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ON-LINE INSTRUMENTATION FOR N-BALANCE: INSTALLATION AND CHARACTERISATION

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TABLE OF CONTENTS

1 INTRODUCTION

Trade-off of the on-line instrumentation for Nitrogen balance has already been presented in TN 52.21. The work presented in the present Technical Note is focused on the optimisation of the operational conditions of both the ammonium and nitrate analysers once installed in the MELISSA pilot plant.

A new ammonium analyser, whose performance is based on the same principles as the Nitrate analyser *Aquanitra*®, has been purchased. In the present technical note an extensive description of the new on-line ammonium analyser (*Aquamonia*®) and the on-line nitrate analyser (*Aquanitra*®) is presented. The decision to purchase a new ammonium analyser was made after meeting further problems with the old ammonium analyser ***(Amtax, Dr. Lange)***, which had in principle been selected as the best alternative in technical note 52.21.

The main parameters of the nitrate analyser were already adjusted during preliminary tests performed with a prototype analyser and discussed in previous technical note 52.21. Therefore, only a summary of the main operational characteristics set in that previous work, and an update on these conditions is presented for the nitrate analyser.

In order to test the performance of the analysers they were operated off-line with the selected conditions before their connection to the outlet of compartment III. For this set of experiments several dissolutions were selected:

First of all, the analysers have been tested with standard solutions containing only a known concentration of sodium nitrate (Aquanitra®) and ammonium chloride (Aquamonia®) in deionized water.

Once the correct performance of the analysers has been tested with standard solutions of nitrate and ammonium, the next step is to reproduce the characteristics of the real samples from the nitrifying reactor, by using solutions of nitrate and ammonium dissolved in fresh nitrifying medium instead of deionized water. This second set of experiments is important in order to

identify any interference that could be found in real samples. The use of fresh medium allows us to reproduce the composition of real samples from the reactor with the advantage that any other components, such as HCO_3^- originated from the Na_2CO_3 added by the pH control system, are not present in the sample. The experiments with fresh medium are particularly important in the characterisation of the on-line nitrate analyser, due to the fact that HCO_3^- was identified as the main interference in the analysis of NO_3^-. An exhaustive study concerning interference by carbonate/bicarbonate was already presented in TN 52.21.

Once the correct performance of both analysers has been proved with standard solutions, and previous to connecting the analysers on-line to compartment III, a set of experiments was carried out using real sample collected from the outflow of the reactor. The concentrations of nitrate and ammonium measured by Aquanitra® and Aquamonia® were compared to those obtained by an off-line reference method (LCK339/LCK305, Dr Lange).

Finally, the design of the connection between the outflow of compartment III pilot reactor and the analysis system is presented, as well as some preliminary tests performed with this configuration. Possible improvements to this system will also be discussed in this technical note.

2 DESCRIPTION OF THE ANALYSERS AND THE SAMPLE

2.1 Description of the detection system of the selected analysers

The measuring technique used by Aquanitra® is a potentiometry-based method. Some of the advantages of using an ISE are its low maintenance requirements and its capability to work unattended.

The analysers presented in this technical note are based on the use of a solid-state ISE on a flow injection analyser.

2.2 Composition and main characteristics of an ISE

The main components of any ISE are a membrane selective to the analyte, coupled with an internal reference that provides both sides of the membrane with a constant potential.

The difference between a conventional ISE and a solid-state ISE is the absence of a liquid internal reference in the latter, which is replaced with a conductor material deposited over the surface of the selective membrane.

The material used as a solid internal reference is an epoxi resin that covers the surface of the different materials that constitute the membrane providing a constant potential.

The composition of the membranes of both the ammonium and the nitrate analysers described in this technical note are presented in table 2.1.

Table 2.1: Composition of the selective membranes of the ammonium and nitrate ISEs. (Alegret *et al*., 1989; Beltran *et al*., 2002)

Aquamonia	**Aquanitra**
1% Ionophore	6.5%Ionophore (Tridodecylmethylammonium nitrate)
67% plastifiant agent	61% 2-Nitrophenyl octyl ether
32 % PVC (high molecular weight)	32.5% PVC (high molecular weight)

The relation between the concentration of the analyte and the potential detected by an electrode based on a selective membrane is described by the Nickolskii-Eisenman equation. (Equation 2.1).

$$E = E^0 \pm S \cdot \log\left(a_x + \sum_y K_{x,y}^{pot} \cdot a_y^{z_x/z_y} \right) \quad (2.1)$$

where: E^0 is the cell constant;

S is the slope of the Nernst equation (S=0.059/n);

$K_{x,y}^{pot}$ is the potentiometric selectivity coefficient and can be described as the ratio analyte/interference under which 100% of the response of the ISE is due to interference.

a_x i a_y are the activities of the analyte and the interferent ion;

z_x i z_y are the charges of the analyte and the interferent ion.

2.3 Composition of the input medium of compartment III

The use of ISEs to quantify the ammonium and nitrate concentrations makes it necessary to study the possible interferences. Although the selectivity of these sensors is high, they are not

specific, and thus the use of complex media may have a negative effect on the performance of an ISE.

Interference from the different ions present in the culture medium currently entering compartment III was studied for each of the analysers. Interference is usually due to ions that have the same charge as the analyte. Cl^-, HCO_3^- and NO_2^- were the main studied interferences of the NO_3^- ISE, whereas K^+ and Na^+ are the main species known to interfere with the NH_4^+ ISE.

In addition to the compounds in table 2.2, also NO_2^- from partial oxidation and HCO_3^- due to the addition of Na_2CO_3 by the pH control system, are known to interfere on the detection of NO_3^-. Their interference has been studied during the characterisation process of the nitrate analyser.

Table 2.2:
Composition of the medium for a mix culture of *N. europaea* i *N. winogradskyi*.
pH must be set to 8.1-8.2 with Na_2CO_3.
$MgSO_4 \cdot 7H_2O$ i $CaCl_2 \cdot 2H_2O$ sterylised separately and added by filtration (0.22μm)

COMPOUND	g/L distilled water
$(NH_4)_2SO_4$	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

2.4 Physical description of the nitrate analyser

The electric 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 to a concentration

value by means of the previous three-point calibration. The electric potential due to the presence of nitrate is compared to the base line potential given by the carrier and the reagent modifier. A detailed scheme of the analyser and its main components describing the flow diagram is presented in figure 2.1.

