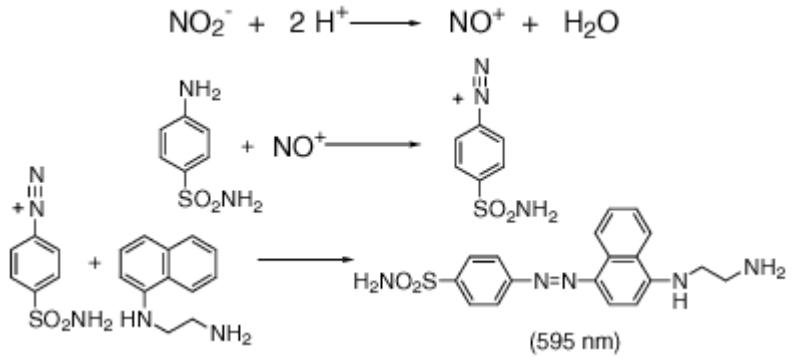


Figure 7.5 Reactions leading to the formation of the detectable azo-dye.

7.3.5.2 Interference

The main dissolved compound known to cause interference with this method is NCl_3, which imparts a false red colour when the colour reagent is added to the sample. In addition, the following ions interfere because of precipitation under test conditions: Sb^{3+}, Au^{3+}, Bi^{3+}, Fe^{3+}, Pb^{2+}, Hg^{2+}, Ag^{+}, chloroplatinate ($PtCl_6^{-}$) and metavanadate (VO_3^{-}). None of these compounds is provided in the feed medium of CIII and thus interference is very unlikely to happen. However, several off line tests were performed to evaluate the performance of the analyser with samples containing all the compounds added to the CIII feed medium.

Any suspended solids present in the sample should also be removed by filtration through a 0.45 μm-pore-diameter membrane filter to avoid interference when the sample reaches the detector.

7.3.5.3 Reagents

To ensure an accurate performance of the analyser it is important to take into account the preparation protocol as well as the storage conditions applicable to each one of the reagents and update the reagent solutions accordingly (Clesceri et al., 1998).

NEDD Colour reagent: An aqueous solution is prepared by adding 100 mL of 85% phosphoric acid and 10 g of sulphanilamide to 800 ml of water. After completely dissolving the sulphanilamide, 1 g of NEDD and water are added to reach a total volume of 1L. The NEDD colour reagent solution is stable for approximately one month when stored in the dark at 4ºC.

Sodium Oxalate: 0.025M sodium oxalate is prepared by dissolving the required weight of $Na_2C_2O_4$ in 1000 mL of de-ionised water.

Ferrous ammonium sulphate: 0.05M ferrous ammonium sulphate is prepared by dissolving 19.607g $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ and 20 mL of concentrated H_2SO_4 in water and diluting it to a total volume of 1000 mL.

Stock nitrite solution: A stock solution of nitrite must be prepared and standardized. This stock solution is used to prepare standard solutions used to calibrate the analysers.

7.3.5.4 Sample analysis and calibration

The tested nitrite analyser (Applikon® Analytical, ADI 2019 HD) had been adapted for the detection of high nitrite concentrations, up to 20 mg $N\text{-}NO_2^- \cdot L^{-1}$. This level of nitrite is above the detection range of the standard method described in section 7.3.5.1, and thus a sample injection loop is used that makes it possible to inject small amounts of sample (sampling loops of 0.2 mL and 0.5 mL were tested) that are diluted using an automatic dilution system. The sample is introduced to the analyser by means of an injection loop. This configuration reduces considerably the amount of sample consumed by the nitrite analyser when compared to other techniques such as Flow Injection Analysis (FIA).

The experimental layout of the ADI 2019 HD nitrite analyser is presented in Figure 7.6. The analyser operates batchwise with the chemical reaction taking place in the colorimetric cell that houses the mixing cuvette, the thermostat, a LED and the detector. This configuration allows high precision additions as well as low reagent and sample consumption. Addition of such a small amounts of sample is made by installing an injection loop of the required volume, which is pumped into the mixing cuvette where the reagents will be added. Dilution water can be added either with a micro burette or with a peristaltic pump if the volume of dilution water that needs to be added is larger.

Physically, the analyser consists of several modules (Figure 7.6) that are assembled according to the required application. Reaction and the subsequent colorimetric measurement take place in the cuvette module, which consists of a reaction cell (cuvette), placed in a holder, where the analytical reagents and the sample are added by means of different wet part modules such as pumps, pipettes or burettes. The analyser uses a LED as a light source. The limited-bandwidth light is partly absorbed by the solution and a photo-detector is then used to detect the amount of light not absorbed by the solution, which is correlated with the nitrite concentration present in the sample.

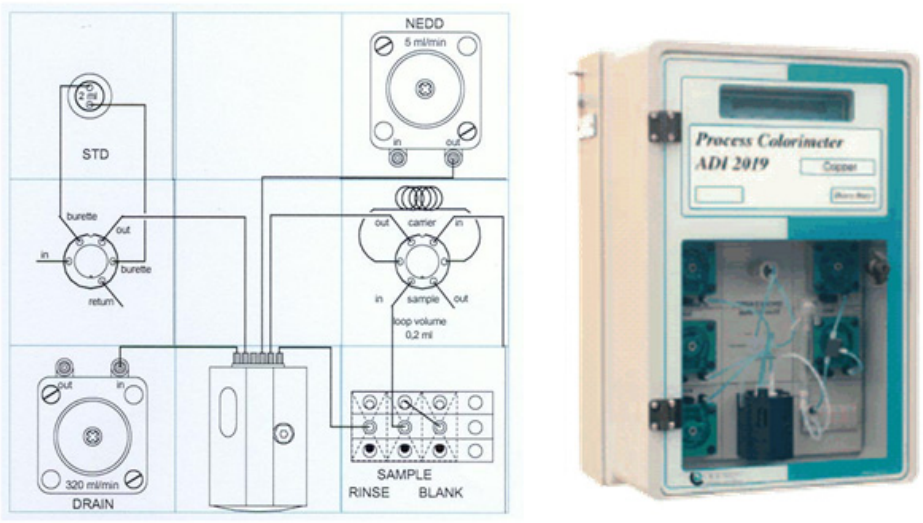


Figure 7.6 Layout of the ADI-2019 heavy duty process analyser used to perfom preliminary tests at the MPP.

Regular calibration of the analyser with nitrite standard solutions needs to be performed periodically, depending on the conditions under which the analyser is operated. The nitrite standard solution used for calibration has to be prepared from a nitrite stock. The stock solution should be prepared with fresh reagent, to ensure the absence of moisture, and should be protected from contact with air. Standardization of the stock solution should be carried out before preparing the standard solution to determine the NO_2^- content. The number of standards used to calibrate the analyser can be selected depending on the requirements of the analysis. Standard solutions of different concentrations are prepared by automatic dilution of a single standard by means of a burette module, allowing the performance of automatic calibration at pre-set intervals.

The analysis cycle starts with the sample being supplied to the cuvette module by the sampling system by means of a rotary type valve that has two positions. The valve either allows the sample to flush the sample loop or switches a precise amount of sample to the reaction cell. Dilution water is added at this point by means of a peristaltic pump or a pipette (as previously mentioned, depending on the required precision). The analyser configuration used in the tests presented in this chapter was adapted for the measurement of nitrite concentration within a wide range (0-20ppm) and hence the selection of a peristaltic pump as an addition system instead of a pipette. Reagent solution was added to the cuvette by means of a peristaltic pump. Rinsing and draining the cuvette module between sample analyses were also carried out by two peristaltic pumps.

7.4 RESULTS AND DISCUSSION

The selected ammonium and nitrate analysers, as described in section 7.3, use solid state ISEs as the detection system, considerably reducing the reagent consumption and the maintenance requirements of the analyser. Their performance was carefully tested and optimised and the results will be presented in the following sections of this chapter.

7.4.1 CHARACTERISATION OF THE AMMONIUM AND NITRATE ANALYSERS

Due to the complexity of the matrix of the feed solution of CIII, there is a great deal of possibility that some of the components of this medium may interfere with the ISEs. Therefore, off-line testing was carried out using samples with different known concentrations of ammonium and nitrate in order to characterise the performance of the analysers. The potential interferences of the ammonium and nitrate on-line analysers have been listed in previous sections of this chapter. The actual compounds causing intereference will be identified for each analyser and the levels leading to interference will be quantified. In addition, a solution will be provided if necessary to avoid their effect guaranteeing the correct performance of the analysers in their presence.

7.4.1.1 Ammonium analyser

The feed medium used in CIII contains several salts with single positive charges (Na^+, K^+) that are likely to interfere with the NH_4^+ analysis, thus allowing the assessment of potential interference. However, the configuration of the ammonium analyser was designed to avoid the effect of interference. The ammonium (NH_4^+) dissolved in the sample is converted to NH_3 gas by increasing the pH with a solution of NaOH, and after flowing through a gas membrane the gas is dissolved in an acid solution so that the NH_4^+, free of interference, can be detected by the ammonium ISE. To evaluate the efficiency of this configuration and the initial settings programmed in the analyser and presented in Table 7.6, a series of tests was carried out. The tests were performed over two different concentration intervals: 10-50 mg N-$NH_4^+ \cdot L^{-1}$ (Figure 7.7) and 0.1-10 mg N-$NH_4^+ \cdot L^{-1}$ (Figure 7.8). The samples used in these tests contained different concentrations of nitrite dissolved in a solution with the same composition as the feed medium used in CIII (Chapter 3 , Table 3.1).

The analyser was operated for a period of approximately two hours with each solution (with a frequency of analysis of 16 minutes, 8 analysis were performed for each sample over 2 hours). For operation in the higher range the analyser was calibrated using standard solutions of 10 and 50 mg $N\text{-}NH_4^+ \cdot L^{-1}$ dissolved in distilled water. In the lower concentration range, the two standard solutions used for calibration contained 1 and 10 mg $N\text{-}NH_4^+ \cdot L^{-1}$ respectively.

Table 7.6 Set up of the different parameters of the ammonium analyser during preliminary off-line tests performed with standard samples.

PARAMETER	VALUE
Injection time	11 s
Injection Volume	300 µL
pH conditioning solution	7.48
Concentation of TRIS	0.01 $mol \cdot L^{-1}$

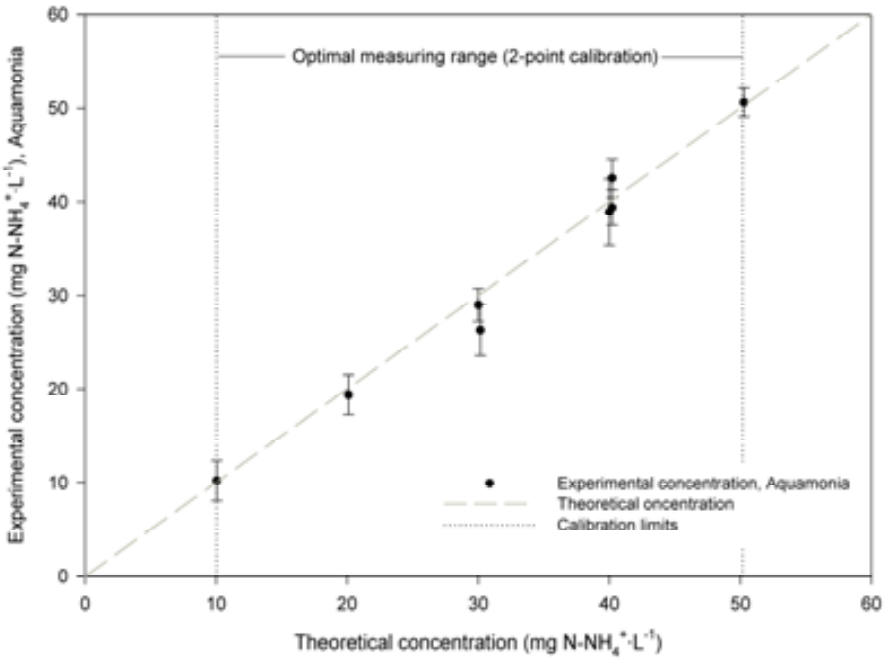


Figure 7.7 Off-line experiments performed with AQUAMONIA® using a solution with the same composition as the feed medium as a solvent, instead of water. Calibration standards 10 and 50 mg $N\text{-}NH_4^+ \cdot L^{-1}$.

The repeatability of the measurements, in terms of relative standard deviation, has a maximum value of 3.5%, with the most common value being around 2% for both concentration ranges. The accuracy of the measurements is between 0.7% and 5.5% of deviation from the theoretical concentration in the higher range and slightly higher (between 1.5% and 14%) in the low range.

During these tests no interference was detected. However, this preliminary evaluation underscored the importance of a good selection of both standard

solutions used for calibration.The calibration range should be adjusted according to the expected range of concentration expected in the sample. The two standard solutions used for calibration should not differ by more than one order of magnitude. Later testing periods proved the good accuracy and repeatability of this analyser at concentration ranges below 1 mg $N\text{-}NH_4^+ \cdot L^{-1}$ during normal on-line operation with the reactor

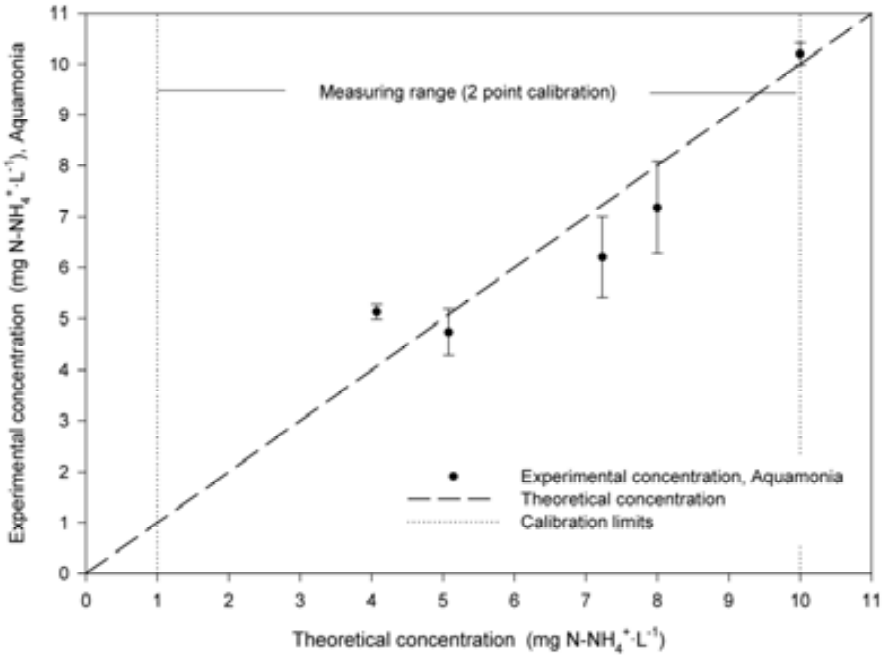


Figure 7.8 Off-line experiments performed with AQUAMONIA® using a solution with the same composition as the feed medium as a solvent, instead of water. Calibration standards 1 and 10 mg $N\text{-}NH_4^+ \cdot L^{-1}$.

7.4.1.2 Nitrate analyser: evaluation and compensation of interference effect on the nitrate ISE

In order to identify interference from different species, the total amount of each one of the compounds as well as effect of pH must be taken into account. The nitrate analyser has been developed to avoid interference from the most common ionic species known to interfere with the nitrate ISE: nitrite (NO_2^-) or bicarbonate (HCO_3^-).

The HCO_3^- concentration cannot be maintained within a constant range of values during reactor operation, due to the additions of Na_2CO_3 carried out by the pH control system. The addition is not constant and it depends on the operational conditions of the reactor, such as ammonium load or flow rate, which determine the quantities of acid (CO_2) or base (Na_2CO_3) required to maintain the pH in the optimal range of 8.0-8.2.

In order to minimise HCO_3^- interference, the pH of the Na_2SO_4 modifier reagent was set to 2.3, which according to Figure 7.9 minimises the presence of HCO_3^- in the carbonate equilibrium. Under these conditions, the estimated value of the selectivity coefficient ($K^{pot}_{NO_3^- - HCO_3^-}$, Equation 7.2) was 0.061. This value of the selectivity constant leads to a maximum admissible ratio between HCO_3^- and $NO_3^-$of 3 mg HCO_3^-/ mg N-NO_3^- in order for the relative error of the measured nitrate concentration to remain under 5%.

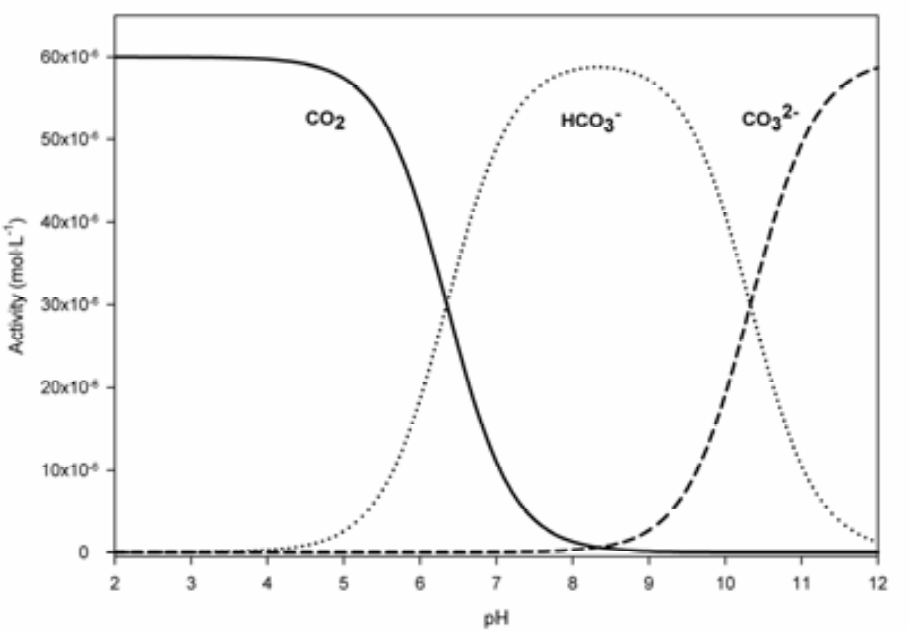


Figure 7.9 Dominance (Bjerrum) plot of carbonate species in solution as a function of pH, at temperature 28ºC and P=1atm.