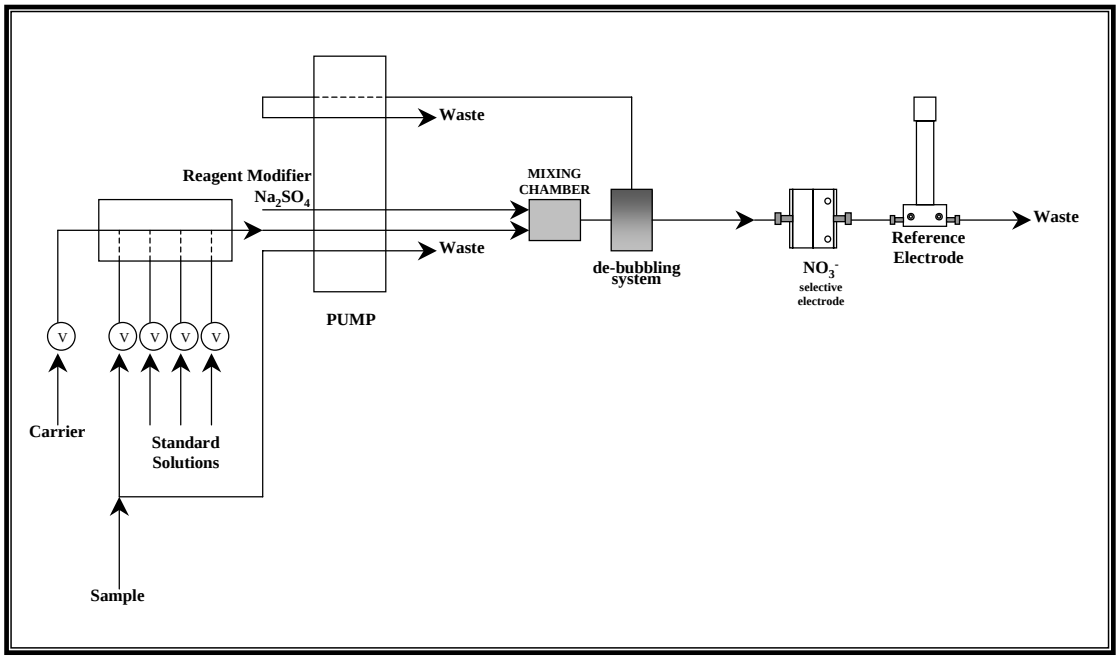

Figure 2.1.- Flow diagram for the Nitrate analyser (Aquanitra®)

The carrier flow is pumped to a mixing chamber, where a flow of reagent modifier is incorporated. The concentration and pH of this solution are selected in such a way that its 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 reached the detection system.

During operation of the analyser a continuous flow of deionised water goes through the system while the standards and the sample are switched to the main carrier flow by means of different electro valves.

Before starting 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 whose frequency is adjustable from the main display of the analyser. The standard

solutions are selected in such a way that the expected concentration of the sample is included within this range.

Before the start of the analysis cycle, sample is injected to the system in order to replace the remaining sample from previous analyses. Periods of time within 0 and 600 seconds can be selected for this purpose.

The analysis cycle starts with the main flow, which contains only the carrier and a solution of reagent modifier, being switched to the nitrate selective electrode 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.

The sample reaches the mixing chamber and is integrated in the main flow, the homogenised flow, whose pH was decreased by the addition of the reagent modifier solution, goes through a de-bubbling chamber, where CO_3^{2-} and HCO_3^- present in the sample are removed as CO_2.

- *The carrier flow* into which the sample and all the reagents are injected is a flow of deionized water.
- *The reagent modifier* is a solution with a high buffer capacity as well as a high ionic strength. The use of a high Na_2SO_4 concentration 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 optimised in order to achieve a good performance of the on-line nitrate analyser. Therefore, further discussion on this topic can be found in the present technical note.

The concentration of the samples is related to the measured peaks of potential by equation 2.2

$$C = 10^{\frac{V-K_1}{K_2}} \qquad (2.2)$$

where:

- V is the peak height in mV
- K_1 is the y-intercept in mV
- K_2 is the slope of the calibration curve in mV/decade. This indicates that, by using this modified constant the concentration is directly obtained in mg/L, instead of obtaining the logarithm of the concentration and subsequently calculating the concentration value from it. However, this calculation is performed by the analyser, which provides us directly with concentration measuments.

2.5 Characterisation of the nitrate analyser:

A prototype of the nitrate analyser now installed at the MELISSA pilot plant was tested off-line with samples from compartment III on a previous phase of this work (TN 52.21). The analyser was optimised to avoid interference from bicarbonate and the results obtained lead to the final conclusion that Aquanitra® is an appropriate analyser to monitor the nitrate concentration in the outlet of compartment III. Interference from other species, mainly nitrite, has also been extensively studied and the conditions to optimise the performance of the analyser under this limitation are documented as well (*Massana et al*., 2001).

After preliminary studies carried out with the prototype analyser, the optimal concentration of the reagent modifier solution was set to 0.5M Na_2SO_4 (containing 10^{-3} M NO_3^-) and a pH of 2.3 was selected to avoid HCO_3^- interference. The effect of nitrite interference has not been observed so far, but it may require further attention when there is nitrite accumulation in the nitrifying reactor. When these preliminary tests were carried out the reactor was operating in steady state with a low flow rate and thus the presence of nitrite was negligible (<1 ppm). Therefore, the estimation of the effect of nitrite on the samples was based on several studies that had been carried out by the researchers who developed the analyser.

Further work has been carried out on the selection of the **optimal buffer capacity** and **ionic potential** of the reagent modifier once the new analyser, as opposed to the prototype, has already been installed in the MELISSA pilot plant.

Another important parameter that also needs to be studied is the **injection time**, which is the time elapsed while the sample is switched by means of a valve into the carrier flow that goes through the detection system. While it is true that the operation of the electrode with a high injection time leads to high reliability and stability in the measurement of the electric potential, it is important as well to take into account that the lifetime of the nitrate selective electrode is significantly reduced, and most importantly, a high injection time significantly increases the overall dead time in the analysis loop. Besides, the use of high injection times will prove to have a negative effect on the performance of Aquanitra® when working with real samples and solutions having the fresh medium composition. The use of long injection times, together with the high sampling frequency required (this sampling frequency has been fixed in a study performed by Adersa and described in TN48.3) will damage the electrode due to constant contact with the sample and hence it needs to be optimised.

Although the optimal conditions had already been adjusted to work with real samples from the nitrifying compartment in the prototype analyser, it was decided to evaluate the performance of the new analyser once installed in the pilot plant. However, before proceeding with optimisation, a few tests were performed using the already set up conditions, presented in table 2.3, as a starting point.

Table 2.3: Nitrate analyser parameters set-up during preliminary tests (Values before optimisation)

PARAMETER	**VALUE**
Frequency of analysis	16 min
Initial time	300 s
Injection time	100 s
Injection Volume	1.8 mL

Expected NO_3^- concentration in sample	240-440 mg/L N-NO_3^-
pH conditioning solution	2.3
Na_2SO_4 concentration	0.5M Na_2SO_4 + 10^{-3}M $NaNO_3$

2.6 Testing of the analyser with nitrate standards

As a first approach to the evaluation and characterisation of the new analyser several tests were performed with samples containing only nitrate in deionised water in order to verify the precision and repeatability of the analyser.

In figure 2.2 it can be observed how a certain period of time is required before the analyser reaches a stable measurement after a disturbance.

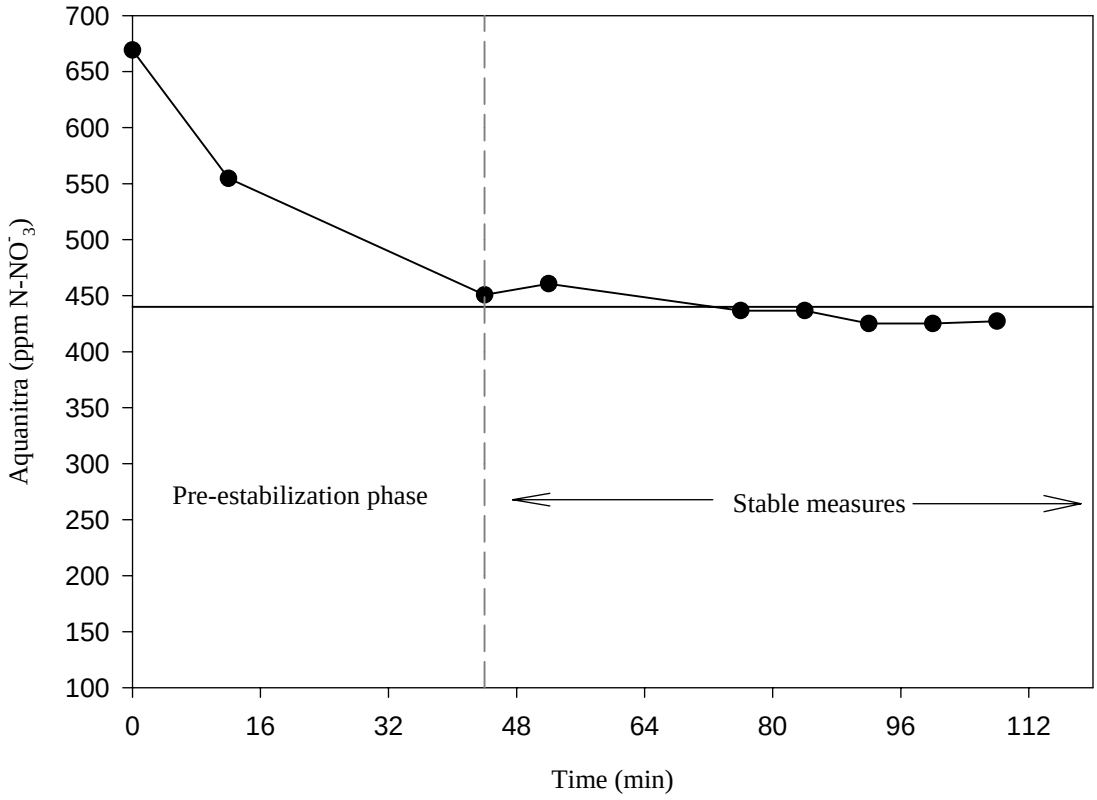

Figure 2.2.- Off-line analysis of a standard solution containing only nitrate in deionised water with Aquanitra. Expected concentration of the sample (—) different measurements performed by the analyser (•).

This test was carried out for one solution of 440 ppm N-NO_3^-, which was analysed over a period of 112 minutes with a sampling frequency of 8 minutes. Further experiments were performed with samples containing all the components of the culture medium as well as with real samples.

During this experiment the analyser was operated with a reagent modifier solution containing 0.5M Na_2SO_4 with a pH of 2.3, that is, the conditions set up during preliminary tests performed with the prototype (table 2.3)

A summary of the statistical results obtained from the experiment performed with nitrate standards are presented in Table 2.4.

Table 2.4.- Statistical results obtained during the experiment shown in figure 2.

Real Concentration (ppm N-NO_3^-)	Aquanitra (ppm N-NO_3^-)	Standard Deviation (n=20)	Relative SD (% final value)	Relative error (%)
440	439.93	14.1	3.21	0.02

2.7 Interference identification

When identifying interference of different species, not only the total amount of each one of the compounds, but also the effect of pH must be taken into account. As stated above, the main interferences of the nitrate ISE are those of NO_2^- and HCO_3^-.

The nitrate analyser has been developed to avoid interference from several potentially interfering ionic species, mainly nitrite (NO_2^-) or bicarbonate (HCO_3^-). Samples from compartment III were studied in detail and the main interference was found to be that of HCO_3 whose concentration cannot be maintained within a constant range of values because of the pH

control of compartment III. The HCO_3^- concentration can increase notably during operation of the pilot reactor due to the addition of Na_2CO_3 by the pH control system. The addition is not constant and it depends on the operational conditions of the reactor, which will determine the quantities of acid (CO_2) or base (Na_2CO_3) required for maintaining pH in the optimal range of 8.0-8.2. Therefore, carbonate addition and hence HCO_3^- interference, are dependent on the operational conditions of the reactor, such as ammonium load or flow rate, which lead to different pH control situations.

On the other hand, interference due to nitrite is usually minimised due to its low concentration (usually below 10 mg $N\text{-}NO_2^-/L$) during normal operation of compartment III. However, NO_2^- is known to interfere with the NO_3^- ISE at higher concentrations, and thus it is important to quantify the effect of this interference. The effect of nitrite concentration on nitrate measurements had been widely studied and data were provided by the supplier of the analyser concerning the selectivity of the nitrate ISE to nitrite. The presence of high nitrite concentrations in the sample, leads to a decrease on the peak height, and thus to an error on the nitrate concentration measurements, which was quantified.

Before the purchase of the nitrate analyser, several tests were carried out with the previously mentioned prototype that led to the operational conditions presented in table 2.3. These values were used as a starting point for optimisation when the new nitrate analyser was purchased and installed in the MELISSA pilot plant. Preliminary tests performed with this prototype analyser revealed the significance of the HCO_3^- interference when its performance was tested off-line with real samples from the pilot reactor of compartment III (TN 52.21). Nitrite interference was not detected during this set of experiments, as the samples had been obtained during a steady state and the nitrite concentration was very low. The selected analyser had been developed to monitor a wastewater stream containing high nitrate concentrations in which the main interference was due to nitrite, and thus the interference of this species had been widely studied.

In order to minimise HCO_3^- interference the pH of the Na_2SO_4 conditioning solution was set to 2.3, which according to figure 2.3 minimises HCO_3^-.