On the other hand, interference due to nitrite was not expected to be significant, as its concentration usually remains below 10 mg N-NO_2^-·L^{-1} during normal operation of CIII. Results from previous studies performed with high nitrite levels and provided by the analyser suppliers (AQUATEC S.A., Spain) concluded that the optimal pH of the conditioning solution (Na_2SO_4) should be set to a value of 4 in order to minimise nitrite interference. When the sample was buffered at pH=2.3 (the optimal value to avoid HCO_3^- interference), the selectivity coefficient ($K^{pot}_{NO_3^- - NO_2^-}$) was 0.55. This value indicates that the nitrate concentration should always remain at least 6 times higher than that of nitrite in order to obtain errors lower than 8%. According to this estimation and assuming the typical range of nitrogen load in the reactor, the highest nitrite concentrations that the samples can contain while still avoiding a deviation > 7% are presented in Table 7.7 alongside the admissible limits of HCO_3^-.

Table 7.7 Bicarbonate and nitrite limits within the normal range of nitrate concentration in CIII. Values obtained with the analyser configuration optimised for the minimisation of the HCO_3^- interference (i.e. pH=2.3).

Nitrate concentration (mg $N\text{-}NO_2^-\cdot L^{-1}$)	Admissible HCO_3^- concentration (mg $HCO_3^-\cdot L^{-1}$)	Admissible NO_2^- concentration (mg $N\text{-}NO_2^-\cdot L^{-1}$)
300	937	66
600	1875	131

Chloride (Cl^-), which is one of the main interferences of the nitrate ISE, was not a big issue in the present application due to the low ratio Cl^-/NO_3^- present in the samples. The concentration of $CaCl_2\cdot 2H_2O$ contained in the culture medium Table 7.2) leads to a concentration of chloride of 0.35 mg $Cl^-\cdot L^{-1}$. The nitrate concentration in the outlet of the nitrifying reactor of the MELiSSA pilot plant is in the range of 270-587 mg $N\text{-}NO_3^-\cdot L^{-1}$, i.e. ca. 10^4 times higher than that of chloride. In these conditions, it is safe to state that, even with a high value of the selectivity coefficient, no significant interference from chloride should be expected.

The selectivity coefficient for different nitrate selective electrodes ranges between $K^{pot}_{NO_3^- - Cl^-} = 0.01$ and 0.03. Assuming a selectivity coefficient of 0.02, the response of the electrode to the chloride ion is 50 times lower than the response due to nitrate, i.e. only when the chloride concentration is 50 times higher than that of nitrate, the response of the ISE would be mainly due to interference. It was estimated that the chloride concentration should be kept below the expected nitrate concentration in order to avoid interference, which was far from the levels reached in the studied system.

7.4.1.3 Optimisation of the operational conditions of the nitrate analyser

During preliminary tests on a prototype analyser, the concentration of the modifier reagent solution was set to 0.5M Na_2SO_4, 10^{-3}M NO_3^- and pH=2.3. Once the nitrate analyser was installed in the premises of the MPP, a new study was conducted with the goal of fine-tuning the concentration of the conditioning solution concentration (Na_2SO_4) and the injection time of the sample.

A long injection time was found to confer higher stability to the electric signal detected by the selective electrode, due to the fact that a higher sample volume was injected and the potential was measured with higher reliability. However, the longer contact time of the solution with the sensor substantially reduced the lifetime of the nitrate ISE.

The tests performed with different values of the injection time showed that an increase of the injection time led to higher deviation from the theoretical concentration of the sample. With a constant Na_2SO_4 concentration of 0.5M, the injection time was progressively decreased from 100 s down to 11 s. An improvement the accuracy of the analysis was observed with lower injection times.

The accuracy of the measurements was also found to improve with increasing Na_2SO_4 concentration. A reagent solution concentration of 1M Na_2SO_4 produced the best results. However, the use of this concentration of sodium sulphate was avoided to prevent problems such as precipitation or clogging of the reference and the NO_3^- ISE due to the high concentration of the salt.

A compromise was finally attained by selecting a Na_2SO_4 concentration of 0.75 M with an injection time of 11 s. Once the optimal conditions had been established, the analyser was tested with real samples from CIII. The values of all required analyser parameters are summarised in Table 7.8.

Table 7.8 Set up of the different parameters of the nitrate analyser concluded during preliminary tests performed with standard samples.

PARAMETER	VALUE
Injection time	11 s
Injection Volume	200 µL
pH conditioning solution	2.3
Na_2SO_4 concentration	0.75M Na_2SO_4 + 10^{-3}M $NaNO_3$

To evaluate the accuracy of the on-line measurements with real samples, the results were compared to a selected reference method (LCK339, Dr. Lange, Germany). The average deviation given by this reference method when working with samples dissolved in fresh nitrifying medium was found to be around 18% (including the error due to required dilution).

This value of the error leads to the confidence interval of 83% presented in Figure 7.10. All measurements performed with the Aquanitra analyser are included within the confidence interval of the reference method. The typical deviation for the results obtained with the on-line nitrate analyser was found to be 6-9%.

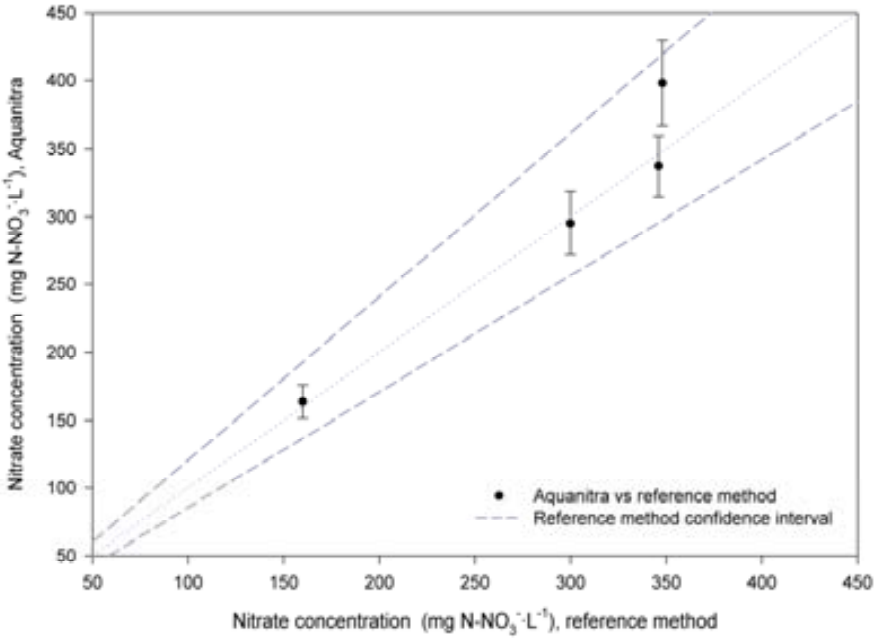


Figure 7.10 Tests performed off-line with real samples from the reactor over different periods of time. The dashed line symbolises the confidence interval (83%) of the reference method (Dr Lange, Germany).

7.4.2 ON-LINE IMPLEMENTATION OF THE AMMONIUM AND NITRATE ANALYSERS

7.4.2.1 Experimental set-up

On-line monitoring of the nitrogen species in the effluent of CIII should guarantee the correct detection of the transient states as well as measuring the concentration under steady state conditions. Therefore, it is essential not only that the values provided by the analysers have a high repeatability and accuracy, but also that the delay between sampling and the performance of the analysis cycle is minimised. Only then does the concentration value provided by the analyser represent an exact picture of the system at sampling time.

The effluent of CIII is obtained with an important fraction of gas, and thus a gas-liquid separation step is required before the sample enters the analysis loop preventing a good performance of the analysers. Modifications in the reactor hardware have been foreseen to overcome these limitations (Chapter 8). However, due to the time consuming start-up process of this packed bed reactor, the testing period of the analysers was carried out with the available hardware and taking special care in not to alter the axenicity in the reactor. A provisional system consisting of a small sterile buffer tank (25 mL) was installed at the outlet of the pilot reactor allowing the separation of the liquid and gas phases (Figure 7.11).

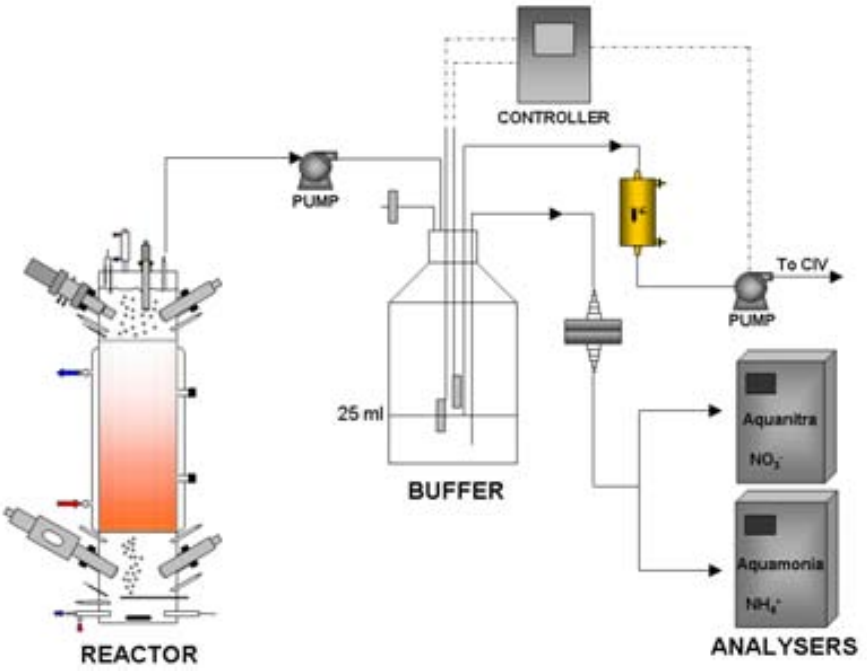


Figure 7.11 Configuration of the sample extraction system and the analysis loop in the effluent of CIII.

In order to obtain a sample free of biomass, once the liquid phase had been separated from the gas phase, a filtration step was performed before the sample was pumped to the analysers. The filtration step was intended to keep the axenic conditions in the reactor and, at the same time, to avoid biomass growth and consequent degradation in the tubing of the analysis loop. Initially an Opticap® disposable filter (Millipore, USA) filter with a pore size of 0.22 μm was used. However, the high liquid retention in this filter (approximately 75 mL) incorporated a very high dead time in the filtration step. Therefore, the 0.22 μm filter was replaced with a filtration unit consisting of an autoclavable 5 mL Nalgene polysulfone filter holder (Thermo Fisher Scientific, UK) coupled with a 0.22 μm filter (Millipore, USA) that was replaced periodically, under axenic conditions, in order to avoid clogging (Figure 7.12). The filtration cell was duplicated to allow its replacement when necessary without altering the frequency of the analysis cycles.

The liquid sample thus obtained from the gas-liquid separator is subsequently filtered before entering the analysis loop. In order to avoid cross contamination between samples the analysis loop is flushed with new sample before a new analysis cycle is started. However, the sample consumption needs to be minimised.

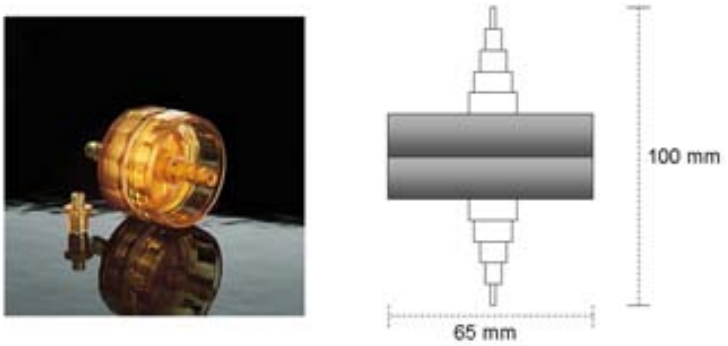


Figure 7.12 Filter used to test the performance of the analysers connected on-line to the pilot reactor of CIII. The set-up was conceived as a provisional way of minimising the dead time in the analysis circuit.

7.4.2.2 Sample consumption and dead time estimation

Sample consumption is a critical issue due to the use of the effluent of CIII in the subsequent compartments of the MELiSSA loop. Besides, the experimental concentration data provided by the ammonium and nitrate analysers will be used for control purposes and thus it is essential that these values are representative of the measured state at a certain sampling time i.e. the dead time must be minimised. In order to minimise the dead time as well as the sample consumption it is essential to decrease the dead volumes in the sampling loop as much as possible by reducing the diameter of all tubing and minimising the distance between the sampling point and the analysers. By solving the mass balances in the reactor and in the different elements of the sampling loop, the response time of the analysis system can be estimated. The total sample requirements both for the ammonium and for the nitrate analysers, including the volume required to replace the remaining sample from the previous analysis, are summarised in Table 7.9. The sample requirements are affected by the settings of a number of parameters that will need to be tuned according to the system demands:

- **Initial time:** During this period of time prior to the actual analysis, sample flows through the analysis loop in order to replace the remaining sample from the previous analysis cycle. During this elapse of time the sample does not reach the detection system (i.e. the ammonium or the nitrate ISE).
- **Stabilising time:** de-ionised water flows through the analyser in order to stabilise the electrode before each analysis. This step ensures that the baseline is correctly set-up when a new analysis starts.
- **Injection time:** the sample is injected into the detection system in order to obtain the maximum potential value from which the concentration is computed.

It is important to optimise these different time parameters to achieve the best possible performance. While the initial time has been set to the highest possible value in order to replace the sample remaining in the system, it is also important to take into account that, as the injection time increases, there is a negative effect on the lifetime of the electrode as well as a significant increase on the sample consumption, both of which should be avoided.

Table 7.9 Settings of the main parameters of the on-line ammonium and nitrate analysers. Sample and reagent consumption.

AQUANITRA		AQUAMONIA	
Total analysis time	16 min	Total analysis time	16 min
Initial time	5 min	Initial time	5 min
Stabilisation time	4 min	Stabilisation time	4 min
Injection time	11 s	Injection time	11 s
Time elapsed between samples	4 min	Time elapsed between samples	4 min
Total sample volume/ analysis	6 mL/ analysis	Total sample volume/ analysis	9.5 mL/ analysis
Na_2SO_4	7 mL/ analysis 21 mL/ calibration	TRIS-HCl	4 mL/ analysis 16 mL/ calibration cycle
Deionised water	7 mL/ analysis 21 mL/ calibration	Deionised water	8 mL/ analysis 32 mL/ calibration cycle
Nitrate standards	0.5 mL/calibration	NaOH	2 mL/ analysis 8 mL/ calibration
		Ammonium standards	7.5 mL/calibration

The residence time in the buffer tank used for phase-separation purposes is determined by the flow rate at which the nitrifying reactor is operated, and thus the dead time in the buffer tank can be estimated as a function of the volume of buffer tank used a gas-liquid separator (V_{buffer}) and the flow rate in CIII (Q_{CIII}) according to Equation 7.8. Taking into account the normal range of flow rate in the reactor (0.15 L·h-1-0.6 L·h-1) and using a 25 mL buffer tank, the value of the dead time in the gas-liquid separation step ranged between 10 min and 2.5 min.

$$\tau_{buffer} = \frac{V_{buffer}}{Q_{CIII}} = \frac{V_{buffer}}{Q_{CIVa} + Q_{analysers}} \qquad \textbf{7.8}$$

In order to evaluate the dead time due to the sample filtration step, the total sample flow rate pumped through the two analysers during the analysis cycle was calculated. For this calculation it was assumed that the analysis cycles of both analysers took place simultaneously and that the analysers were operated with the configuration presented in Table 7.9. By solving the mass balances in the filter the delay due to the filter could be calculated (Equation 7.9).

$$\tau_{filter} = \frac{V_{filter}}{Q_{analysers}} \qquad \textbf{7.9}$$

The volume of all the tubing used to carry the sample from the reactor outlet to the analyser must also be estimated so that its dead time can be taken into account. As previously mentioned, one of the ways to reduce the dead time is by minimising the distance between the sampling point and the analyser. The dead time due to the capillary tubing connecting the analyser to the reactor outlet can be calculated by means of Equation 7.10, where A_S is the cross sectional area of the capillary tubing connection; L is the total length of the tubing and $Q_{analysers}$ is the total sample flow rate required by the two analysers operating simultaneously.

$$\tau_{tubing} = \frac{A_S \cdot L}{Q_{analysers}} \qquad \textbf{7.10}$$

The results of the dead time estimation in the different elements of the analysis loop are presented in Table 7.10. The total dead time with the configuration of the sampling loop used in this preliminary study ranged between 4.4 min and 11.9 min. Improvements are foreseen in the reactor liquid sampling system (Chapter 8) in order to minimise the dead time in the sampling loop and the sample consumption. Therefore, it can be stated at this point that on-line monitoring of both ammonium and nitrate in the effluent of CIII has been satisfactorily resolved.

Table 7.10 Description of the main elements of the connection between the reactor and the nitrogen analysis loop.

Element	Flow rate ($mL \cdot min^{-1}$)	Liquid volume (mL)	Dead time (min)
Reactor	2.5 – 10	3800	-
Ammonium analyser	1.8	-	-
Nitrate analyser	1.15	-	-
Gas-liquid separator	2.5 – 10	25	10 – 2.5
Filter	2.95	5.1	1.7
Tubing (D_i=0.4 mm)	2.95	0.5	0.17

7.4.3 VALIDATION OF THE NITRITE ESTIMATOR

During the early stages of its development, the nitrite predictor performance had been validated with experimental off-line measurements of ammonium and nitrate concentration obtained during a set of experiments performed with different levels of oxygen limitation, i.e. the performance during a period of high nitrite accumulation was tested.

After the installation of the ammonium and nitrate on-line equipment, a second validation process became necessary to evaluate the performance of the predictor in this new scenario. The predictor was validated by imposing a set of different disturbances in process variables. The performance of the ammonium and nitrate analysers was then studied and the suitability of the results for use in conjunction with the nitrite predictor was evaluated.