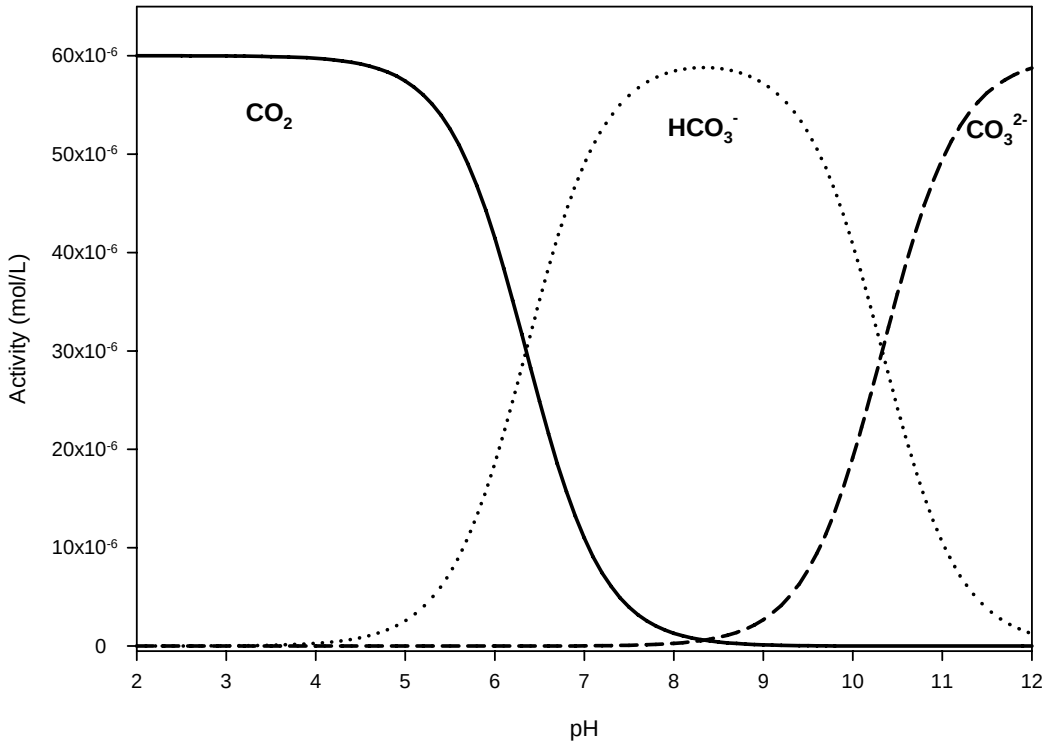

Figure 2.3: Activities of carbonate species as a function of pH

By mixing the sample flow with the conditioning solution, the pH is reduced from 8-8.2, at which HCO_3^- is the predominant species, to a pH around 2.3.

In these conditions, the estimated selectivity coefficient (see equation 2.1) was $K^{pot}_{NO_3^- - HCO_3^-} = 0.061$, and the HCO_3^- concentrations on table 2.5 cannot be exceeded in order to maintain a deviation between the expected nitrate concentration and the concentration measured by the analyser lower than 5%.

Table2.5: HCO_3^- limits within the normal range of NO_3^- concentration in compartment III. Analyzer configuration parameters as in table 2.3.

Nitrate concentration (mg/L NO_3^-)	**Maximum HCO_3^- concentration (mg/L HCO_3^-)**
1328 (300 ppm N-NO_3^-)	937
2657 (600 ppm N-NO_3^-)	1875

The amount of HCO_3^- present in the inlet of the nitrifying reactor is 580 mg/L HCO_3^-, and thus the expected deviation caused by the inlet HCO_3^- is below 3%, according to the results that we have obtained and the estimated selectivity coefficient. The amount of Na_2CO_3 added by the pH control system depends on the operating conditions of the reactor, and according to the equilibrium $CO_3^{2-}/HCO_3^-/CO_2$, at a pH=8 the predominant species is the interferent ion HCO_3^-. Therefore, the deviation on the final concentration measurement depends on both the concentration of HCO_3^- in the inlet of the reactor, and the amount of HCO_3^- introduced in the system by the pH control, which is difficult to quantify and is not constant.

From previous work it was concluded that the optimal pH of the conditioning reagent solution should be set to 4 to minimise the nitrite interference. Several experiments were performed with different nitrite concentrations at different pH values. When the sample was buffered at pH=2.3 (the optimal value to avoid HCO_3^- interference), the selectivity coefficient (see equation 2.1) was of $K^{pot}_{NO_3^- - NO_2^-} = 0.55$. With this selectivity coefficient it can be estimated that when the NO_3^- concentration is 6 times higher than the NO_2^- concentration, the error between the expected concentration value and the nitrate measurement performed by the online analyser has a value of 8%. Therefore, the nitrate concentration should always be at least 6 times higher than that of nitrite in order to obtain errors lower than 8%. Taking this into account and assuming the typical range of nitrogen load in the reactor, the maximum nitrite concentrations

that can be contained in the samples to prevent a deviation higher than 7% due to interference, are presented in table 2.6.

Table 2.6: Nitrite limits within the normal range of NO_3^- concentration in compartment III. Analyser configuration set-up to minimise HCO_3^- interference.

Nitrate concentration (mg/L N-NO_3^-)	**Maximum nitrite concentration (mg/L N-NO_2^-)**
300	66
600	131

The interference of Cl^-, which is one of the main species known to interfere with the nitrate ISE, has not been given special attention in the characterization process due to the low concentration of chloride present in the medium currently used in compartment III.

In table 2.2 the concentration of all the compounds present in the medium of compartment III was presented. The concentration of $CaCl_2 \cdot 2H_2O$ stands for a concentration of chloride of 0.35 mg Cl^-/L. Chloride is usually identified as one of the main interferences in the analysis of NO_3^- with an ISE in stream waters, in which the nitrate concentration is very low. However, the nitrate concentration in the outlet of the nitrifying reactor of the MELISSA pilot plant is in a range of 1200-2600 mg NO_3^-/L, i.e. approximately 10^4 times higher than that of chloride. In these conditions, it is safe to state that no interference from chloride is likely to be detected even if the selectivity coefficient was quite important. Although we did not perform any experiments concerning the chloride interference, there are data available for other commercial nitrate selective electrodes in the literature that can be used as approximate values to estimate the maximum amount of this ion before it negatively affects the nitrate measurement performed by the ISE. The selectivity coefficient for different nitrate selective electrodes ranges between $K^{pot}_{NO_3^- - Cl^-} = 0.01$ and 0.03 and thus if a selectivity coefficient of 0.02 is assumed, the response of the electrode to the chloride ion is 50 times lower than the response due to nitrate. Therefore, when the chloride concentration is 50 times higher than that of nitrate, the response of the ISE is mainly due to interference. In order to control the effect of

chloride interference, its concentration in mg /L should be kept below the expected nitrate concentration. However, some tests should be performed in the future in order to set up the chloride concentration threshold of the actual ISE used by Aquanitra in case it was necessary to use this analyzer for further applications in which compartment III would be working with higher chloride concentrations.

2.8 Tests with samples containing a known concentration of NO_3^- and HCO_3^-

Given that carbonate was identified as the main interference, a series of experiments working with different concentrations of this ion were carried out. To this effect, different concentrations of HCO_3^- were added to standard solutions of nitrate. The results obtained in one of these experiments are presented in figure 2.4. This experiment consisted in testing the continuous operation of the analyser over a period of time and it was run with the experimental settings presented in table 2.7.

The expected nitrate concentration was of 245 mg/L $N\text{-}NO_3^-$ and an added concentration of bicarbonate of 580 mg/L HCO_3^-. The quantity of bicarbonate added to these samples is equal to the concentration present in the fresh nitrifying medium.

Table 2.7: Nitrate analyser parameters before optimisation

PARAMETER	**VALUE**
Frequency of analysis	16 min
Initial time	300 s
Injection time	100 s
Injection Volume	1.8 mL
Expected NO_3^- concentration in sample	245 mg/L $N\text{-}NO_3^-$
pH conditioning solution	2.3

Na_2SO_4 concentration	0.5M Na_2SO_4 + 10^{-3}M $NaNO_3$

The effect of interference is known to increase as the ratio between analyte concentration and interference concentration decreases.

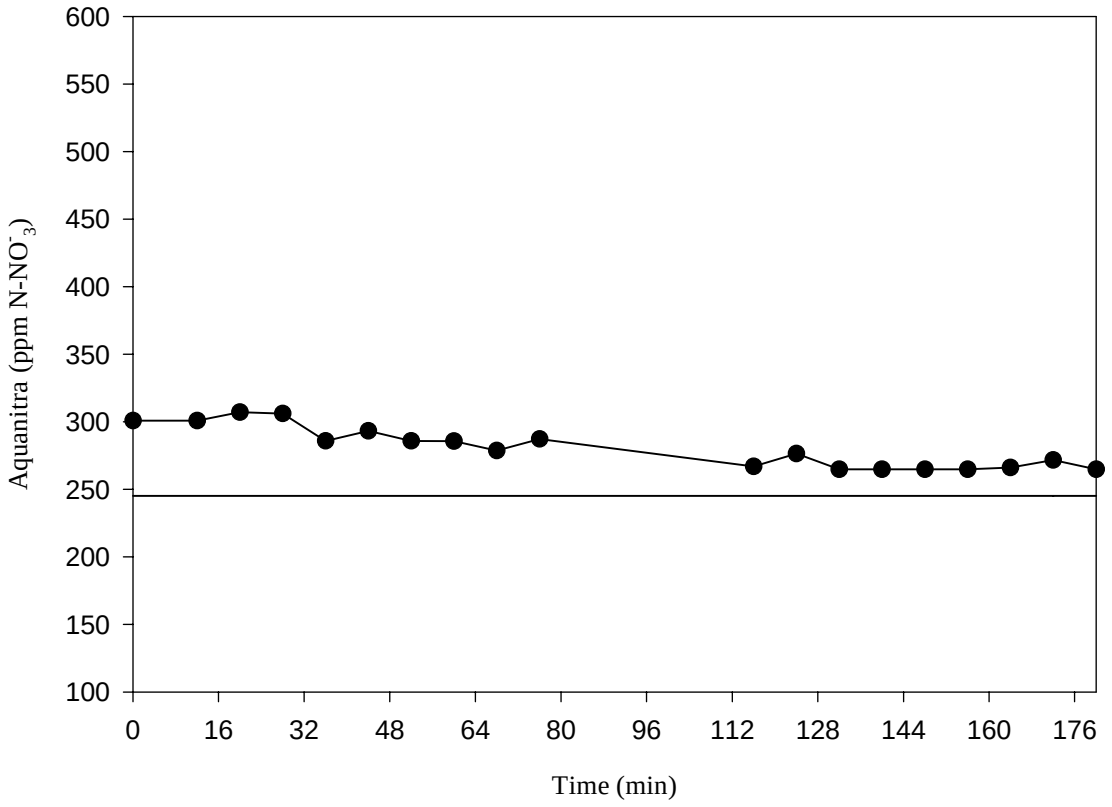

Figure 2.4.- Analysis of a nitrate standard containing 580 ppm HCO_3^- (the same concentration contained in the fresh culture medium) and 245 ppm N-NO_3^-. Real concentration of the sample (—) and values obtained with Aquanitra (•) .

Repeatability, in terms of relative standard deviation, is in the range of 6-7% and relative errors of 9-15% can be expected at HCO_3^- levels found within the normal range of operation of the reactor. (table 2.8)

Table 2.8.- Results obtained with samples containing HCO_3^-

Real (ppm N-NO_3^-)	**Aquanitra (ppm N-NO_3^-)**	**Standard Deviation (n=20)**	**Relative SD (%)**	**Relative Error (%)**
245.02	266.77	15.28	5.73	8.88

According to these results, bicarbonate interferes in the correct operation of the analyser, leading to a relative error of approximately 9% when the bicarbonate concentration, while the error obtained when working with nitrate standards was only of around 0.2%.

2.9 Tests using fresh medium from the nitrifying column

Samples were prepared by dissolving a known concentration of nitrate with a solution containing all the same components as the culture medium except for ammonium.

No ion interference had been detected during the first preliminary tests using this kind of solution with the prototype analyser. However, a deviation of around 10% was found when this type of samples were analysed with the new analyser installed at the MELISSA pilot plant. Results are shown in figure 2.5.

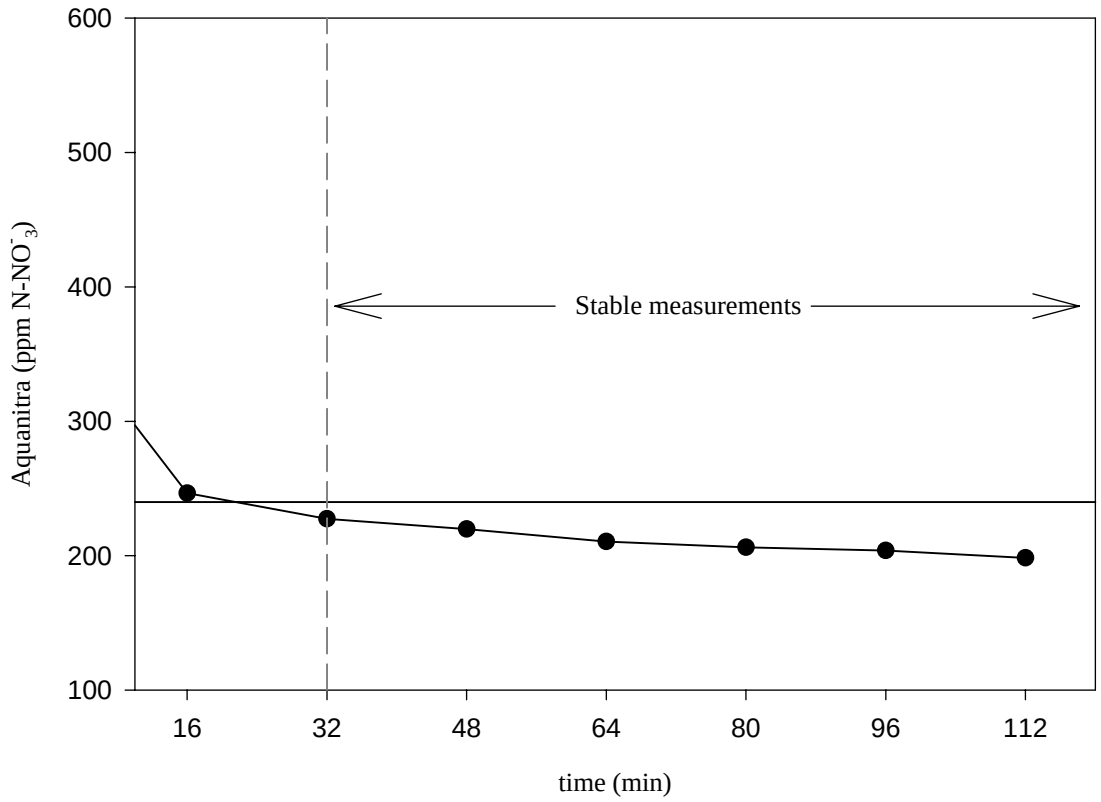

Figure 2.5- Results obtained with Aquanitra® (•) for fresh culture medium with an added concentration of 240 ppm $N\text{-}NO_3^-$

The results shown in figures 2.4 and 2.5 are not in accordance with the results obtained with the prototype analyser, and thus further studies were considered and eventually new experiments concerning optimisation of the main parameters were carried out. The new experiments were focused not only on the optimisation of the reagent modifier composition, but also on the optimisation of the injection time.