7.4.3.1 On-line analysers settings

The main operating parameters of the analysers (frequency of analysis, injection volume, injection time, concentration of the different reagents) used during this set of experiments are the result of previous studies performed off-line in the MPP to characterise their performance in the required operational conditions (section 7.4.1). The values that were eventually assigned to the different parameters are summarised in Table 7.11. Other parameters such as the stabilisation time or the initial time, required to remove the sample from previous analyses and to stabilise the baseline, are subject to change depending on the final configuration of the CIII hardware and the sampling loop i.e. the distance between the reactor and the analysers once installed in their final position in the new location of the MPP.

Table 7.11 Optimised operating conditions for the ammonium and nitrate on-line analysers.

AMMONIUM ANALYSER		NITRATE ANALYSER	
Frequency of analysis	16 min	Frequency of analysis	16 min
Injection Volume	300 µL	Injection Volume	200 µL
Injection Time	11 s	Injection Time	11 s
pH conditioning reagent (TRIS)	7.48	pH conditioning reagent (Na_2SO_4)	2.3
Concentration TRIS	0.01 mol·L^{-1}	Concentration Na_2SO_4	0.75 mol·L^{-1}

7.4.3.2 Validation tests

The validation of the nitrite predictor with the measurements provided by the ammonium and nitrate on-line analysers was carried out during a late stage of the reactor operation, approximately 4 years after start up. The steady state attained in the reactor after long term continuous operation provides a suitable scenario for the validation of the nitrite predictor in conditions of low nitrite accumulation.

Two main parameters are changed in the set of experiments proposed for the validation of the nitrite predictor: (i) the ammonium input concentration and (ii) the residence time used in the reactor.Three stepwise increases in flow rate were performed (3.6 L·d^{-1} to 6 L·d^{-1}, 6 L·d^{-1} to 9.6 L·d^{-1} and 9.6 L·d^{-1} to 14.4 L·d^{-1}) followed by one step down (from 14.4 L·d^{-1} back to 3.6 L·d^{-1}). A constant ammonium

concentration in the feeding medium of 300 mg $N\text{-}NH_4^+ \cdot L^{-1}$ was maintained during these experiments. Following the steps in flow rate, and keeping the input flow rate constant at 3.6 $L \cdot d^{-1}$, a step in the input ammonium concentration was done from the initial concentration of 300 mg $N\text{-}NH_4^+ \cdot L^{-1}$ up to 600 mg $N\text{-}NH_4^+ \cdot L^{-1}$. The steps described above are depicted in Figure 7.13.

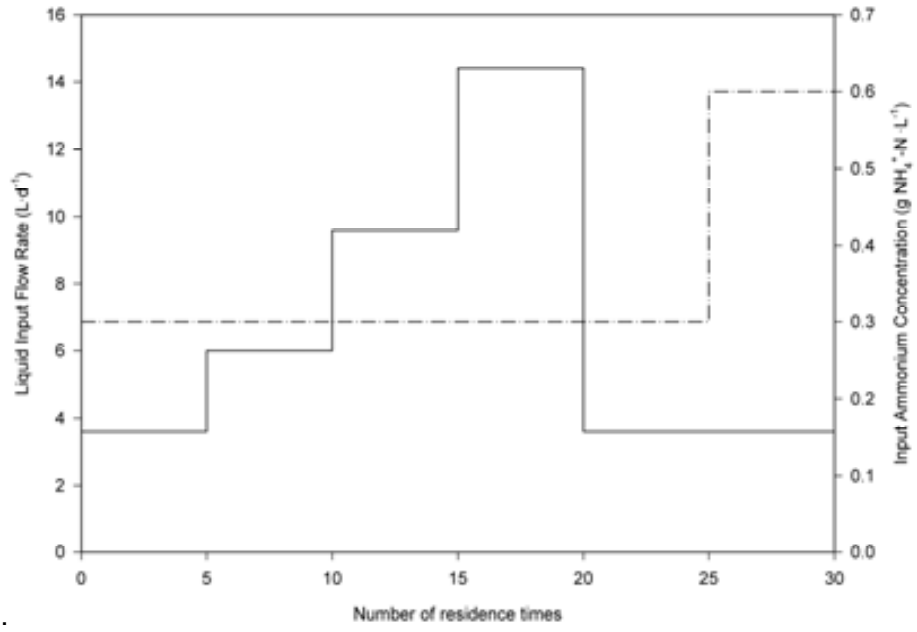


Figure 7.13 Operating conditions imposed to the CIII pilot reactor for the validation of the nitrite predictor. The solid line indicates the steps of flow rate. The dashed line indicates the ammonium concentration.

The sampling frequency was established at the beginning of this set of experiments and was kept constant throughout. The lowest frequency required is governed by a correlation between the nitrite concentration and the ammonium and nitrate concentrations as established by the nitrite predictor. The sampling period was adjusted according to the residence time used in the reactor, lower residence times leading to lower sampling frequencies.

A minimum sampling frequency of 30 min was established to ensure that (i) the sampling frequency does not become limiting in the correct performance of the control system and (ii) the selected sampling frequency is adequate under all the different tested operational conditions. Since both the ammonium and nitrate analysers were capable of performing one analysis cycle every 16 min, this was the selected sampling frequency used for the validation tests.

The ammonium, nitrite and nitrate concentration values obtained off-line by means of ready to use probes (Dr Lange, Germany) are represented alongside the results obtained with the on-line ammonium and nitrate analysers. Concentration

data generated by the analysers were registered at intervals of 60s by the MELiSSA General Purpose Station (GPS).

The first of the perturbations used on the system consisted of increasing the liquid flow rate from 3.6 $L \cdot d^{-1}$ to 6 $L \cdot d^{-1}$. In these conditions the nitrite peak reached during the transient state was around 0.4 mg $N\text{-}NO_2^- \cdot L^{-1}$ (Figure 7.14A). The on-line analyser measured the nitrate concentration with an estimated repeatability of around 16%. Ammonium measurements were obtained with relative standard deviation values that ranged between 3% and 8%.

Similar results were obtained in terms of accuracy and relative standard deviation for the rest of the validation tests carried out with different flow rate steps (Figure 7.14B and C). The analysers correctly monitored the evolution of the ammonium and the nitrate concentrations over the transient state as well as during the steady state with acceptable accuracy. However, the amplitude of the noise is rather high, most importantly in the nitrate measurements.

The last of the experiments planned for the validation of the nitrite predictor was a step in the incoming ammonium concentration, keeping a constant flow rate, from an initial value of 300 mg $N\text{-}NH_4^+ \cdot L^{-1}$ to 600 mg $N\text{-}NH_4^+ \cdot L^{-1}$ (Figure 7.14D). A good correlation between on-line data and the off-line reference method was observed during both the transient state and the steady state. Again the on-line measurements correctly predicted the increase in the NO_3^- concentration in the reactor outlet However, the repeatability in the nitrate measurements (relative standard deviation of 10-15%) was again too low to guarantee an admissible noise in the estimated NO_2^- concentration. The high noise levels in the estimated nitrite concentration are expected to produce a significant oscillation of the manipulated variable (flow rate) once the control law was implemented.

7.4.3.3 Efficiency and robustness of the nitrite predictor

The accuracy of the main parameters and the noise in the data of the main measured variables (dissolved oxygen, ammonium and nitrate) play important roles in the stability of the nitrite estimations. Inaccurate values could compromise the robustness of the nitrite control.

A sensitivity study performed by Sherpa Engineering (France) showed that the estimator has a high sensitivity towards the noise in the NH_4^+ and NO_3^- measurements. The noise of the estimated nitrite concentration in the effluent is amplified by the required calculations, leading to a noise of 10% of the mean value when the ammonium and nitrate measurements are estimated with a relative

standard deviation of 0.5%. From simulations performed with different levels of noise, it was concluded that a relative standard deviation in the ammonium and nitrate measurements as high as 5% was acceptable. Significantly higher amplitudes of the noise would lead to unstable behaviour of the manipulated variable (flow rate), which would constantly oscillate between 0 and the requested value. According to this sensitivity analysis, a relative standard deviation of up to 5% in the NH_4^+ and NO_3^- measurements would be acceptable. The ammonium measurements obtained during the validation tests would thus fulfil this requirement. However, the significant noise of the nitrate measurements (around 15%) would compromise the correct estimation of the nitrite concentration by means of the nitrite predictor.

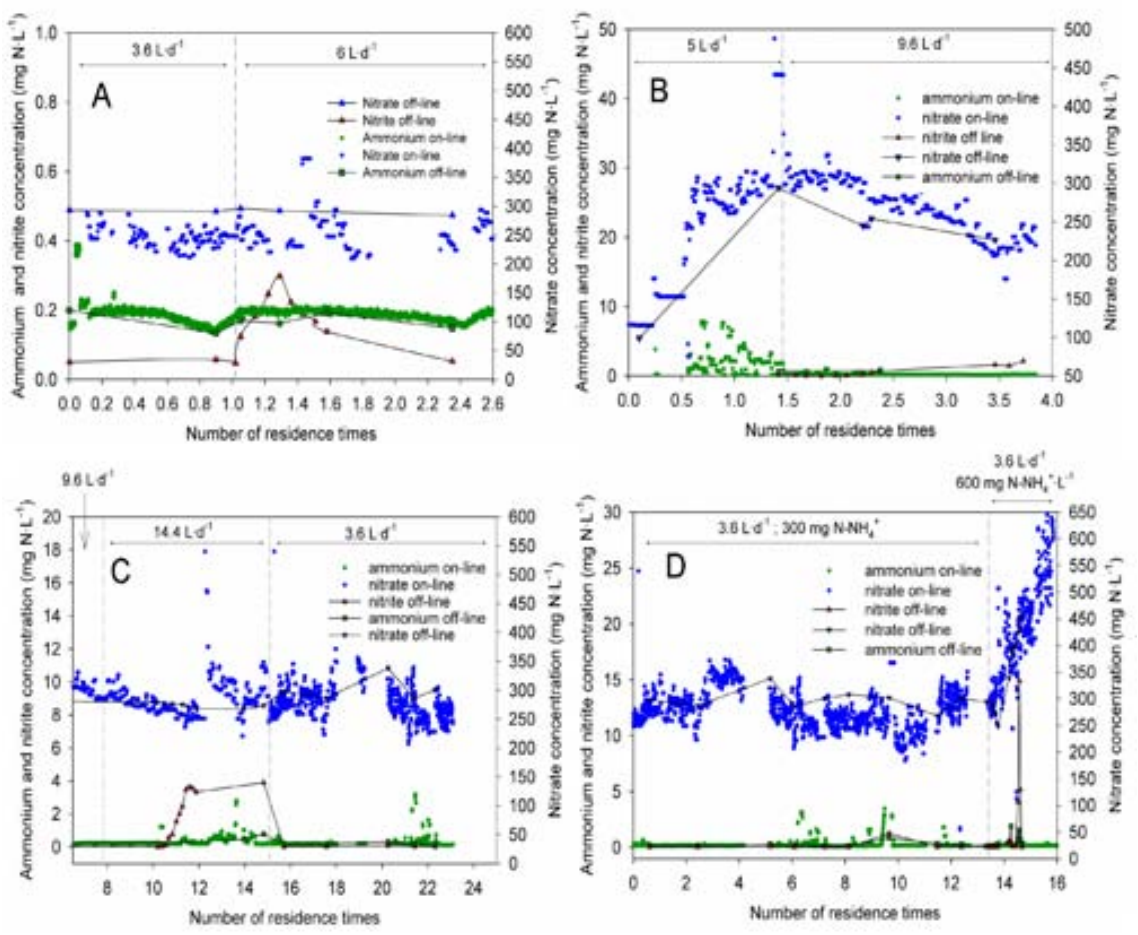


Figure 7.14 Time courses of the ammonium, nitrite and nitrate concentrations in the effluent. (A) during a step in the flow rate from 3.6 to 6 $L \cdot d^{-1}$; (B) during a step in the flow rate from 5 to 9.6 $L \cdot d^{-1}$; (C) during a step in the flow rate from 9.6 to 14.4 $L \cdot d^{-1}$ followed by a subsequent decrease to 3.6 $L \cdot d^{-1}$ and (D) during a step in the ammonium concentration in the input from 300 to 600 mg $N\text{-}NH_4^+ \cdot L^{-1}$. The ammonium and nitrate results obtained with the tested on-line analysers are represented next to the off-line measurements of ammonium, nitrite and nitrate carried out periodically using Dr Lange test tubes.

The ammonium and nitrate experimental data were first used to test the efficiency of the internal model used by the control system to predict the nitrite concentration. Ammonium and nitrate estimations were found to be in agreement with the experimental results. However, the nitrite concentration value was overestimated in the steady state and the experimental nitrite concentration peak observed in the transient states following an ammonium load step was lower than the predicted peak and decreased faster.

This discrepancy could be due to the fact that the nitrite predictor had previously been calibrated using off-line experimental data obtained during a period of oxygen limitation, with a consequent nitrite accumulation. In order to achieve a higher accuracy in the nitrite concentration estimation, the tuning of the different parameters should be improved to ensure a good performance under a wider range of operational conditions.

The experiments presented above also provide evidence of the noise of the ammonium and nitrate measurements. Noise amplitudes above 5% in the ammonium and nitrate measurements are above the acceptable values for the use of the nitrite predictor.

An experiment carried out to evaluate the response of the control system demonstrated that, as foreseen, the high noise in the nitrite estimation makes the estimator inefficient. The experiment consisted of a step in the requested flow rate from 0.4 $L \cdot h^{-1}$ to 0.6 $L \cdot h^{-1}$, with a constant NO_2^- constraint of 4 mg $N\text{-}NO_2^- \cdot L^{-1}$. High amplitude noise was observed in the manipulated variable, i.e. the flow rate, so that this variable tends to its requested value very slowly (Figure 7.15).

The use of a Kalman filter was proposed in order to balance the weight of the measurements at the input of the nitrite estimator. A Kalman filter is calibrated based on the characteristics of the noise signals of the measrurements and was expected to improve the performance of the nitrite predictor. However, the use of the filter was not sufficient to compensate the inaccuracy in the prediction of the NO_2^- concentration, due to the number of other parameters that affected its estimation.

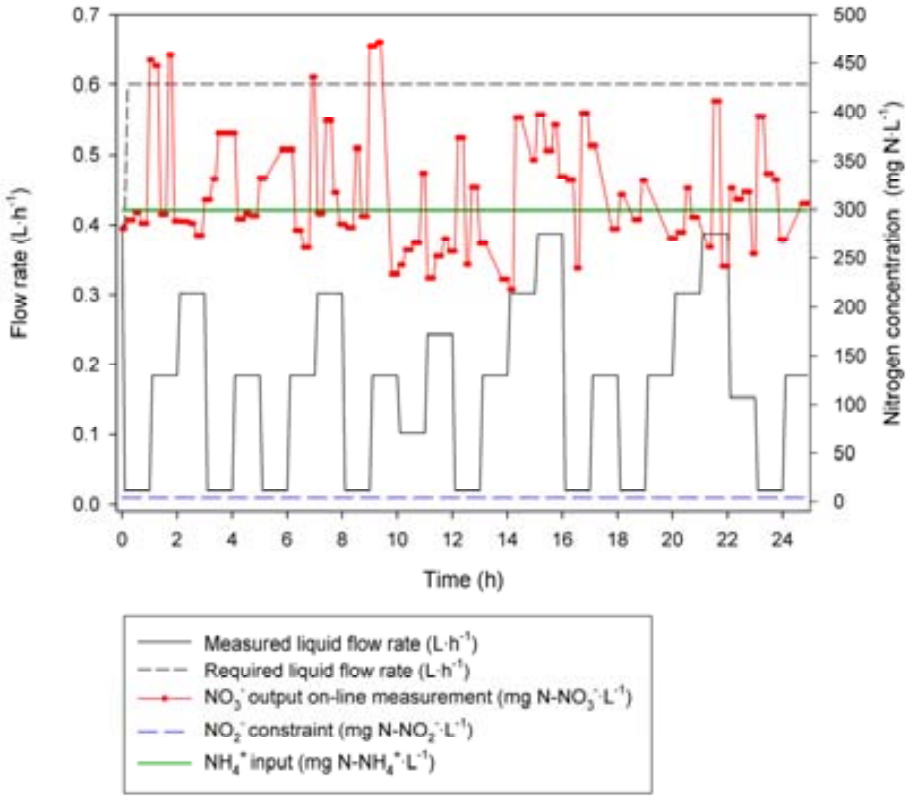


Figure 7.15 Test for the evaluation of the control system response.

7.4.4 DIRECT ON-LINE MEASUREMENT OF THE NITRITE CONCENTRATION IN CIII

The accuracy and repeatability of the ammonium and nitrate measurements obtained with a system based on ISEs were not good enough for the correct performance of the nitrite predictor, particularly when the system was working under low nitrite accumulation conditions. In conclusion, the first working hypothesis for the on-line nitrite concentration analysis proved to be unsuccessful for fine tuning control of nitrite in CIII.

The low precision of the nitrite estimations requires the installation of an on-line nitrite analyser for the direct monitoring of this nitrogen species in the effluent of CIII with high accuracy. Therefore, the combined data of the ammonium and nitrate analysers will mainly be used to monitor the global nitrification efficiency in the reactor, while a new analyser will be installed for the direct on-line analysis of nitrite.

7.4.4.1 Technical requirements for the on-line nitrite analyser

The results obtained from the experiments performed at the MPP (section 7.4.3.2) for the validation of the nitrite predictor software developed by SHERPA Engineering (France) provided some useful information on the requirements for the

measurement of nitrite. The analysis of these results yielded a number of requirements regarding the accuracy, measurement range and sampling frequency that an adequate analyser should have. These requirements are summarised in Table 7.12.

The range of measurement was selected by taking into account that the analyser should be able to measure the nitrite concentration not only under normal operational conditions (low range) but also during irregular operation or transient states following a load increase (high range).

The optimal configuration should allow for the monitoring of the nitrite peak indicating a transient state up to the highest possible concentration, that was fixed at 20 mgN-NO_2^-·L^{-1}. To this end, the optimal configuration should allow for the monitoring of nitrite from a regular value of below 0.01 mg N-NO_2^--·L^{-1} up to 20 mg N-NO_2^-·L^{-1}. It must be underscored that the optimisation of the analyser performance at high concentration ranges may result in a decrease in the accuracy of the equipment when working at lower ranges.

Table 7.12 Requirements for the on-line nitrite analyser.

Range of measurement	Accuracy	Response time
0.02-0.5 mg N-NO_2^-·L^{-1} 0.5-20 mg N-NO_2^-·L^{-1}	5%-10%	Maximum 12 min

In addition to the usual constraints predetermined by the control system (frequency of analysis, repeatability, accuracy), other considerations dealing with the operational conditions and limitations of the process need to be taken into account (range of measurement, sample consumption).

7.4.5 PRELIMINARY TESTS WITH A PROTOTYPE ANALYSER

A number of samples containing different nitrite concentrations within the requested measurement range (0-20 mg N-NO_2^-·L^{-1}) in a matrix with the same composition as the culture medium used in CIII plus an excess of either HCO_3^- or NO_3^- was analysed. The aim of these tests was to identify the potential interference by any of these two species that are present at a high concentration in the samples from the nitrifying reactor and might have an effect on nitrite determination at certain levels.

The composition of the different samples is listed in Figure 7.2: samples A-F contain 600 mg N-NO_3^-·L^{-1}, 580 mg HCO_3^-·L^{-1} and different concentrations of nitrite dissolved in the same sample matrix as the feeding medium while samples 1-4

contain 300 mg $N\text{-}NO_3^-\cdot L^{-1}$ and 1160 mg $HCO_3^-\cdot L^{-1}$. These two sets of samples were used to evaluate the possible interference from high nitrate concentrations and from bicarbonate respectively.

Table 7.13 Composition of the samples used for the preliminary tests performed with a nitrite analyser prototype.

	CONCENTRATION (mg·L)											
	A	B	C	D	E	F	1	2	3	4		
$(NH_4)_2SO_4$	1320	1320	1320	1320	1320	1320	1320	1320	1320	1320	$N\text{-}NO_2^-$	0	0.02	0.1	1	10	20	0	0.02	0.1	10
$FeSO_4.7H_2O$	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	$N\text{-}NO_3^-$	600	600	600	600	600	600	300	300	300	300
Na_2HPO_4	710	710	710	710	710	710	710	710	710	710	HCO_3^-	580 952	580 952	580 952	580 952	580 952	580 952	1161 905	1161 905	1161 905	1161 905
KH_2PO_4	680	680	680	680	680	680	680	680	680	680											
$(NH_4)_6Mo_7O_{24}.4H_2O$	177	177	177	177	177	177	177	177	177	177											
$MgSO_4.7H_2O$	52	52	52	52	52	52	52	52	52	52											
$NaNO_3$	3642 857	3642 857	3642 857	3642 857	3642 857	3642 857	1821 429	1821 429	1821 429	1821 429											
$CuSO_4.5H_2O$	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004											
$ZnSO_4.7H_2O$	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004											
$CaCl_2.2H_2O$	0.740	0.740	0.740	0.740	0.740	0.740	0.740	0.740	0.740	0.740											
$NaHCO_3$	800	800	800	800	800	800	1600	1600	1600	1600											
$NaNO_2$	0.000	0.099	0.493	4.929	49.286	98.571	0.000	0.099	0.493	49.286											

7.4.5.1 Results obtained with a 0.2 mL injection loop

The prototype nitrite process analyser used to perform these tests had been adapted for the determination of this compound in a wide concentration range (0-20 mg $N\text{-}NO_2^-\cdot L^{-1}$). Due to the wide measuring range that had to be covered, the volume of dilution water added in each analysis was rather high (17 mL dilution water / 0.2 mL sample). Nitrite concentration measurements were obtained with an accuracy between 5% and 9% for samples in the range 1-10 mg $N\text{-}NO_2^-\cdot L^{-1}$ (Figure

7.16A and Figure 7.16B). However, the accuracy obtained at concentrations below 0.5 ppm N-NO_2^- was much lower, as can be observed in Figure 7.16C and Figure 7.16D.

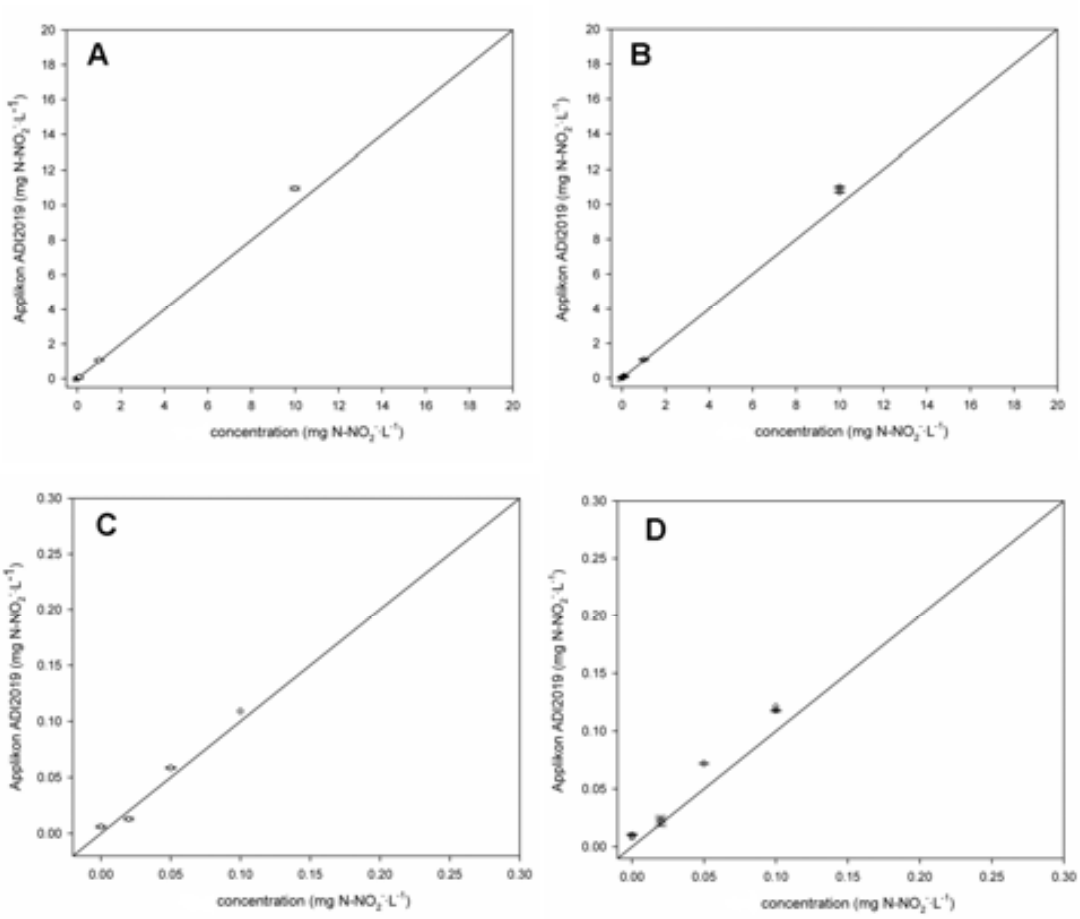


Figure 7.16 Tests performed with the Applikon ADI2019 nitrite process analyser using a 0.1 mL injection loop. (A) samples diluted in water (B) samples containing the same composition as the feeding medium. (C) Samples diluted in water, zoomed in at lower concentration range (D) samples containing the same composition as the feeding medium, zoomed in at lower concentration range.

7.4.5.2 Results obtained with 0.5mL injection loop

The observed lack of accuracy at the low concentration range was attributed to the dilution conditions imposed by the high value of the maximum concentration range that was set as a target. Therefore, it should be possible to attain a higher accuracy by narrowing the concentration range. To this effect, a sample loop with a volume of 0.5 mL was used instead of the 0.2 mL loop used in the previous tests. Finally, a second batch of test samples containing the same nitrite concentrations were used and the analyser was calibrated using a standard of 0.05 mg N-NO_2^-·L^{-1} in order to optimise its performance within the range 0-0.5 ppm N-NO_2^- .

By implementing these changes, the dilution was reduced 10 times (from 1/100 to 1/10). As in previous tests, the samples were prepared in a solution containing all the components of CIII feeding medium and also in water, in order to check the influence of potential interferences on the analysis error, and to discriminate the error due to the dilution from that due to potential interferences.

The results are provided in Figure 7.17A (samples prepared in water). And Figure 7.17B (samples prepared in CIII medium). Although a linear trend is observed for the complete range, when the data at the lower concentrations are examined in detail, the error increases to a high level. The results are similar when a full solution with all the compounds present in CIII medium was used in the preparation of the samples, or when only water is employed, thus suggesting that the observed error at low concentrations can not, in principle, be attributed to the possible interferences of any compound present in the liquid medium.

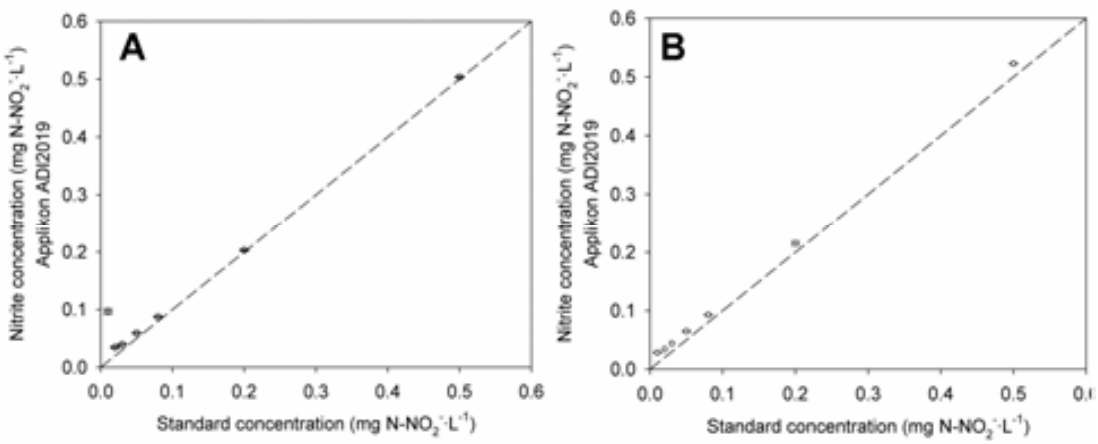


Figure 7.17 Tests performed with the Applikon ADI2019 process analyser using a 0.5 mL injection loop. (A) samples diluted in water (B) samples containing the same composition as the feeding medium.

7.4.5.3 Analysis of the results: deviation between experimental and theoretical concentrations.

The evolution of the relative error of the results obtained with the Applikon analyser is shown in Figure 7.18A for the range between 0-20 mg $N\text{-}NO_2^-\cdot L^{-1}$ (using the 0.5 mL sampling loop i.e. a dilution of 1/10) and Figure 7.18B for the range between 0-0.5 mg $N\text{-}NO_2^-\cdot L^{-1}$ (using a 0.1 mL sampling loop) It can be clearly observed how the error increases exponentially at concentrations below 0.1 mg $N\text{-}NO_2^-\cdot L^-$.

When the results of the analysis of nitrite standards prepared using the culture medium were compared to the values obtained with the samples diluted in water, only a slightly higher deviation was detected in the case of the samples containing the medium composition.

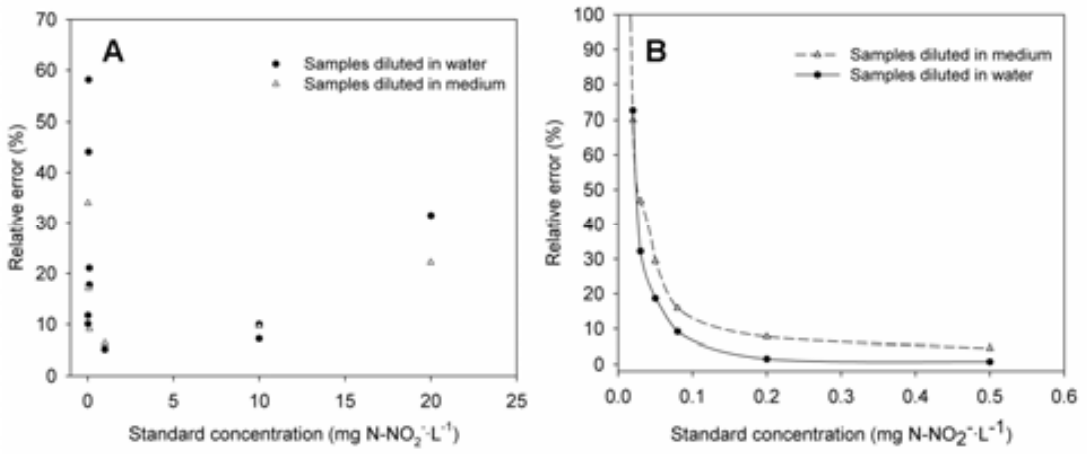


Figure 7.18 Evolution of the relative error of the nitrite measurements as a function of the sample nitrite concentration. (A) dilution 1/100, sample loop 0.1 mL (B) dilution 1/10, sample loop 0.5 mL.

7.4.5.4 Design of an optimal configuration for operation in a wide concentration range

From the tests done with the prototype ADI2019 analyser (Applikon, The Netherlands) it was concluded that a high accuracy in the measurement of both very low nitrite concentrations (0.005 to 0.1 mg $N\text{-}NO_2^-$ L^{-1}) and peak concentrations (up to 20 mg $N\text{-}NO_2^-$ L^{-1}), was not achievable with a conventional process analyser.

The implementation of an automatic dilution system allows for the analyser operation in a higher concentration range. However, the dilution system significantly increases the error in the values of the lower ranges samples. The solution was a dual analyser that would switch to a different analysis routine when the lower range of analysis was exceeded, providing accurate nitrite concentration values in the whole measuring range. The dual analyser was based on the adaptation of the model ADI2040 (Applikon, The Netherlands) to this particular application.

The layout of the analyser designed to monitor nitrite in the two required concentration ranges can be found in Figure 7.19. The analyser consists of two different sampling loops and their respective injection system. The sample pump is activated when the analysis cycle is due to start and the sample subsequently flows through the sampling loop.

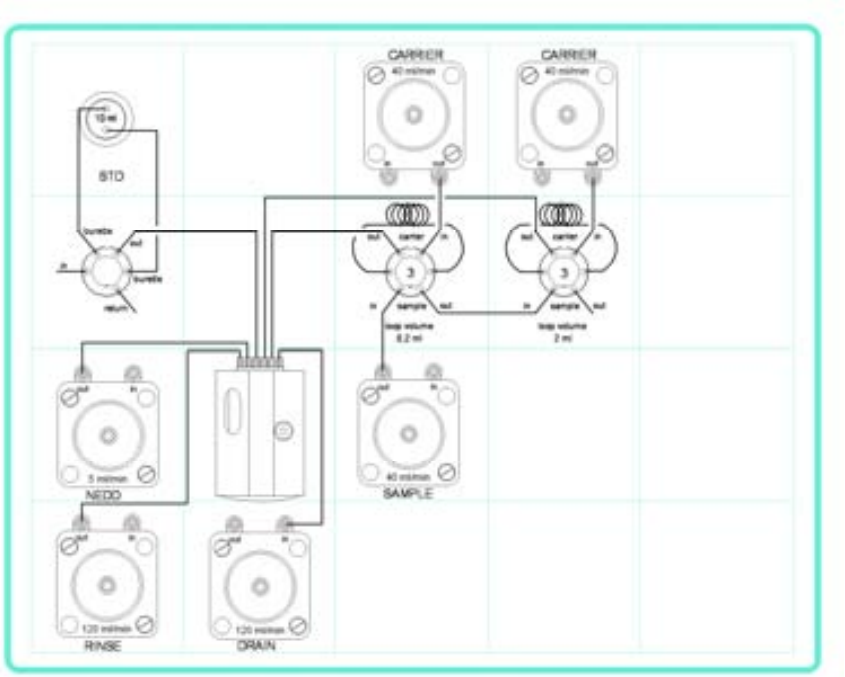


Figure 7.19 Layout of the wet parts of the dual analyser (ADI2040, Applikon, The Netherlands) designed for the monitoring of the nitrite concentration in the two required concentration ranges.

Depending on the expected concentration of the sample, one of the two sampling systems (with either a 2 mL or a 0.2 mL sampling loop) is activated and a precise amount of sample is dosed into the cuvette module by means of a carrier. The use of a 2 mL sampling loop, i.e. a volume higher than the tested 0.5 mL, was proposed to further increase the accuracy of the measurements in the lower range. The initial absorbance measured before the reaction takes place in the cuvette module is measured. The NEDD reagent (0.8 mL) is subsequently added to the cuvette and the final absorbance is measured after the required amount of time has elapsed for the reaction to take place. The cuvette is then drained and rinsed so that the following cycle can take place.

7.5 CONCLUSIONS

In this chapter a strategy for the on-line monitoring of ammonium, nitrite and nitrate in the effluent of CIII has been developed. In the process of defining the best approach to nitrogen monitoring, the following partial goals and conclusions were achieved:

- Ammonium and nitrate on-line analysis equipment was successfully configured and tested off-line for correct performance with samples having the same composition as the effluent of CIII.

- The experimental set-up was designed to minimise the delay in the on-line concentration measurements. Tests performed on-line with this provisional configuration of the sampling loop indicate that the analysers will be suitable for the monitoring of the total ammonium conversion to nitrate in the reactor. Further adjustments were foreseen for the sampling loop in order for the concentration data to fulfil the control system requirements.
- A strategy based on the possible use of the ammonium and nitrate data provided by the on-line analysers for the indirect measurement of nitrite was investigated. The frequency of analysis of the ammonium and nitrate analysers (16 min with the used parameter configuration) fulfilled the requirements of the nitrite predictor. However, the amplitude of the noise of the nitrate concentration was too high, leading to a noisy estimation of the nitrite concentration that was not admissible for control purposes. The control strategy based on the estimation of the nitrite predictor using experimental ammonium and nitrate concentration data was thus unsuccessful for fine tuning control in CIII.
- The bases for the implementation of an experimental nitrite monitoring system were set with the selection of a nitrite analyser and the design of its configuration for the fulfilment of the requirements of the CIII nitrite control system.