Table 2.9.- Deviation and repeatability summary for the experiments shown in figure 5.

Real (ppm N-NO_3^-)	**Aquanitra (ppm N-NO_3^-)**	**Standard Deviation (n=20)**	**Relative SD (%)**	**Relative Error (%)**
240	216.06	16.64	7.7	-10.00

2.10 Optimisation of the operational conditions of the nitrate analyser

In order to improve these results a series of experiments were carried out concerning two of the main parameters of the analyser: concentration of the conditioning solution and injection time, the latter of which determines the volume of injection at a given flow rate.

During preliminary tests with the prototype analyser, the concentration of the reagent modifier solution was set to 0.5M Na_2SO_4, 10^{-3}M NO_3^- and pH=2.3. The same conditions have been used during the first experiments performed with the new analyser.

A new series of experiments has been carried out with the new analyser and is presented in this section. The sample used in these experiments contains 361.5 mg/L N-NO_3^- as well as the rest of the compounds that constitute the feeding of compartment III, except for NH_4^+ including 580 mg/L HCO_3^-.

The Na_2SO_4 as well as the injection time have been submitted to optimisation. During the optimisation of the Na_2SO_4 concentration the injection time was maintained at 100 seconds, the same injection time used in the previous experiments.

The lifetime of the nitrate selective electrode considerably decreases when long injection times are selected. A long injection time leads to higher stability of the electric signal detected by the selective electrode, due to the fact that a higher sample volume is injected and the potential is measured with higher reliability. However, it is important to take into account that the use of long injection times combined with a high sampling frequency considerably reduces the

lifetime of the nitrate selective electrode. In addition, from the results previously presented in this technical note it can be concluded that the precision of the measurements once they have been translated into concentration by means of the calibration, is not enhanced by the use of a high injection time (100 seconds). By the contrary, the higher the injection time, the more important the deviation from the reference method is. The selection of a high sampling frequency also contributes to reducing the lifetime of the sensor, which leads to the final conclusion that the injection time should be minimised.

In figure 2.6 it can be observed how the deviation between the expected value of 361.5 mg/L N-NO and the measured values slightly decreases as the concentration of the conditioning solution increases.

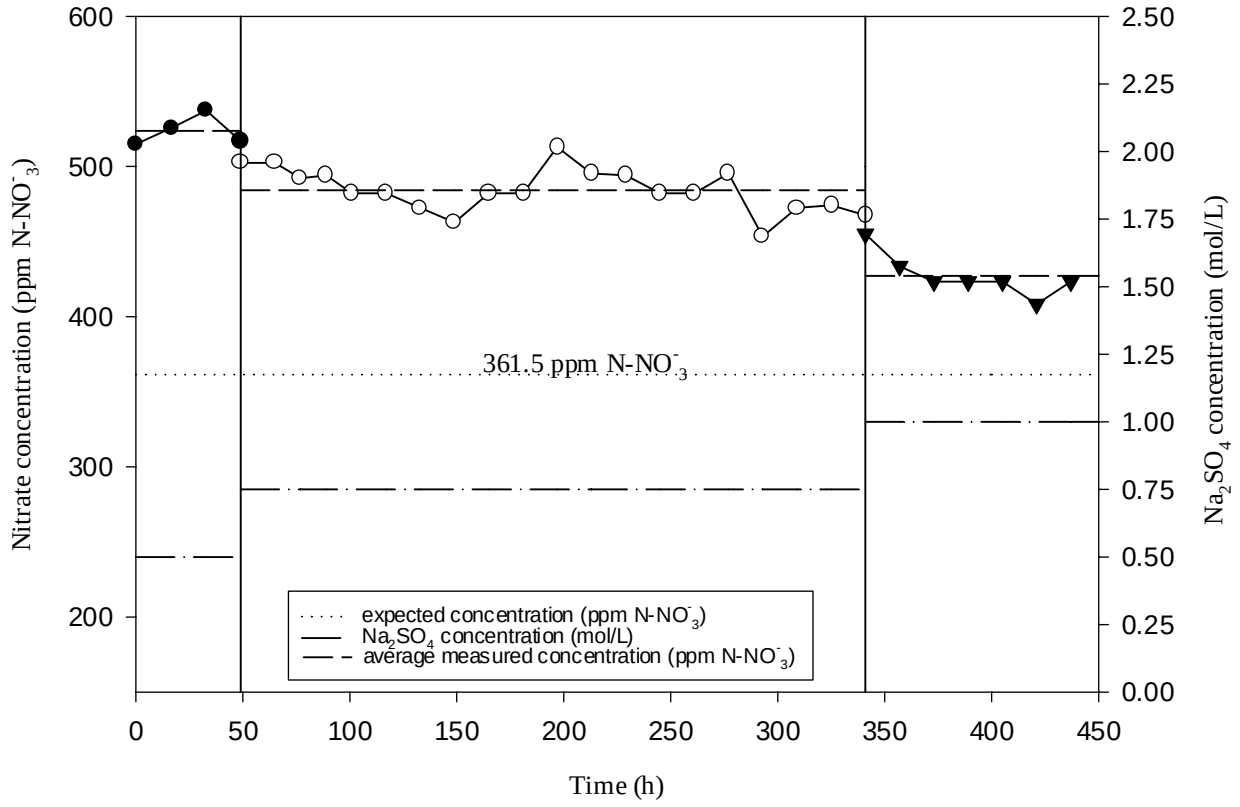

Figure 2.6.- Optimisation of the conditioning solution concentration. Reagent modifier (Na_2SO_4) concentration (— · —); real concentration (·····); Nitrate concentration with a Na_2SO_4 concentration of 0.5M (•), 0.75M (o) and 1M (∇).

Although the best results were obtained when working with a 1M Na_2SO_4 solution, the use of this concentration of sodium sulphate was avoided to prevent problems such as precipitation or clogging of the reference and the nitrate selective electrodes due to the high concentration of the salt.

Even when working with the highest buffer capacity of the conditioning solution (1 M) a high deviation (18 %) between the expected and the measured values is observed. This is attributed to the damaged state of the selective electrode, which had already been in use for a long period of time when these experiments were carried out. However, as the aim of this study was comparative, the old electrode has been used for optimisation of the parameters and has been

changed before resuming normal operation of the analyser with samples. Indeed, when the concentration of the conditioning solution is increased a high deviation is still obtained.

The initial concentration of 0.5M was selected to carry out the experiments concerning optimisation of the injection time. With a constant Na_2SO_4 concentration of 0.5M, the injection time was subsequently decreased from 100 seconds until the optimum value of 11 seconds was attained. The results of these experiments are shown in figure 2.7.