PART V

FUTURE PERSPECTIVE AND GENERAL CONCLUSIONS

CHAPTER 8

Bases for the re-design of the MELiSSA compartment III pilot reactor

CHAPTER 9

General conclusions

CHAPTER 8

BASES FOR THE RE-DESIGN OF THE MELISSA COMPARTMENT III PILOT REACTOR

Foreword

Following the characterisation of the pilot scale nitrifying reactor of the MELiSSA project and taking advantage of a general upgrade of the MELiSSA Pilot Plant and its compartments, the re-design of the hardware of CIII is considered. This upgrade should help to ensure the optimal operation and better control of the pilot reactor of the MELiSSA CIII. The knowledge gained in this thesis through experimental analysis and simulation, and information available from previous studies performed with this reactor is used in this chapter to set the bases for the reactor re-design.

8.1 INTRODUCTION: MOTIVATION FOR THE RE-DESIGN OF CIII

The nitrifying pilot reactor of the MPP was operated over extensive periods of time, under different experimental conditions. Taking advantage of the removal of this compartment into a new site of the MPP, the re-design of its hardware is envisaged, in order to improve its performance. Special attention is dedicated to some parts of the equipment that need to be replaced due to their intensive use, and to the performance requirements for the final MELiSSA loop closure.

The main elements to be addressed in the re-design work are discussed in this chapter. Major changes from the previous hardware have not been envisaged, since the overall operation of the reactor in the previous experiments was satisfactory. Changes in sizing are not required either, since the compartment was designed to fit the needs of the MELiSSA loop. Therefore, the final goal is to optimise the existing design, improving the necessary elements according to the knowledge yielded by the present thesis as well as previous studies related to the operation of this reactor (Pérez et al., 2004).

The main reason for the re-design of this compartment is to guarantee the continuous operation over long periods of time, under well controlled conditions, and in fully axenic conditions. Indeed, it has to be remembered that full steam sterilisation must be undertaken to guarantee the axenicity of the reactor. Additionally, the need for chemical (or radiation) sterilisation of the support particles currently used (BIOSTYR®) should also be borne in mind.

Other important features related to long term operation and axenicity of the reactor are the selection of pumping devices, retractable probes, online measurements and the prevention/control of potential clogging of the packed bed bioreactor. To this end, an on-line biomass detection system is being tested at the moment in a parallel study (NTE; Lliçà d'Amunt, Spain), and will allow the monitoring of the biomass within the packed bed during its long term operation to be monitored.

8.2 TECHNICAL SPECIFICATIONS FOR THE RE-DESIGN OF CIII

The technical specifications for the re-design of the CIII pilot reactor have been divided into three main categories: (i) General hardware and auxiliary equipment (ii) instrumentation up-grade and (iii) specific operation requirements.

8.2.1 GENERAL HARDWARE AND AUXILIARY EQUIPMENT

The new hardware should have the same dimensions and basic characteristics as the existing one, but some particular features should be modified in order to improve reactor operation.

The whole reactor will be built in stainless steel 316L to allow sterilisation using pressurised steam, and to avoid the exposure of the nitrifying bacteria to external light sources, which are known to inhibit their activity (Prosser, 1989). For the sterilisation requirements, the tightness of all seals should be effectively guaranteed. The incorporation of a spy hole along the packed bed height in the frontal face of the reactor to allow (i) the observation of the biofilm and (ii) the observation of the lower part (mixing region) and upper part (level of liquid, gas separation) of the reactor will provide further useful information. Similarly, illumination from the top lid will also allow examination of the top section of the reactor.

Mixing will be provided in the bottom section with a magnetically coupled stirrer with variable speed, which will ensure axenicity in long term operation due to the lack of mechanical seals. The BIOSTYR® beads will be kept within the central section (packed bed) by means of stable stainless steel 316L or Delrin grids, preferably removable (for cleaning purposes).

In order to guarantee the continuous operation of the reactor over extended periods, as well as the adequate sterilisation and handling of the whole equipment, stainless steel 316L vessels will be provided for the feed and outlet liquid, with a total volume of 40 L (working volume 30 L). The vessels will allow steam sterilisation and will be connected by means of pipelines built of the same material. The remaining vessels and pipelines (acid and base storage vessels; acid, base, culture medium feeding, reactor exhaust and recirculation pipelines) will also be constructed in stainless steel 316L.

Alternatives to the use of peristaltic pumps for the culture medium, broth removal and broth recirculation have been considered to guarantee the operating flow ranges during extended periods of time. A list of the main elements of auxiliary equipment of the reactor is shown in Table 8.1.

Table 8.1 List of new auxiliary equipment requirements. The different items can be located by their references in Figure 8.2 (P&ID diagram).

ITEM	QUANTITY	EQUIPMENT	FUNCTION
C-01	1	BIOREACTOR	Fermentation
AC-01+FV01	1	Bioreactor mixer + Frequency variator	Mixing
D-03	1	Vessel	Medium feeding vessel
D-04	1	Vessel	Medium outlet vessel
D-05	1	Expansion vessel	Heating/cooling
E-02	1	Exchanger	Heating
E-03	1	Exchanger	Cooling
CC-01	1	Compressor	Gas recirculation
P-01+FV02	1	Pump+ Frequency variator	Recirculation
P-04	1	Pump	Medium feed
P-05	1	Pump	Heating/cooling Water circulation
P-06+FV06	1	Pump+ Frequency variator	Backwashing
F-01	1	Filter	Sterile filtration of acid
F-02	1	Filter	Sterile filtration of base
F-03	1	Filter	Sterile filtration of medium
F-04	1	Filter	Sterile filtration of medium load
F-05	1	Filter	Sterile venting filter of D-03
F-06 A/B	2	Filter	Gas sample filtration
F-07	1	Filter	Sterile filtration of compressed air
F-08	1	Filter	Sterile venting filter of D-04
F-09 A/B	2	Filter	Outlet gas filtration
F-10 A/B	2	Filter	Sample filtration

8.2.2 INSTRUMENTATION UP-GRADE

8.2.2.1 Current on-line instrumentation and control elements

The main elements of instrumentation associated with the CIII pilot reactor and currently installed in the MPP are shown in Table 8.2 together with the required number of units.

Table 8.2 Description of the main instrumentation associated to the compartment III pilot scale bioreactor.

Monitored parameter	Measuring range	Number of units
pH	0-14	2
Oxygen	0-100%	2
Temperature	4-250ºC	2
Pressure	0-1000 mbar	1
Level	-	1
O_2 mass flowmeter	0-500 mL·min^{-1}	1
CO_2 mass flowmeter	0-50 mL·min^{-1}	1
N_2 mass flowmeter	0-20 L·min^{-1}	1
Gas flowmeter	0-10 L·min^{-1}	1
NH_4^+	0.01-255 mg N-NH_4^+ ·L^{-1}	1
NO_2^-	0.005-0.5 mg N-NO_2^-·L^{-1} 0.5-20 mg N-NO_2^-·L^{-1}	1
NO_3^-	50-600 mg N-NO_3^-·L^{-1}	1

In Table 8.3 the different controlled parameters along with a brief description of the main control loops with the values of the set points and the related precision are listed. The control loops are embedded in the general control architecture of the MPP. To this end, the electrical connections of all sensors and actuators need to be compatible with those of the quantum Schneider PLC used in the MPP.

Table 8.3 Description of the action of the main control loops associated to the compartment III pilot scale bioreactor.

Control loop	Set point	Precision
pH	8.0	± 0.1
Liquid flow rate	2.5-10 ml/min	To be defined
Gas flow rate	3000 ml/min	To be defined
Temperature	28.0 ºC	± 0.1ºC
Pressure	<80 mbar	1 mbar
Dissolved Oxygen	80%	± 5%

8.2.2.2 New instrumentation requirements

In addition to the basic instrumentation already installed in the reactor, new parameters have been identified that require monitoring. The new instrumentation requirements will be enumerated in this section.

The liquid level control should be improved in order to increase its precision. The sensor for high level will be redundant to avoid any potential flooding, and a low level sensor will be incorporated to guarantee a minimal liquid level in the reactor. Flow meters will be incorporated in the most critical liquid lines (feeding, outlet and recirculation).

A differential pressure measurement will be implemented in the reactor in order to detect the clogging of the packed bed due to excessive biofilm generation. This measurement will be used to predict the necessity of preventive backwashing procedures, in order to remove excess of biofilm, before severe clogging occurs. Moreover, the installation of a newly developed biomass sensor will allow monitoring the biofilm thickness. The combination of the data recorded by analytical instrumentation (e.g. differential pressure, nitrogen analysers, biomass sensor) with the mathematical model developed in previous chapters of this thesis, can be used to simulate clogging in the packed bed, optimising the frequency and intensity of the backwash events.

A new biomass sensor will be installed in CIII, allowing the monitoring of the biomass concentration in the packed bed. The principle of this biomass sensor is the measurement of the electrical impedance at different frequencies. The relationship between impedance at low frequencies and the impedance at high frequencies is proportional to the concentration of cells in the medium. A scan to measure the electrical impedance at different frequencies is initially carried out to determine the minimum and maximum impedance values. The initial biomass concentration is calculated as a function of the ratio between the minimum and maximum frequencies. At later stages an adjustment of the expression that defines the dependence of the impedance on the frequency is carried out, yielding the values of some characteristic parameters related to the type of biomass. The biomass concentration can then be calculated from the impedance measurements as a function of the ratio between the values of these estimated characteristic parameters.

The sensor consists of a main unit housing all the electronic elements, connected to a computer and provided with several reading probes, each one containing at least four impedance electrodes, which are placed into the reactor ports at three different heights of the packed bed (Figure 8.1).

Retractable housing will also be provided in the reactor for the dissolved oxygen, pH probes and for any other sensor, allowing sterilisation without interrupting the process. A complete list of all the instrumentation of CIII, including the new elements as well as the elements already available in the MPP is shown in Table 8.4.

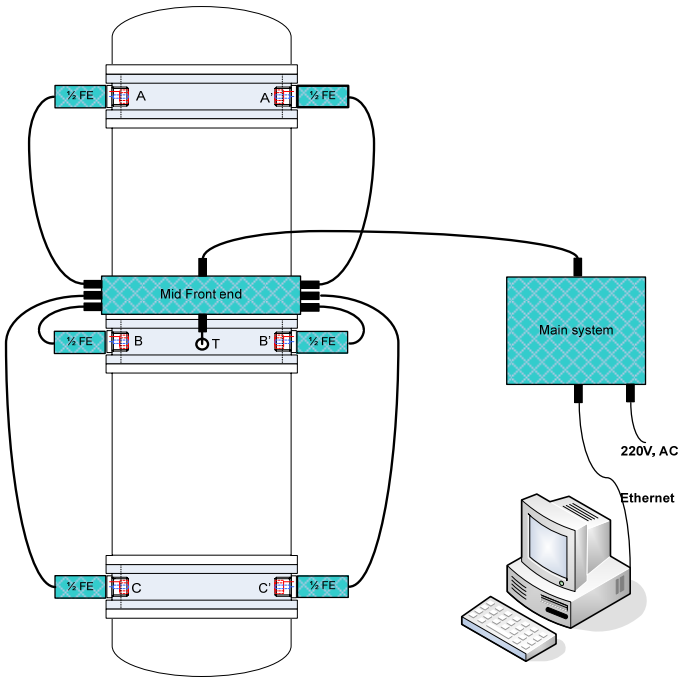


Figure 8.1 Schematic view of the biomass sensor system concept.

8.2.3 SPECIFIC OPERATION REQUIREMENTS

In previous sections of this chapter the basic requirements of the reactor during normal continuous operation have been defined. However, there are a number of specific situations (e.g. start-up, maintenance operations) that require the definition of exhaustive protocols.

8.2.3.1 Sterilisation

Two main features of the reactor sterilisation have to be distinguished: (i) the sterilisation of the hardware, including auxiliary equipment such as vessels or pipelines and (ii) the sterilisation of the material used as a support for the biofilm development (BIOSTYR® beads).

The hardware is steam sterilised following a well defined protocol carried out in different steps: emptying of the facility, a first sterilisation with steam flowing through all the elements of the hardware followed by a second sterilisation step with steam at 121ºC (P=1.2 barg) for 20-30 minutes. The hardware sterilisation is finalised after drying with compressed air and cooling of all the elements, including filters.

The BIOSTYR® beads used as support material do not resist high temperature; hence a different sterilisation method is required. The contents of the packed bed will sterilised by radiation and kept in an addition bag. The bag has to be provided with the passive part of a buck-valve. The other end of the bulk valve will be installed in a compressed air pipeline so that the contents of the bag will be added to the reactor when the bag is connected to the line.

8.2.3.2 Inoculation

Inoculation of the reactor must be performed in such a way that axenicity is not compromised. The reactor will incorporate an inoculation port that allows steam sterilisation and that guarantees a proper connection with the sterile bottle containing the inocule. Inculation will be carried out using the recirculation line.

8.2.3.3 Backwashing and long term operation

In all the modifications described above it must be borne in mind that the reactor will operate over long periods of time. As previously mentioned, extended periods of continuous operation in the packed bed reactor inevitably lead to a progressive clogging of the packed bed and the need for maintenance operations. The new reactor hardware will incorporate the necessary elements (e.g. pumps) that allow reversal of the liquid flow in order to enhance biomass detachment from the biofilm without compromising the axenicity in the reactor.

8.2.3.4 Analysis loop

The reactor will incorporate a cellular retention device in the output line in order to retain any free biomass detached from the biofilm (although only minor concentrations are expected in normal operational conditions). The fitered liquid will be pumped to the subsequent MELiSSA compartments while an additional line of the filtered solution will be used to feed the on-line analysers. The sampling device installed in the output should guarantee both the correct performance of the on-line analysers and the axenicity in the bioreactor. On-line sampling will be required for NH_4^+, NO_3^- and NO_2^-.

The complete process and instrumentation diagram (P&ID) of the MELiSSA CIII and its auxiliary instrumentation is shown in Figure 8.2, with all instrumentation elements listed in Table 8.4.

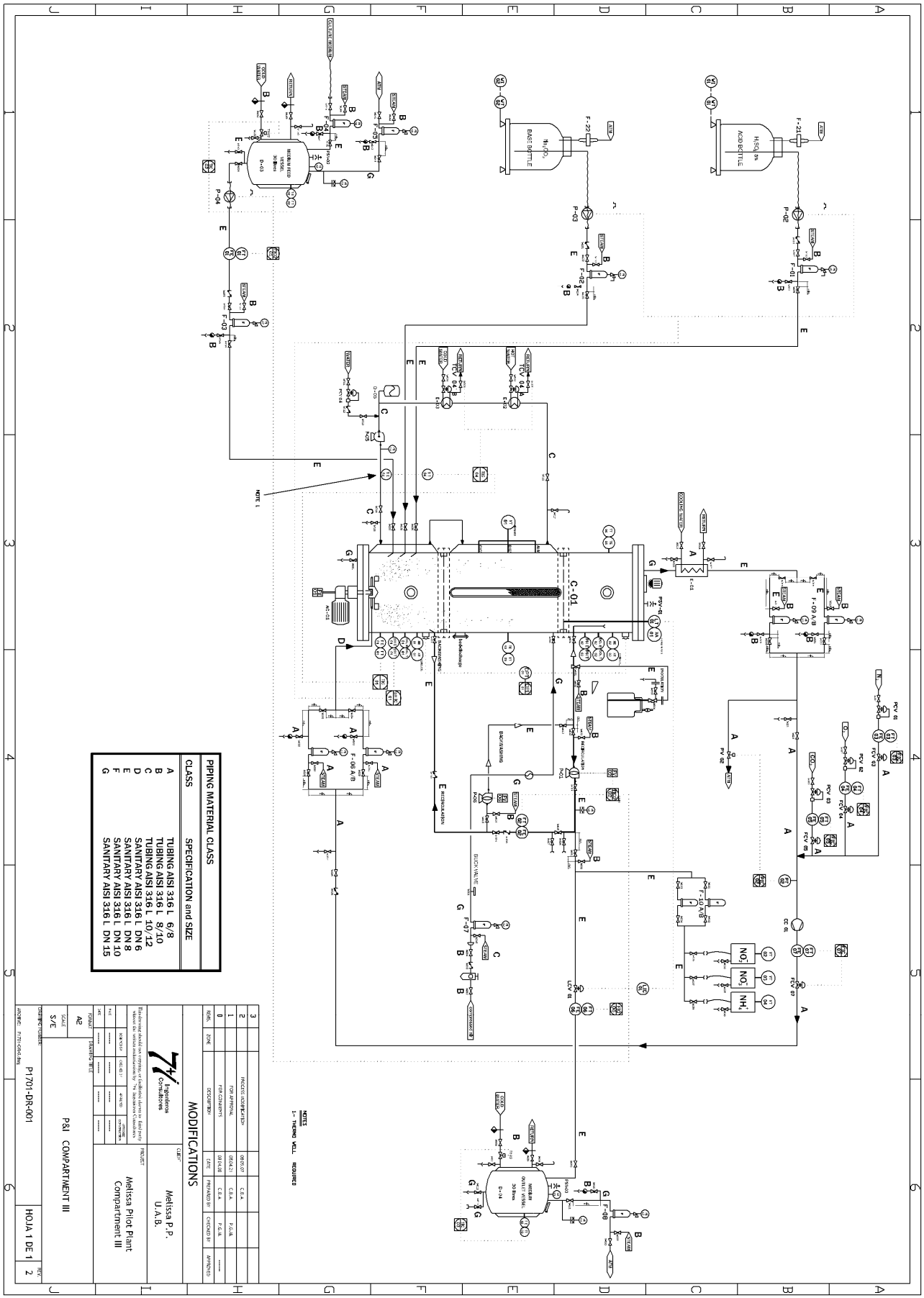
Table 8.4 List of auxiliary equipment requirements. The different items can be located by their references in Figure 8.2 (P&ID diagram).

LOOP	TAG	INSTRUMENT	FUNCTION
LEVEL			
LIC 01	LT 01 LIC 01 LCV 01	Level transmitter PID controller Level control valve	Bioreactor
LI 02	LI 02	Level indicator	Feeding medium vessel
LI 03	LI 03	Level indicator	Effluent medium vessel
FLOW			
FICQ 01	FE 01 FT 01 FICQ 01	Flow sensor (Coriolis) Flow transmitter PID controller	Feeding medium
FICQ 02	FE 02 FT 02 FICQ 02	Mass flow sensor Flow transmitter PID controller	Bioreactor recirculation
FIC 06	FE 06 FT 06 FIQ 06	Flow sensor (Coriolis) Flow transmitter Flow totalizer	Effluent medium
FIC 07	FE 07 FT 07 FIC 07 FCV 07	Vortex mass flow sensor Flow transmitter PID controller Flow control valve	Outlet gas
pH			
pHIC 01	pH E 01 pHT 01 pHIC 01	pH electrode pH transmitter PID controller	Bioreactor (bottom section)
pHIC 02	pH E 02 pHT 02 pHIC 02	pH electrode pH transmitter pH indicator	Bioreactor (top section)
WEIGHT			
WI 01	WI 01 WT 01	Weight indicator Weight transmitter	Acid addition
WI 02	WI 02 WT 02	Weight indicator Weight transmitter	Base addition
DISSOLVED OXYGEN			
DO2 I 01	DO2 E 01 DO2 T 01	DO2 electrode (bottom section) DO2 transmitter	Bioreactor
DO2 I 02	DO2 E 02 DO2 T 02	DO2 electrode (bottom section) DO2 transmitter	Bioreactor

LOOP	TAG	INSTRUMENT	FUNCTION
PRESSURE			
DPI 01	DPI 01 DPT 01	Differential pressure indicator Differential pressure transmitter	Bioreactor
PIC 02	PT 02 PIC 02 PV 02	Pressure transmitter Pressure controller On/off pressure valve	Outlet gas
PI 03	PI 03	Pressure indicator	Vessel D-03
PI 04	PI 04	Pressure indicator	Vessel D-04
PI 05	PI 05	Pressure indicator	F-01
PI 06	PI 06	Pressure indicator	F-02
PI 07	PI 07	Pressure indicator	F-03
PI 08	PI 08	Pressure indicator	F-04
PI 09	PI 09	Pressure indicator	F-05
PI 10	PI 10	Pressure indicator	F-06A
PI 11	PI 11	Pressure indicator	F-06B
PI 12	PI 12	Pressure indicator	F-07
PI 13	PI 13	Pressure indicator	F-08
PI 14	PI 14	Pressure indicator	Heating/cooling Water circulation
PI 15	PI 15	Pressure indicator	Liquid phase recycling and outlet
PI 16	PI 16	Pressure indicator	F-09A
PI 17	PI 17	Pressure indicator	F-09B
AUTOREGULATION PRESSURE DEVICES			
PCV 04	PCV 04	Pressure valve water	Heating/cooling Water circulation
PSV 01	PSV 01	Rupture disc	Bioreactor
PSV 02	PSV 02	Rupture disc	Feeding medium vessel
PSV 03	PSV 03	Rupture disc	Effluent medium vessel

LOOP	TAG	INSTRUMENT	FUNCTION
TEMPERATURE			
TIC 01	TE 01 TT 01 TIC 01	PT100 temperature sensor Temperature transmitter PID controller	Bioreactor (bottom section)
TIC 02	TE 02 TT 02 TIC 02 TV 02	PT100 temperature sensor Temperature transmitter PID controller On/off temperature valve	Feeding medium vessel
TIC 03	TE 03 TT 03 TIC 03 TV 03	PT100 temperature sensor Temperature transmitter PID controller On/off temperature valve	Effluent medium vessel
TIC 04	TE 04 TT 04 TIC 04 TCV 04A TCV 04B	PT100 temperature sensor Temperature transmitter PID controller Temperature control valve Temperature control valve	Heating/cooling Water circulation
TI 05	TE 05 TT 05	PT100 temperature sensor Temperature transmitter	Bioreactor (middle section)
TI 06	TE 06 TT 06	PT100 temperature sensor Temperature transmitter	Bioreactor (top section)
FOAM			
XA 01	XA 01	Foam alarm	Bioreactor
BIOMASS			
YT 01	YE 01 to 06 YT 01	Biomass sensor Biomass transmitter	Bioreactor
CONDUCTIVITY			
XI 01	XE 01 XT 01	Conductivity sensor Conductivity transmitter	Bioreactor (bottom section)
XI 02	XE 02 XT 02	Conductivity sensor Conductivity transmitter	Bioreactor (top section)
ANALYSERS			
YT 02	YT 02	NO_2^- analyser	Effluent liquid phase
YT 03	YT 03	NO_3^- analyser	Effluent liquid phase
YT 04	YT 04	NH_4^+ analyser	Effluent liquid phase

Figure 8.2 Process and instrumentation diagram (P&ID) of the MELiSSA compartment III.



CHAPTER 9

GENERAL CONCLUSIONS

The work developed in the present thesis has contributed to increase the understanding on the performance of the pilot reactor of the MELiSSA nitrifying compartment (CIII). The development of a new mathematical model, in combination with the study of the biofilm composition through molecular analysis and the implementation of an on-line monitoring system of the nitrifying efficiency, have provided the necessary tools for a comprehensive study of the reactor performance.

The stability of the packed bed biofilm reactor over a long operational period has been assessed. Analysis of the biofilm using molecular techniques demonstrated that the two initial strains used to inoculate the reactor (*N. europaea* and *N. winogradskyi*) were still the dominant species in the biofilm after 1750 days of operation. A small deviation from ideal well-mixed tank behaviour in the liquid phase of the reactor was identified as the cause of the heterogeneity experimentally assessed along the packed bed height of both the biofilm thickness and the relative distribution of AOB and NOB. The good correlation of the results obtained through model simulation with the experimental biomass profiles yielded by molecular analysis (FISH and Q-PCR) makes the model a useful tool to simulate the reactor status under a wide range of operational conditions. The availability of a mathematical model is particularly valuable to study the reactor behaviour given the difficulty to harvest biomass samples to be used for FISH and Q-PCR analysis.

The mathematical model was used to design a strategy to optimise the intensity and frequency of the backwash events in the packed bed reactor, minimising nitrite accumulation in the reactor as established by the requirements of the MELiSSA loop. The results obtained pointed towards stronger backwash events as the recommended strategy rather than weaker, more frequent, events. A biomass sensor developed within the MELiSSA project and tested at the MELiSSA pilot plant, will allow monitoring the biofilm thickness and quantifying the detachment during backwashing, contributing to further improve the maintenance strategy. In addition,

a strategy to monitor ammonium, nitrite and nitrate on-line in the reactor effluent with high frequency and accuracy has been defined in this thesis, providing the necessary data to fulfil the requirements of the nitrite control system in the MELiSSA compartment III.

The bases for a re-design of the reactor hardware have been set taking into consideration all the necessary improvements identified in this work. The mathematical model, together with the installation of the up-graded hardware and instrumentation will provide the necessary tools to understand and improve the performance of the nitrifying compartment of the MELiSSA pilot plant, guaranteeing its stable operation over long periods of time.

REFERENCES

Abe K, Ishikawa Y, Kibe S and Nitta K (2005) Simulation model for the closed plant experiment facility of CEEF. Advances in Space Research 35, 1597-1608.

Albiol J (1994) Study of the MELiSSA photoheterotrophic compartment. Kinetics and effects of C limitation. ESA/YCL/2148.JAS ESTEC Working Paper. ESA-EWP-1808.

Aleem MIH and Sewell DL (1984) Oxydoreductase system in Nitrobacter agilis. In: Microbial Chemoautotrophy . Strhol WR and Tuovinen OH (editors). Ohio State University Press. Columbus (USA). p. 185.210.

Alegret J, Alonso J and Martínez-Fàbregas E (1989) Flow injection system for on-line potentiometric monitoring of ammonia in freshwater streams. Analyst 114, 1443-1447.

Alleman JE (1984) Elevated nitrite occurrence in biological wastewater treatment systems. Water Science and Technology 17, 409-419.

Allen JP, Nelson M and Alling A (2003) The legacy of Biosphere 2 for the study of biospherics and closed ecological systems. Advances in Space Research 31, 1629-1639.

Amann RI (1995) In situ identification of micro-organisms by whole cell hybridization with rRNA-targeted nucleic acid probes. In Akkermann, ADC, van Elsas JD and de Bruijin FJ (ed.) Molecular Microbiology Ecology Manual. Kluwer Academic Publishers, Dordrecht. P. 1-15.

Amann RI, Krumholz L and Stahl DA (1990) Fluorescent oligonucleotide probing of whole cells for determinative, phylogenetic, and environmental studies in microbiology. Journal of Bacteriology 172, 762-770.

Anderson KK and Hooper AB (1983) O_2 and H_2O are each the source of one O in NO_2^-. ^{15}N-NMR evidence. Federation of European Biochemical Societies (FEBS) Letters 164, 236-239.

Anthonisen A, Loehr RC, Prakasam TBS and Srinath EC (1976) Inhibition of nitrification by ammonia and nitrous acid. Journal of the Water Pollution Control Federation 48, 835-852.

AQUATEC S.A. (2001) AQUAMONIA A-103. Equipment for the analysis of total ammonium concentration in aqueous media. User's Manual version 7.0.

AQUATEC S.A. (2002) AQUANITRA N-103. Equipment for the analysis of nitrate in aqueous media. User's Manual version AB 7.1

Barquero Cáceres EJ (2001). Disseny, construcció i avaluació d'analitzadors per a la monitorització mediambiental. Ph.D. Thesis. Universitat Autònoma de Barcelona (in Catalan).<http://www.tdx.cat/TDX-1122104-161956>

Beccari M, Marani D and Ramadori R (1979) A critical analysis of nitrification alternatives. Water Research 13, 185-192.

Becker EW (1994) Microalgae:biotechnology and microbiology. Cambridge University Press. Cambridge, UK.

Belkin S and Boussiba S (1991) High internal pH conveys ammonia resistance in *Spirulina platensis*. Bioresource Technology 38, 167-169.

Beltran A, Artigas J, Jiménez C, Mas R, Bartrolí J and Alonso J (2002) Development of durable nitrate-selective membranes for all-solid state ISE and ISFET sensors based on photocurable compositions. Electroanalysis 14, 213-220.

Benthack C, Srinivasan B and Bonvin D (2001) An optimal operating strategy for fixed-bed bioreactors used in wastewater treatment. Biotechnology and Bioengineering 72, 34-40.

Bernet N, Sanchez O, Cesbron D, Steyer JP and Delgenès JP (2005) Modeling and control of nitrite accumulation in a nitrifying biofilm reactor. Biochemical Engineering Journal 24, 173-183.

Blackburne R, Vadivelu VM, Yuan Z and Keller J (2007a) Kinetic characterisation of an enriched *Nitrospira* culture with comparison to *Nitrobacter*. Water Research 41, 3033-3042.

Blackburne R, Vadivelu VM, Yuan Z and Keller J (2007b) Determination of growth rate and yield of nitrifying bacteria by measuring carbon dioxide uptake rate. Water Environment Research 79, 2437-2445.

Bock E, Koop HP, Harms H and Halers B (1991) The biochemistry of nitrifying organisms. In: Variations in Autotrophic Life. Shively JM and Burton, (editors). Harcourt Brace Jovanovich Publ., New York (USA).

Bock E. and Wagner M. (2001) Oxidation of inorganic nitrogen compounds as an energy source. In: The Prokaryotes: an evolving electronic resource for the microbiological community, 3rd edition, release 3.7. Dworkin M. (editor), Springer-Verlag.

Boller M, Kobler D and Koch G (1997) Particle separation, solids budgets and headloss development in different biofilters. Water Science and Technology 36, 239-247.

Boyd A and Chakrabarty AM (1994) Role of alginate lyase in cell detachment of *Pseudomonas aeruginosa*. Applied and Environmental Microbiology 60, 2355-2359.

Bron S and Venema G (1972) Ultraviolet inactivation and excision-repair in Bacillus subtillis. Construction of a transformable 8-fold auxotrophic strain and to Ultra-Violet sensitive derivatives. Mutation Research 15, 1-10.

Brown JC, Snoeyink VL, Raskin L and Lin R (2003) The sensitivity of fixed-bed biological prechlorate removal to changes in operating conditions and water quality characteristics. Water Research 37, 206-214.

Bruning-Fann CS and Kaneene JB (1993) The effects of nitrate, nitrite and N-nitroso compounds on human health: a review. Veterinary and Human Toxicology 35, 521-538.

Cabello F (2007) Cultivo en bioreactores de *Rhodospirillum rubrum* en condiciones fotoheterotróficas. Ph.D. Thesis. Universitat Autònoma de Barcelona (in Spanish). < http://www.tdx.cat/TDX-0123108-165234>

Caldwell DE, Korber DR and Lawrence JR (1992). Confocal laser scanning microscopy and digital image analysis in microbial ecology. Advances in Microbial Ecology 12, 1-67.

Carrera J (2001) Biological nitrogen removal from high load wastewater. Analysis of process parameters and design of a full-scale wastewater treatment plant. Ph.D. Thesis. Universitat Autònoma de Barcelona (in Spanish). <http://www.tdx.cat/TDX-1027104-162956/index_an.html>

Carrera J, Jubany I, Carvallo L, Chamy R and Lafuente J (2004) Kinetic models for nitrification inhibition by ammonium and nitrite in a suspended and an immobilised biomass systems. Process Biochemistry 39, 1159-1165.

Carvalho JCM, Francisco FR, Almeida KA, Sato S and Converti A (2004) Cultivation of *Arthrospira (Spirulina) platensis* (Cyanophyceae) by fed-batch addition of ammonium chloride at exponentially increasing feeding rates. Journal of Phycology 40, 589-597.

Chain, P, Lamerdin, J, Larimer, F, Regala, W, Lao, V, Land, M, Hauser, L, Hooper, A, Klotz, M, Norton, J, Sayavedra-Soto, L, Arciero, D, Hommes, N, Whittaker, M and Arp, D (2003) Complete genome sequence of the ammonia-oxidizing bacterium and obligate chemolithoautotroph *Nitrosomonas europaea*. Journal of Bacteriology 185, 2759-73.

Chambless JD and Stewart PS (2007) A three-dimensional computer model analysis of three hypothetical biofilm detachment mechanisms. Biotechnology and Bioengineering 97, 1573-1984.

Choi YC, Li X, Raskin L and Morgenroth E (2007) Effect of backwashing on perchlorate removal in fixed bed biofilm reactors. Water Research 41, 1949-1959.

Clesceri LS, Greenberg AE and Eaton AD (1998) Standard Methods for the examination of water and wastewater, 20th edition. Published by American Public Health Association, American Water Works Association and Water Environment Federation, USA.

Compas JM, Decarreau P, Lanquetin G, Estival JL, Fulget N, Martin R and Richalet J (1994) Industrial applications of predictive functional control to rolling mill, fast robot, river dam. In: The third IEEE conference on control application. Glasgow, UK, p. 1643-1655.

Converti A, Scapazzoni S, Lodi A and Carvalho CM (2006) Ammonium and urea removal by *Spirulina platensis*. Journal of Industrial Microbiology and Biotechnology 33, 8-16.

Cornet JF and Albiol J (2000) Modelling photoheterotrophic growth kinetics of *Rhodospirillum rubrum* in rectangular photobioreactors. Biotechnology Progress 16, 199-207.

Costa E, Pérez J and Kreft JU (2006) Why is metabolic labour divided in nitrification? Trends in Microbiology 14, 213-219.

Costa JAV, Cozza KL, Oliveira L and Magagnin G (2001) Different nitrogen sources and growth responses of Spirulina platensis in microenvironments. World Journal of Microbiology and Biotechnology 17, 439-442.

Creus N, Cabello F, Pérez J, Albiol J, Gòdia F and Lasseur C (2001) Connection between different compartments of the MELiSSA biological life support system. Proceedings of the 31st International Conference on Environmental Systems, Orlando (USA).

Creus N (2003) Estudi d'un bucle de bioreactors pel desenvolupament d'un sistema de suport de vida biològic. Ph.D. Thesis. Universitat Autònoma de Barcelona. (In Catalan) < http://www.tdx.cat/TDX-0621104-153210>.

Degrange V and Bardin R (1995) Detection and counting of *Nitrobacter* populations in soil by PCR. Applied and Environmental Microbiology 61, 2093-2098.

Dionisi HM, Layton AC, Robinson KG, Brown JR, Gregory IR, Parker JJ and Sayler GS (2002) Quantification of *Nitrosomonas oligotropha* and *Nitrospira* spp. using competitive polymerase chain reaction in bench-scale wastewater treatment reactors operating at different solids retention times. Water Environment Research 74, 462-469.

Dorigo U, Volatier L and Humbert JF (2005) Review. Molecular approaches to the assessment of biodiversity in aquatic microbial communities. Water Research 39, 2207-2218.

Drysdale AE, Ewen MK and Hanford AJ (2003) Life Support approaches for Mars missions. Advances in Space Research 31, 51-61.

Egorov VV, Borisenko ND, Rakhman'ko EM, Lushchik YF and Kacharsky FF (1997) The effect of the ion exchanger site-counterion complex formation on the selectivities of ISEs. Talanta 44, 1735-1747.

Elenter D, Milferstedt K, Zhang W, Hausner M and Morgenroth E (2007) Influence of detachement on substrate removal and microbial ecology in a heterotrophic/autotrophic biofilm. Water Research 41, 4657-4671.

Ensafi A and Kazemzadeh A (1999) Simultaneous determination of nitrite and nitrate in various samples using flow injection with spectrophotometric detection. Analytica Chimica Acta 382, 15-21

Fdz-Polanco F, Méndez E, Urueña MA, Villaverde S and García PA (2000) Spatial distribution of heterotrophs and nitrifiers in a submerged biofilter for nitrification. Water Research 34, 4081-4089

Fliermans CB, Bohlool BB and Schmidt EL (1974) Autoecological study of the chemoautotroph *Nitrobacter* by immunofluorescence. Applied Microbiology 27, 124-129.

Forler C (1994) Development of a fixed bed pilot reactor for a continuous axenic co-culture of *Nitrosomonas europaea* and *Nitrobacter winogradskyi*. YGT ESA/YCL-X-997

Gheewala SH, Pole RK and Annahhatre AP (2004) Nitrification modelling in biofilms under inhibitory conditions. Water Research 38, 3179-3188.