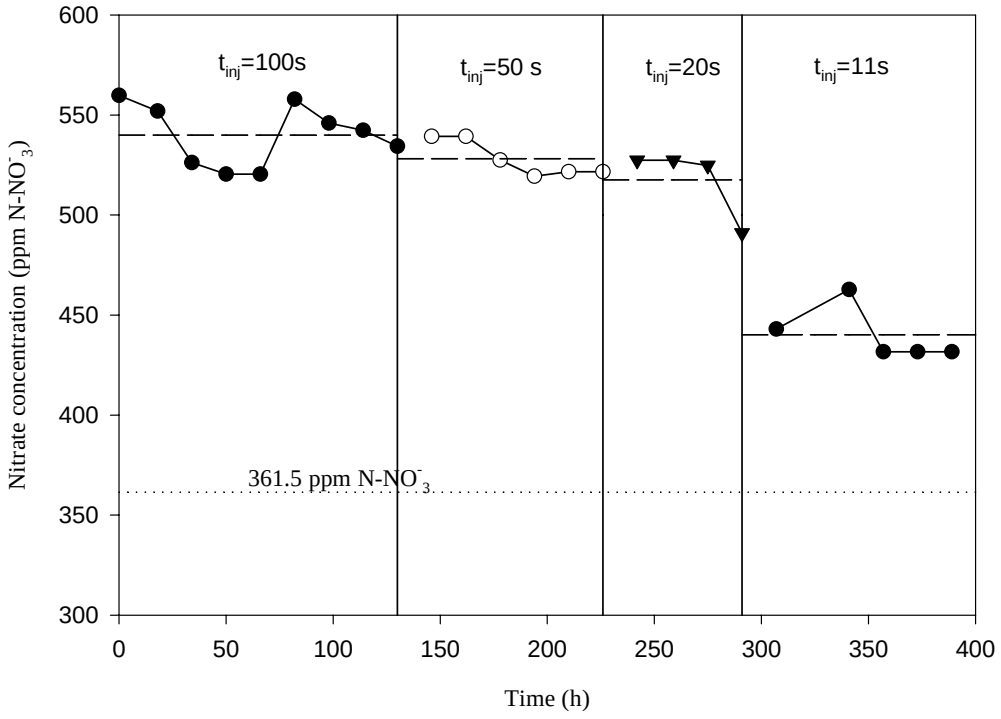

Figure 2.7.- Optimisation of the injection time. Concentrations measured with Aquanitra® with different injection times.

It becomes clear how the highest accuracy is achieved when using an injection time of 11 seconds, which was the injection time recommended by the suppliers of the analyser. The initial injection time of 100 seconds leads to an important deviation that explains the poor results already discussed. Moreover, the use of high injection times for long periods of time has been proved to considerably reduce the lifetime of the electrode as already stated.

As a result of these experiments it was decided to operate the analysers with a combination of the conditions presented above. The poor precision of some results obtained can be attributed, as it has already been discussed, to the damaged state of the nitrate selective electrode, which leads to very low signals being measured.

In figure 2.7 it can be observed that the precision can be notably improved by the decrease of the injection time, although a total concordance is not attained due to the state of the electrode. It is concluded that a combination of the optimum reagent modifier concentration with the optimum injection time will lead to better performance of the analyser, especially when the nitrate selective electrode is replaced for a new one. Therefore, a Na_2SO_4 concentration of **0.75 M** was selected and an injection time of **11 seconds**.

Once these conditions had been selected, the analyser was tested with real samples from compartment III.

2.11 Tests performed off-line with real sample from compartment III

Once the optimal conditions have been implemented on the analyser, the nitrate selective electrode is replaced and several samples have been analysed before the online connection is done.

Concentration of these samples was measured with Aquanitra and the results were compared to a selected reference method. (*LCK339, Dr. Lange, Germany, ready-to-use probes*).

Figure 2.8 shows how all measurements obtained with the new analyser over different periods of time are within the confidence interval of concentrations given by the estimated error of the reference method. The average deviation given by this reference method working with the fresh nitrifying medium used in compartment III was found to be around 18% (TN 52.21).

The expected concentrations of the samples, as determined by analysis with reference method, are presented in table 2.10 along with the number of replicates for each sample.

Table 2.10: Description of the samples used in figure 2.8

Expected concentration (mg/L N-NO_3^-)	number of replicates Aquanitra
160	8
299	16
346	8
348	8

The typical interval of standard deviation for the results obtained with the online nitrate analyser was found to be 6-9%, which is in accordance to the average repeatability attained in the experiments carried out during the preliminary tests with the prototype analyser (relative standard deviation interval: 2-19%).

The average deviation of the selected reference method was found to be around 18% when working with the nitrifying medium used in compartment III.

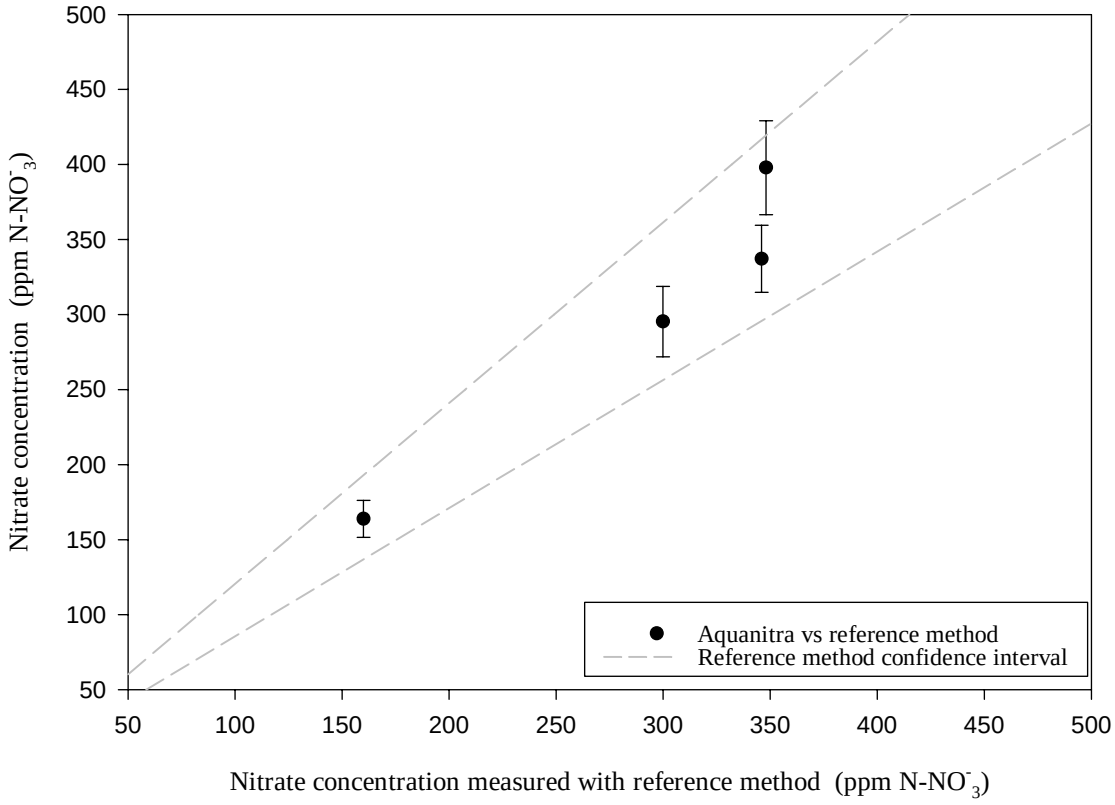

Figure 2.8.- Tests performed off-line with real samples from the nitrifying compartment over different periods of time (•). The dashed line symbolises the confidence interval of the reference method used for off-line measurements.