Gill P, Murray W and Wright M (1981) Practical optimisation. Academic Press. London.

Gitelson II, Terskov IA, Kovrov BG, Sidko FY, Lisovsky GM, Okladnikov YN, Belyanin VN, Trubachov IN and Rerberg MS (1976) Life support system with autonomous control employing plant photosynthesis. Acta Astronautica 3, 633-650.

Gitelson II, Terskov IA, Kovrov BG, Lisovskii GM, Okladnikov YN, Sid'ko FY, Trubachev IN, Shilenko MP, Alekseev SS, Pan'kova IM and Tirranen LS (1989) Long term experiments on man's stay in biological life support system. Advances in Space Research 9, 65-71.

Gjaltema A (1996) Biofilm development: growth versus detachment. PhD thesis. Delft University of Technology.

Gòdia F, Albiol J, Montesinos JL, Pérez J, Creus N, Cabello F, Mengual X, Montras A and Lasseur Ch (2002) MELiSSA: a loop of interconnected bioreactors to develop life support in Space. Journal of Biotechnology 99, 319-330.

Guisasola A, Chandran K, Smets BF, Baeza JA, Carrera J and Lafuente J (2006) Observation and mathematical description of the acceleration phenomenon in batch respirograms associated with ammonium oxidation. Water Science and Technology 54, 181-188.

Hanaki K, Wantawin C and Ohgaki S (1990a) Effects of the activity of heterotrophs on nitrification in a suspended-growth reactor. Water Research 24, 289-296.

Hanaki K, Wantawin C and Ohgaki S (1990b) Nitrification at low level of dissolved oxygen with and without organic loading in a suspended-growth reactor. Water Research 24, 297-302.

Hansen TA and Gemerden H (1972) Sulphide utilisation by purple nonsulfur bacteria. Archives of Microbiology 86, 49-56.

Hartmann, A, Lawrence JR, Assmus B and Schloter M (1998) Detection of microbes by laser scanning confocal microscopy. Molecular Microbial Ecology Manual 4, p. 1-34.

Hendrickx L and Wuertz S (2004) Investigating *in situ* natural genetic transformation of *Acinetobacter* sp. BD413 in biofilms with confocal laser scanning microscopy. Genetic Engineering 26, 159-173.

Hendrickx L, De Wever H, Hermans V, Mastroleo F, Morin N, Wilmotte A, Janssen P and Mergeay M (2006) Microbial ecology of the closed artificial ecosystem MELiSSA (Micro-Ecological Life Support System Alternative): Reinventing and compartmentalizing the Earth's food and oxygen regeneration system for long-haul space exploration missions. Research in Microbiology 157, 77-86.

Hendrickx L and Mergeay M (2007) From the deep sea to the stars: human life support through minimal communities. Current Opinion in Microbiology 10, 231-237.

Hendrikus J, Laambroek HJ and Gerards S (1993) Competition for limiting amounts of oxygen between *Nitrosomonas europaea* and *Nitrobacter winogradskyi* grown in mixed cultures. Microbiology 159, 453-459.

Henze M, Gujer W, Mino T and van Loosdrecht MCM (2000) Activated sludge models ASM1, ASM2, ASM2D and ASM3. Scientific and technical report n. 9. London: IWA publishing.

Hugenholtz P, Tyson GW and Blackall L (2001) Design and evaluation of 16S rRNA-targeted oligonucleótido probess for fluorescence *in situ* hybridization. Methods in Molecular Biology 176, 29-41

Hunik JH, Tramper J and Meijer GH (1992) Kinetics of *Nitrosomonas europaea* at extreme substrate, product and salt concentration. Applied Microbiology and Biotechnology 37, 802-807.

Hunik JH, Meijer GH and Tramper J (1993) Kinetics of *Nitrobacter agilis* at extreme substrate, product and salt concentration. Applied Microbiology and Biotechnology 40, 442-448.

Hunik JH (1993) Engineering aspects of nitrification with immobilized cells. PhD Thesis.

Hunik JH, Bos CG, Den Hoogen MP, De Gooijer CD and Tramper J (1994). Co-immobilized *Nitrosomonas europaea* and *Nitrobacter agilis* cells: validation of a dynamic model for simultaneous substrate conversion and growth in K-carrageenan gel beads. Biotechnology and Bioengineering 43, 1153-1163.

Hyman MR and Arp DJ (1992) $^{14}C_2H_2$- and $^{14}CO_2$-labeling studies of the de novo synthesis of polypeptides by Nitrosomonas europaea during recovery form acetylene and light inactivation of ammonia monooxygenase. The Journal of Biological Chemistry 267, 1534-1545.

Jassby A (1988) *Spirulina:* A model for microalgae as human food. In: Algae and human affairs. Lembi and Waaland (editors). Cambridge University Press. Cambridge (UK).

Kim DJ and Kim SH (2006) Effect of nitrite concentration on the distribution and competition of nitrite-oxidizing bacteria in nitratation reactor systems and their kinetic characteristics. Water Research 40, 887-894.

Kindaichi T, Kawano Y, Ito T, Satoh H and Okabe S (2006) Population dynamics and in situ kinetics of nitrifying bacteria in autotrophic nitrifying biofilms as determined by real-time quantitative PCR. Biotechnology and Bioengineering 94 (6), 1111-1121.

Kirisits MJ, Snoeyink VL, Inan H, Chee-Sanford JC, Raskin L and Brown JC (2001) Water quality factors affecting bromate reduction in biologically active carbon biofilters. Water Research 35, 891-900.

Klotz MG, Alzerreca J and Norton JM (1997) A gene encoding a membrane protein exists upstream of amoA/amoB genes in ammonia oxidizing bacteria: a third member of the amo operon? FEMS Microbiology Letters 150, 65-73.

Kompatseva EI (1981) Utilisation of sulphide by nonsulfur purple bacteria *Rhodopseudomonas capsulata.* Microbiologia 50, 429-436.

Koops HP, Böttcher B, Möller UC, Pommerening-Röser A and Stehr G (1990) Description of a new species of *Nitrosococcus*. Archives of Microbiology 154, 244-248

Kowalchuk GA, Stephen JR, De Boer W, Prosser JI, Embley TM and Woldendorp JW (1997) Analysis of ammonia-oxidizing bacteria of the *beta* subdivision of the class *Proteobacteria* in coastal sand dunes by denaturing gradient gel electrophoresis and sequencing of PCR-amplified 16S ribosomal DNA fragments. Applied and Environmental Microbiology 63, 1489-97.

Lackner S, Terada A and Smets BF (2008) Heterotrophic activity compromises autotrophic nitrogen removal in membrane-aerated biofilms: Results of a modelling study. Water Research 42, 1102-1112.

Laspidou CS and Rittmann B (2004a) Modeling the development of biofilm density including active bacteria, inert biomass, and extracellular polymeric substances. Water Research 38, 3349-3361.

Laspidou CS and Rittmann B (2004b) Evaluating trends in biofilm density using the UMCCA model. Water Research 38, 3362-3372.

Laurent P, Kihn A, Andersson A and Servais P (2003) Impact of backwashing on nitrification in the biological activated carbon filters used in drinking water treatment. Environmental Technology 22, 277-287.

Lasseur Ch, Verstraete W, Gros JB, Dubertret G and Rogalla F (1996) MELiSSA: a potential experiment for a precursor mission to the Moon. Advances in Space Research 18, 111-117.

Lasseur Ch and Vieira da Silva L (2005) Yearly report for 2004 activity. Prepared by the MELiSSA partners. Memorandum of understanding TOS-MCT/2002/3161/In/CL.

Lawrence JR, Neu TR and Swerhone GDW (1998) Application of multiple parameter imaging for the quantification of algal, bacterial and exopolymer components of microbial biofilms. Journal of Microbiological Methods 32, 253-261.

Lehninger A, Nelson DL and Cox MM (2004) Principles of biochemistry. 4th edition. WH Freeman. ISBN 0-7167-4339-6.

Le Tallec X, Zeghal S, Vidal A and Lesouëf A (1997) Effect of influent quality variability on biofilter operation. Water Science and Technology 36, 111-117.

Lewandowski Z (2000) Notes on biofilm porosity. Water Research 34, 2620-2624.

Lobo M and Lasseur C (2003) Yearly report for 2002 activity. Prepared by the MELiSSA partners. Memorandum of understanding TOS-MCT/2002/3161/In/CL.

Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadhukumar, Buchner, A., Lai, T., Steppi, S., Jobb, G., Forster, W., Brettske, I., Gerber, S., Ginhart, A., Gross, O., Grumann, S., Hermann, S., Jost, R., Konig, A., Liss, T., Lussmann, R., May, M., Nonhoff, B., Reichel, B., Strehlow, R., Stamatakis, A., Stuckmann, N., Vilbig, A., Lenke, M., Ludwig, T., Bode, A. and Scheleifer, K. (2004) ARB: a software environment for sequence data. Nucleic Acids Research 32, 1363-1371.

Lydmark P, Lind M, Sörensson F and Hermansson M (2006) Vertical distribution of nitrifying populations in bacterial biofilms from a full-scale nitrifying trickling filter. Environmental Microbiology 8, 2036-2049.

Masot A (2007) Engineering photosynthetic systems for bioregenerative life support. Ph.D. Thesis. Universitat Autònoma de Barcelona. <http://www.tdx.cat/TDX-1107107-130048/>

Matsumoto S, Terada A and Tsuneda S (2007) Modelling of membrane aerated biofilm: effects of C/N ratio, biofilm thickness and surface loading of oxygen on feasibility of simultaneous nitrification and denitrification. Biochemical Engineering Journal 37, 98-107.

McTavish H, Fuchs JA and Hooper AB (1993) Sequence of the gene coding for ammonia monooxygenase in Nitrosomonas europaea. Journal of Bacteriology 175, 2436-2444.

Meleshko GI, Sheoelev YY, Averner M and Volk T (1991) Biological life support systems, in: Nicogossian AE, Mohler SR, Gazenco OG and Grigoryev AI (Eds), Space Biology and Medicine. American Institute of Aeronautics Publishers, Washington DC, pp. 357-394.

Mergeay M, Verstraete W, Dubertret G, Lefort-Tran M, Chipaux C and Binot R (1988) MELiSSA – a microorganisms based model for CELSS development.

Proceedings of the 3rd symposium on space thermal control and life support systems. Noordwijk, The Netherlands, 3-6 October 1988, p. 65-68.

Mitchell CA (1994) Bio-regenerative life support system. American Journal of Clinical Nutrition 60, 820-824.

Moorcroft MJ, Davis J and Compton RJ (2001) Detection and determination of nitrate and nitrite: a review. Talanta 54, 785-803.

Montràs A, Pycke B, Boon N, Gòdia F, Mergeay M, Hendrickx L and Pérez J (2008) Distribution of *Nitrosomonas europaea* and *Nitrobacter winogradskyi* in an autotrophic nitrifying biofilm reactor as depicted by molecular analyses and mathematical modelling. Water Research 42, 1700-1714

Morgenroth E and Wilderer PA (2000) Influence of detachment mechanisms on competition in biofilms. Water Research 34, 417-426.

Mulder A, Van de Graaf AA, Robertson LA and Kuenen JG (1995) Anaerobic ammonium oxidation discovered in a denitrifying Fluidized bed reactor. FEMS Microbiology Ecology 16, 177-183.

Ni BJ and Yu HQ (2008) Kinetic modelling microbial storage process in activated sludge under anoxic conditions. Chemical Engineering Science 63, 2785-2792.

Nogueira R, Melo LF, Purkhold U, Wuertz S and Wagner M (2002) Nitrifying and heterotrophic population dynamics in biofilm reactors: effects of hydraulic retention time and the presence of organic carbon. Water Research 36, 469-481.

Noto K, Ogasawara T, Suwa Y and Sumino T (1998) Complete oxidation of high concentration of ammonia by retaining incompatible nitrification activities in three-vessel system. Water Research 32, 769-773.

Okabe S, Satoh H and Watanabe Y (1999) In situ analysis of nitrifying biofilms as determined by in situ hybridization and the use of microelectrodes. Applied and Environmental Microbiology 65, 3182-3196.

Olson TC and Hooper AB (1983) Energy consumption in the bacterial oxidation of small molecules: an extracytoplasmatic dehydrogenase in Nitrosomonas. FEMS Microbiology Letters 19, 47-50.

Pan PHC (1971) Lack of distinction between *Nitrobacter agilis* and *Nitrobacter winogradskyi*. Journal of Bacteriology 108, 1416-1418.

Pérez J (2001) Utilización de *Nitrosomonas* y *Nitrobacter* en forma de biopelícula para la nitrificación biológica en reactores de lecho fijo. PhD Thesis. Universitat Autònoma de Barcelona (in Spanish) <http://www.tdx.cat/TDX-0802101-093242/index_an.html>.

Pérez J, Montesinos JL, Albiol J and Gòdia F (2004) Nitrification by immobilized cells in a micro-ecological life support system using packed-bed bioreactors: an engineering study. Journal of Chemical Technology and Biotechnology 79, 742-754.

Pérez J, Poughon L, Dussap CG, Montesinos JL and Gòdia F (2005) Dynamics and steady state operation of a nitrifying fixed bed biofilm reactor: mathematical model based description. Process Biochemistry 40, 2359-2369.

Pérez J, Montesinos JL and Gòdia F (2006) Gas-liquid mass transfer in an up-flow cocurrent packed-bed biofilm reactor. Biochemical Engineering Journal 31, 188-196.

Persson F, Wik T, Sörensson F and Hermansson M (2002) Distribution and activity of ammonia oxidizing bacteria in a large full-scale trickling filter. Water Research 36, 1439-1448.

Picioreanu C, van Loosdrecht MCM, Heijnen JJ (1997) Modeling the effect of oxygen concentration on nitrite accumulation in a biofilm airlift suspensión reactor. Water Science and Technology 36,147-156.

Pirsing A, Wiesman G, Kelterbach G, Schaffranietz U, Röck H, Eichner B, Skuzal S and Schulze G (1996) On-line monitoring and modelling based process control of high rate nitrification – lab scale experimental results. Bioprocess Engineering 15,181-188.

Poughon L, Dussap CG and Gros JB (1999) Dynamic model of a nitrifying fixed bed column: simulation of the biomass distribution of Nitrosomonas and Nitrobacter and of transient behaviour of the column. Bioprocess Engineering 20, 209-221.

Poughon L, Duchez JF, Cornet CG and Dussap CG (2003) Kla determination: comparative study for a gas mass balance method. Bioprocess and Biosystems Engineering 25, 341-348.

Prosser JI (1989) Autotrophic nitrification in bacteria. Advances in Microbial Physiology 30, 125-181.

Ralston M and Jennrich R (1978) DUD – a derivative-free algorithm for non-linear least squares. Technometrics 20, 7-14.

Reichert P (1998) AQUASIM 2.0-Computer program for the identification and simulation of aquatic systems. EAWAG, Dübendorf, Switzerland.

Richalet J (1993) Industrial applications of model based predictive control. Automatica 29, 1251-1274.

Ruzicka J and Hansen EH (1980) Flow injection analysis. Principles, applications and trends. Analytica Chimica Acta 114, 19-44.

Sandblom J, Eisenman G and Walker JL (1967) Electrical phenomena associated with the transport of ions and ion pairs in liquid ion-exchange membranes. I. zero current properties. The Journal of Physical Chemistry 71, 3862-3870.

Sastry BN, DeLosReyes AA Jr, Rusch KA and Malone RF (1999) Nitrification performance of a bubble-washed bead filter for combined solids removal and biological filtration in a recirculating aquaculture system. Aquacultural Engineering 19, 105-117.

Schramm A, de Beer D, van den Heuvel JC , Ottengraf S and Amann R (1999) Microscale distribution of populations and activities of *Nitrosospira* and *Nitrospira spp*. Along a macroscale gradient in a nitrifying bioreactor: quantification by in situ hybridization and the use of microelectrodes. Applied and Environmental Microbiology 65, 3690-3696.

Schramm A. (2003) In situ analysis of structure and activity of the nitrifying community in biofilms, aggregates and sediments. Geomicrobiological Journal 20, 313-333.

Schumpe A, Quicker G and Deckver WD (1982) Gas solubilities in microbial culture media. Advances in Biochemical Engineering 24, 1-38.

Sekizawa S, Fujie K, Kubota H, Kasakura T and Mizuno A (1985) Air diffuser performance in activated sludge aeration tanks. Journal of the Water Pollution Control Federation 57, 53-59.

Sinha B and Annachhatre AP (2007) Partial Nitrification – operational parameters and microorganisms involved. Reviews in Environmental Science and. Biotechnology 6, 285-313.

Soejima K, Matsumoto S, Ohgushi S, Naraki K, Terada A, Tsuneda S and Hirata A (2008) Modelling and experimental study on the anaerobic/aerobic/anoxic process for simultaneous nitrogen and phosphorus removal: The effect of acetate addition. Process Biochemistry 43, 605-614.

Soletto D, Binaghi L, Lodi A, Carvalho JCM and Converti A (2005) Batch and fed-batch cultivations of *Spirulina platensis* using ammonium sulphate and urea as nitrogen sources. Aquaculture 243, 217-224.

Starkenburg SR, Chain, PS, Sayavedra-Soto, LA, Hauser, L, Land, ML, Larimer, FW, Malfatti, SA, Klotz, MG, Bottomley, PJ, Arp, DJ and Hickey, WJ (2006) Genome sequence of the chemolithoautotrophic nitrite-oxidizing bacterium *Nitrobacter winogradskyi* Nb-255. Applied and Environmental Microbiology 72, 2050-63.

Strathmann M, Wingender J and Flemming H (2002) Application of fluorescently labelled lectins for the visualisation and biochemical characterization of polysaccharides in biofilms of *Pseudomonas aeruginosa.* Journal of Microbiological Methods 50, 237-248.

Tamponnet C and Savage C (1994) Closed ecological systems. Journal of Biological Education 28, 167-173.

Terada A, Lackener S, Tsuneda S and Smets BF (2007) Redox-stratification controlled biofilm (ReSCoBi) for completely autotrophic nitrogen removal: The effect of co- versus counter- diffusion on reactor performance. Biotechnology and Bioengineering 97, 40-51.

Tokutomi T (2004) Operation of a nitrite-type airlift reactor at low DO concentration. Water Science and Technology 49, 81-88.

Vadivelu VM, Keller J and Yuan Z (2006a) Stoichiometric and kinetic characterisation of *Nitrosomonas spp*. In mixed culture by decoupling the growth and energy generation processes. Journal of Biotechnology 126, 342-356.

Vadivelu VM, Yuan Z, Fux C and Keller J (2006b) Stoichimetric and kinetic characterisation of *Nitrobacter* in mixed culture by decoupling the growth and energy generation processes. Biotechnology and Bioengineering 94, 1176-1188.

Van de Graaf AA, Mulder A, de Bruijn P, Jetten MSM, Robertson LA and Kuenen JG (1995) Anaerobic oxidation of ammonium is a biologically mediated process. Applied and Environmental Microbiology 61, 1246-1251.

Van Loosdrecht MCM and Heijnen JJ (1993) Bioreactors in wastewater treatment. Trends in Biotechnology 11, 117-121.

Vanrolleghem P, Sin G and Gernaey K (2004) Transient response of aerobic and anoxic activated sludge activities to sudden concentration changes. Biotechnology and Bioengineering 86, 277-290.

Vernerey A, Albiol J, Lasseur C and Gòdia F (2001) Scale-up and design of a pilot plant photobioreactor for the continuous culture of *Spirulina platensis*. Biotechnology Progress 17, 431-438.

Wagner M, Horn M and Daims H (2003) Fluorescence *in situ* hybridisation for the identification and characterisation of prokaryotes. Current Opinion In Microbiology 6, 302-309

Wahman DG, Katz LE and Speitel GE Jr (2006) Modeling of trihalomethane cometabolism in nitrifying biofilters. Water Research 41, 449-457.

Wanner O and Gujer W (1986) A multi-species biofilm model. Biotechnology and Bioengineering 28, 314-328.

Wanner O and Reichert P (1996) Mathematical modelling of mixed-culture biofilms. Biotechnology and Bioengineering 49, 172-184.

Wanner O and Morgenroth E (2004) Biofilm modelling with AQUASIM. Water Science and Technology 4, 137-144.

Wanner O, Eberl HJ, Morgenroth E, Noguera DR, Picioreanu C, Rittmann BE and van Loosdrecht MCM (2006) Mathematical Modeling of Biofilms. Series: Scientific and Technical Report Series Report No. 18. IWA Publishing, London, UK.

Waters G, Masot A, Albiol J, Gòdia F and Dixon M (2004) Static mass balance studies of the MELiSSA Pilot Plant: integration of a Higher Plant Chamber.

Proceedings of the 34th International Conference on Environmental Systems (ICES). July 19-22, 2004. Colorado Springs (USA). SAW paper No. 2004-01-2579.

Waters G, Gidzinski D, Dixon M, Masot A, Gòdia F, Paillé C, Lamaze B and Lasseur C (2007) Integration of a Higher Plant Chamber into the European Space Agency's MELiSSA Pilot Plant: The canadian role in Advanced Life Support test-bed development. 2nd International Workshop - Exploring Mars and its Earth analogues. June 19-23, 2007. Trento (Italy).

Watson SW (1974) In: Bergey's manual of determinative bacteriology, 8th Ed.. Buchanan RE and Gibbons NE (Eds.), Williams and Wilkins. Baltimore, USA.

Watson ST, Bock E, Harms H, Koops HP and Hooper AB (1989) In: Bergey's Manual of Systematic Bacteriology. Staley JT, Bryant MP, Pfenning N and Holt JG (editors). Williams and Wilkins, Baltimore (USA), 3 (20), 1807-1835.

Watson SW, and Waterbury JB (1971) Characteristics of two marine nitrite oxidizing bacteria, *Nitrospina gracilis* nov.gen. nov.sp. and *Nitrococcus mobilis* nov.gen.nov.sp. Archives of Microbiology 77, 203-230.

Wik T (1999) On modelling the dynamics of fixed biofilm reactors. PhD thesis. Chalmers University of Technology. ISBN91-7197-797-X. <http://www.s2.chalmers. Se /publications>

Wik T (2003) Trickling filters and biofilm reactor modelling. Reviews in Environmental Science and Biotechnology 2, 193-212.

Wilderer PA and Characklis WG (1989) Structure and function of biofilms. In "Structure and function of biofilms" (eds. WG Characklis and PA Wilderer). John Wiley & Sons, New York, USA. p. 5-17.

Wingender J, Flemming HC and Neu TR (1999) Introduction. What are bacterial extracellular polymeric substances? In Wingender J, Neu TR and Flemming HC (editors). Microbial extracellular polymeric substances. Berlin (Germany). p. 1-19.

Wolfaardt GM, Lawrence JR, Robarts RD and Caldwell DE (1998). In situ characterisation of biofilm exopolymers involved in the accumulation of chlorinated organics. Microbial Ecology 35, 213-223

Wolfaardt GM, Lawrence JR and Korber DR (1999) Function of EPS. In: Wingender J, Neu TR and Flemming HC (editors). Microbial extracellular polymeric substances. Berlin: Springer p. 171-200.

Yang X, Beyenal H, Harkin G and Lewandowsky Z (2001) Evaluation of biofilm image thresholding methods. Water Research 35, 1149-1158.

Yun HJ and Kim DJ (2003) Nitrite accumulation characteristics of high strength ammonia wastewater in an autotrophic nitrifying biofilm reactor. Journal of Chemical Technology & Biotechnology 78, 377-383.

ANNEX

ABBREVIATIONS AND ACRONYMS

Acronyms

AOB	Ammonia Oxidising Bacteria
CII	MELiSSA Compartment II (*Rhodospirillum rubrum*)
CIII	MELiSSA Compartment III (Nitrifying compartment)
CIVa	MELiSSA Compartment Iva (*Arthrospira platensis*)
CIVb	MELiSSA Compartment Ivb (Higher Plant Compartment, HPP)
CLSM	Confocal Laser Scanning Microscopy
CSTR	Continuously Stirred Tank Reactor
DO	Dissolved Oxygen
EPS	Extracellular Polymeric Substances
FA	Free Ammonia
FIA	Flow Injection Analysis
FNA	Free Nitrous Acid
FISH	Fluorescent *in situ* hybridisation
FITC	Fluorescein IsoThioCyanate
HB	Heterotrophic bacteria
HPP	Higher Plant Compartment
ISE	Ion Selective Electrode
MELiSSA	Microbial Ecological Life Support System Alternative
MPP	MELiSSA Pilot Plant
NEDD	N-(1-naphthyl)-EhtylenDiamine Dihydrochloride
NOB	Nitrite Oxidising Bacteria
Q-PCR	Quantitative Polymerase Chain Reaction
RTD	Residence Time Distribution

Model parameters and abbreviations

a	Correction of the AOB kinetics due to a wake-up phenomenon
A	Area
C	Concentration of substrates / products in the bulk liquid (g L^{-1})
D	Reactor diameter (dm)
d_P	Substratum particle diameter (dm)
f	Back mixing coefficient
f_P	Fraction of biomass leading to inert products
LF	Biofilm thickness (µm)
$K_L \cdot a$	Volumetric oxygen transfer coefficient (h^{-1})
Q_{rec}	Recirculation flow rate ($L \cdot d^{-1}$)
Q_{bmix}	Back mixing flow rate ($L \cdot d^{-1}$)
Q	Incoming liquid flow rate ($L \cdot d^{-1}$)
Q_G	Gas flow rate ($L \cdot min^{-1}$)
u_{det}	Detachment rate ($dm \cdot d^{-1}$)
u_F	Growth velocity of the biofilm (dm d^{-1})
u_G	Gas superficial velocity (m s^{-1})
V	Volume (dm^3)
X	Concentration of biomass in the biofilm (g VSS L^{-1})
$Y_{X/S}^{Nts}$	Growth yield coefficient of *N. europaea* (g VSS g^{-1} $N\text{-}NH_4^-$)
$Y_{X/S}^{Ntb}$	Growth yield coefficient of *N. winogradskyi* (g VSS g^{-1} $N\text{-}NO_2^-$)

$Y_{X/S}^{H}$	Growth yield coefficient of heterotrophic bacteria (g VSS g^{-1} VSS)
z	Distance to substratum surface (μm)
ρ	Biofilm density (g VSS L^{-1} biofilm matrix)
ε_P	Solids hold up
ε_G	Gas hold up
ε_L	Liquid hold up
μ	Specific growth rate of biomass (d^{-1})

Subscripts

A	Bottom section of the reactor
B	Packed bed section of the reactor
C	Top section of the reactor for gas-liquid separation
bmix	Back mixing
det	Detachment
F	Biofilm
G	Gas
LF	Biofilm thickness (dm)
in	Incoming
In	Refers to an inhibiting substrate
L	Liquid
N	Number of tanks into which the packed bed was divided
NH_4^+	Ammonium
NO_2^-	Nitrite
NO_3^-	Nitrate
O_2	Oxygen
rec	Recirculation
S	Soluble biodegradable substrate

Superscripts

H	Heterotrophic bacteria
I	Inert biomass obtained from biomass decay
Nts	*N. europaea*
Ntb	*N. winogradskyi*
P	Non-biodegradable products of biomass decay
S	Biodegradable products of biomass decay

BACTERIAL STRAINS AND GROWTH MEDIA

The strains selected to perform the two-step biological nitrification in the MELiSSA nitrifying compartment are *N. europaea* ATCC 19718 and *N. winogradskyi* ATCC 25391, which perform the oxidation of ammonia to nitrite and of nitrite to nitrate respectively.

ATCC GROWTH MEDIA

Recovery of the strains of *N. europaea* ATCC 19718 and *N. winogradskyi* ATCC 25391 was carried out following the protocols provided by ATCC and presented in Table 9.1 and Table 9.3, respectively.

Frozen *N. europaea* samples were recovered by transferring the contents of the vial into and Erlenmeyer flask containing 50 mL of ATCC medium 221 (Table 9.1), and subsequently incubating the suspension at 27ºC in darkness.

Table 9.1 Composition of ATCC medium 221 for *N. europaea.* (*)Composition specified in Table 9.2. (†)Magnesium and calcium solutions are added after sterilisation. Bring to pH 8.2 with 50% K_2CO_3.

Components	Amounts
$(NH_4)_2SO_4$	3.0 g
K_2HPO_4	0.5 g
$NaH_2PO_4 \cdot 2H_2O$	0.78 g
Fe/EDTA solution*	0.1 mL
$MgSO_4$ †	0.05 g
$CaCl_2$ †	$4 \cdot 10^{-3}$ g
cresol red (0.0005%)	25 mL
MilliQ water	Up to 1000 mL

Table 9.2 Composition of the Fe/EDTA solution

Components	Amounts
EDTA	0.14 g
$FeSO_4 \cdot 7H_2O$	0.5 g
H_2SO_4	0.05 mL
MilliQ water	Up to 100 mL

N. winogradskyi was received as a lyophilised form and was recovered by resuspending in 0.5 mL of ATCC 480 medium (Table 9.3).

Table 9.3 Composition of ATCC medium 480 for *N. winogradskyi*. Bring to pH 8.0 with 50% K_2CO_3. (*)Composition specified in Table 9.2. The composition of solutions A, B, D, E and F can be found in Table 9.4.

Components	Amounts (per 1L MilliQ water)
Solution A	0.5 mL
Solution B	0.5 mL
Fe/EDTA solution*	0.1 mL
Solution D	0.5 mL
Solution E	0.5 mL
Solution F	10 μL

Table 9.4 ATCC medium 480 for *N. winogradskyi.:* composition of solutions A, B, D, E and F.

Solution	Components	Amount
Solution A	$CaCl_2$	2.0 g
	MilliQ water	100 mL
Solution B	$MgSO_4 \cdot 7H_2O$	20.0 g
	MilliQ water	100 mL
Solution D	$Na_2MoO_4 \cdot 2H_2O$	0.1 g
	$ZnSO_4 \cdot 7H_2O$	0.1 g
	$CuSO_4 \cdot 5H_2O$	0.02 g
	$CoCl_2 \cdot 6H_2O$	0.002 g
	$MnCl_2 \cdot 4H_2O$	0.2 g
	MilliQ water	1000 mL
Solution E	$NaNO_2$	41.4 g
	MilliQ water	100 mL
Solution F	K_2HPO_4	1.71 g
	MilliQ water	100 mL

N. europaea AND *N. winogradskyi* CO-CULTURE

The composition of the medium used for cultivation of a co-culture of *N. europaea* and *N.winogradskyi*, both in Erlenmeyer flasks and in bioreactor is presented in Table 9.5.

Table 9.5 Composition of the culture medium used in CIII. pH is set to 8.1-8.2 with Na_2CO_3; $MgSO_4 \cdot 7H_2O$ and $CaCl_2 \cdot 2H_2O$ are sterilised separately and subsequently added to the main solution.

Compound	Concentration ($g \cdot L^{-1}$)
$(NH_4)_2SO_4$	1.32
$FeSO_4 \cdot 7H_2O$	0.0025
$CuSO_4 \cdot 5H_2O$	$4 \cdot 10^{-6}$
Na_2HPO_4	0.71
KH_2PO_4	0.68
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.177
$ZnSO_4 \cdot 7H_2O$	$4.3 \cdot 10^{-6}$
$MgSO_4 \cdot 7H_2O$	0.052
$CaCl_2 \cdot 2H_2O$	$7.4 \cdot 10^{-4}$
$NaHCO_3$	0.8

PUBLICATIONS

INTERNAL TECHNICAL REPORTS

Montràs A, Hendrickx, L and Pérez J (2008) Biofilm studies in compartment III pilot reactor: analysis and discussion of the results. MELiSSA Technical Note 78.92. European Space Agency ESA/CCN7 to contract 13292/98/NL/MV.

Montràs A, Hendrickx, L and Pérez J (2007) Biofilm studies in compartment III pilot reactor: experimental set-up and technical description. Technical Note 78.91. European Space Agency ESA/CCN7 to contract 13292/98/NL/MV.

Montràs A, Pérez J, Peiró, E and Gòdia F (2007) Technical specifications for the re-design of the compartment III pilot reactor. MELiSSA Technical Note 78.61. European Space Agency ESA/CCN7 to contract 13292/98/NL/MV.

Montràs A, Pérez J and Gòdia F (2007) Compartment III nitrogen sources instrumentation upgrade. MELiSSA Technical Note 66.1. European Space Agency ESA/CCN7 to contract 13292/98/NL/MV.

Montràs A, Pérez J and Gòdia F (2006). On-line instrumentation for N balance: installation and characterisation. MELiSSA Technical Note 52.22. European Space Agency ESA/ESTEC/CONTRACT13292/98/NL/MV.

Montràs A, Pérez J and Gòdia F (2004).Nitrite predictor complementary tests. MELiSSA Technical Note 52.4. European Space Agency ESA/ESTEC/CONTRACT13292/98/NL/MV.

Montràs A, Pérez J and Gòdia F (2002). On-line instrumentation for N balance. MELiSSA Technical Note 52.21. European Space Agency ESA/ESTEC/CONTRACT13292/98/NL/MV.

Creus N, Montràs A, Masot A, Pérez J, Albiol J and Gòdia F (2002) Pilot design of the MELiSSA solid loop. MELiSSA Technical Note 62.5. European Space Agency ESA/ESTEC/CONTRACT13292/98/NL/MV.

Albiol J, Creus N, Pérez J, Montràs A, Masot A and Gòdia F (2002) Pilot Plant liquid loop review. MELiSSA Technical Note 62.3. European Space Agency ESA/ESTEC/CONTRACT13292/98/NL/MV.

Pérez J, Albiol J, Creus N, Montràs A, Masot A and Gòdia F (2002) Pilot Plant gas loop review. MELiSSA Technical Note 62.4. European Space Agency ESA/ESTEC/CONTRACT13292/98/NL/MV.

PUBLICATIONS IN INTERNATIONAL JOURNALS

Montràs A, Pycke B, Boon N, Gòdia F, Mergeay M, Hendrickx L and Pérez J (2008) Distribution of *Nitrosomonas europaea* and *Nitrobacter winogradskyi* in an autotrophic nitrifying biofilm reactor as depicted by molecular analyses and mathematical modelling. Water Research 42, 1700-1714.

Gòdia F, Albiol J, Pérez J, Creus N, Cabello F, Montràs A, Masot A and Lasseur Ch. (2004) The MELISSA pilot plant facility as an integration test-bed for advanced life support systems. Advances in Space Research 34, 1483-1493.

Gòdia F, Albiol J, Montesinos JL, Pérez J, Creus N, Cabello F, Mengual X, Montràs A and Lasseur Ch (2002) MELISSA: a loop of interconnected bioreactors to develop life support in space. Journal of Biotechnology 99, 319-330.

CONTRIBUTION TO INTERNATIONAL SCIENTIFIC CONFERENCES

Montràs A, Pycke B, Boon N, Gòdia F, Mergeay M, Hendrickx L and Pérez J (2007) Distribution of *Nitrosomonas europaea* and *Nitrobacter winogradskyi* in a packed-bed biofilm reactor as depicted by molecular analyses and mathematical modelling. Proceedings of the II International Conference on Environmental, Industrial and Applied Microbiology (BioMicroWorld2007). Sevilla, Spain.

Montràs A, Pycke B, Boon N, Gòdia F, Mergeay M, Verstraete W, Hendrickx L and Pérez J (2007) Distribution of *Nitrosomonas europaea* and *Nitrobacter winogradskyi* in an autotrophic nitrifying biofilm reactor used in a biological life support system. Proceedings of the 5th International Workshop in Space Microbiology. Tokyo, Japan.

Montràs A, Hendrickx L and Pérez J (2005) Nitrifying biofilms in biological life support systems for Space missions. Poster presented in the 3rd International Workshop in Space Microbiology, Mol, Belgium.

Hendrickx L, Baatout S, Wattiez R, Wilmotte A, Janssen P, Pérez J, Montràs A, Leys N, Marty F, Paillé C and Mergeay M (2004). Monitoring microbiology in the Micro-Ecological Life Support System Alternative (MELiSSA). Proceedings of 10th International Symposium on Microbial Ecology (ISME10), Cancun, Mexico**.**