2.12 Physical description of the ammonium analyser:

Main description of the flow in the ammonium analyser is described in the scheme presented in figure 2.9.

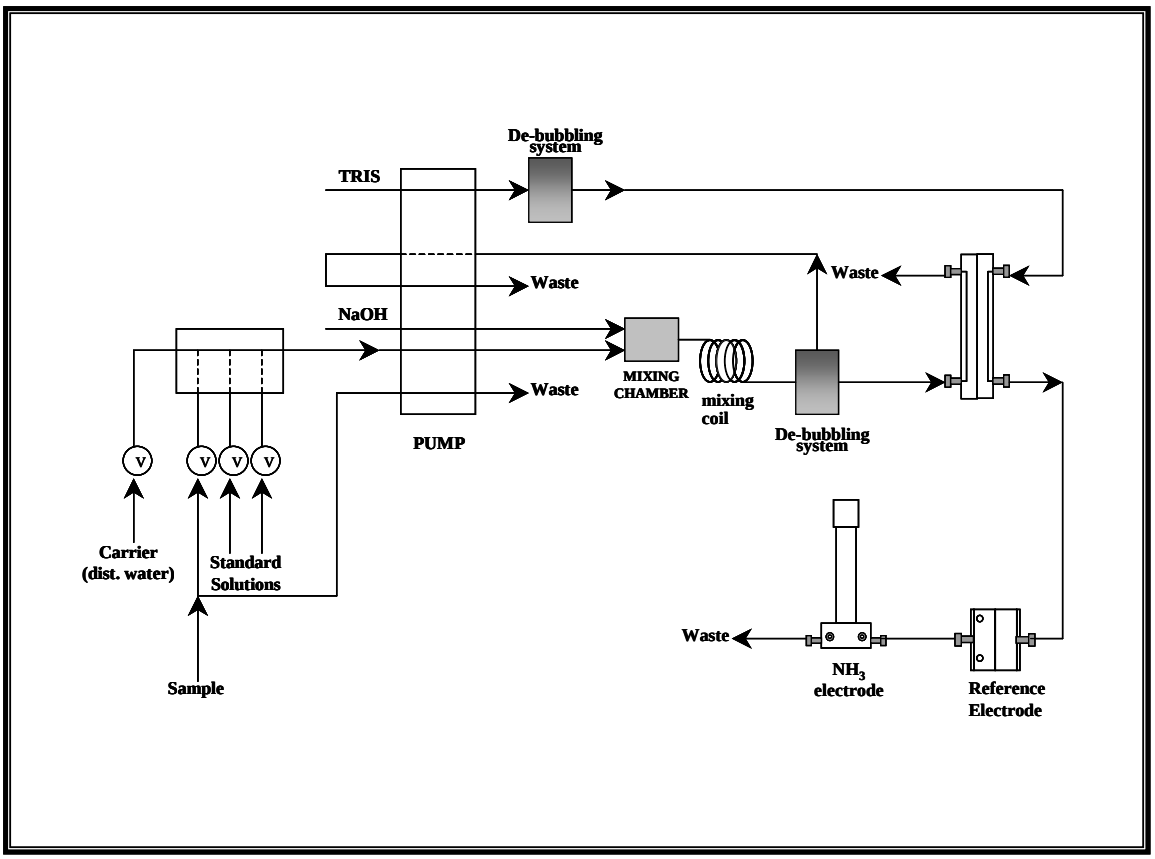

Figure 2.9.- Flow diagram for the ammonium analyser (Aquamonia®)

The new ammonium analyser is also based on the measurement of the electric potential generated by the ions present in a sample. In the following section a detailed description of the analytical sequence of analysis of samples is carried out.

Sample analysis

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

The flow of deionised water is mixed with the sodium hydroxide and flows 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, which can be set on the display of the analysers, has elapsed, the valve switches to allow the sample flow into the system. As the sample is mixed with the sodium hydroxide the pH increases and thus all the dissolved ammonium turns to ammonia. Part of this gas crosses the gas permeable membrane and, as it flows through the diffusion chamber, it gets dissolved in the Tris solution (pH=7.48) and recovers its ionic form, which can be detected by the electrode. The tension 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 tension between the two electrodes, which can be visualised as a peak of tension in the analyser screen. After the injection time (the elapse of time during which the sample is switched through the system is user-defined) the valve switches back to the deionised water position so that the baseline is recovered.

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 the following equation:

$$C = 10^{\left(\frac{V-K_1}{K_2}\right)} - f \qquad (2.3)$$

where:

- C is the NH_4^+ concentration in mg/L
- V the peak height in mV

- K_1 is the y-intercept in mV
- K_2 is the slope of the calibration curve in mV/decade. Like in the nitrate on-line analyser this units indicate that, by using this modified constant the concentration is directly obtained in mg/L, instead of obtaining the logarithm of the concentration and subsequently calculating the concentration value from it.
- *f* is expressed in mg/L and is a parameter that makes it possible to describe the loss of lineality of the ISE response at low concentrations. 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.

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

2.13 Calibration cycle

The standard solution with the lowest concentration is first injected to 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 be computed from the parameters obtained by this calibration procedure.

2.14 Characterisation of the ammonium analyser

The same approach adopted to test the nitrate analyser was applied to the experiments concerning the ammonium analyser. As a result, Aquamonia was first tested with standard solutions containing only the ammonium ion (either ammonium chloride or ammonium sulphate dissolved in deionised water). Afterwards, several tests were performed with solutions containing all other ions of the fresh culture medium, as well as ammonium, in order to simulate the real conditions.

Table 2.11: Ammonium analyser parameters set-up

PARAMETER	VALUE
Frequency of analysis	16 min
Initial time	300 s
Injection time	100 s
Injection Volume	3 mL
pH conditioning solution	7.48
Tris-Hydroxi-methyl aminomethane concentration	0.01M TRIS

2.15 Tests performed with standard solutions of ammonium

The results obtained when ammonium standard solutions are discussed in the present section. A set of analysis were done using standard solutions, the mean value obtained is represented in figure 2.10 Error bar of each set of samples is represented as standard deviation of this set of measurements.

The experiments carried out with standard solutions of ammonium in water made it possible to identify the deviation between the ammonium concentrations obtained with Aquamonia and the real concentration of the standard, represented in the x-axis of figure 2.10.

For the experiments presented in figure 2.10 the ammonium online analyser was calibrated with two standard solutions of 1 and 10 ppm $N\text{-}NH_4^+$. The calibration range must be selected according to the expected range of concentrations to be measured. A decrease in the precision of the analyser was detected when the width of the calibration interval was increased. In addition, samples with concentrations close to the limits of the standards used for calibration are determined with higher errors.

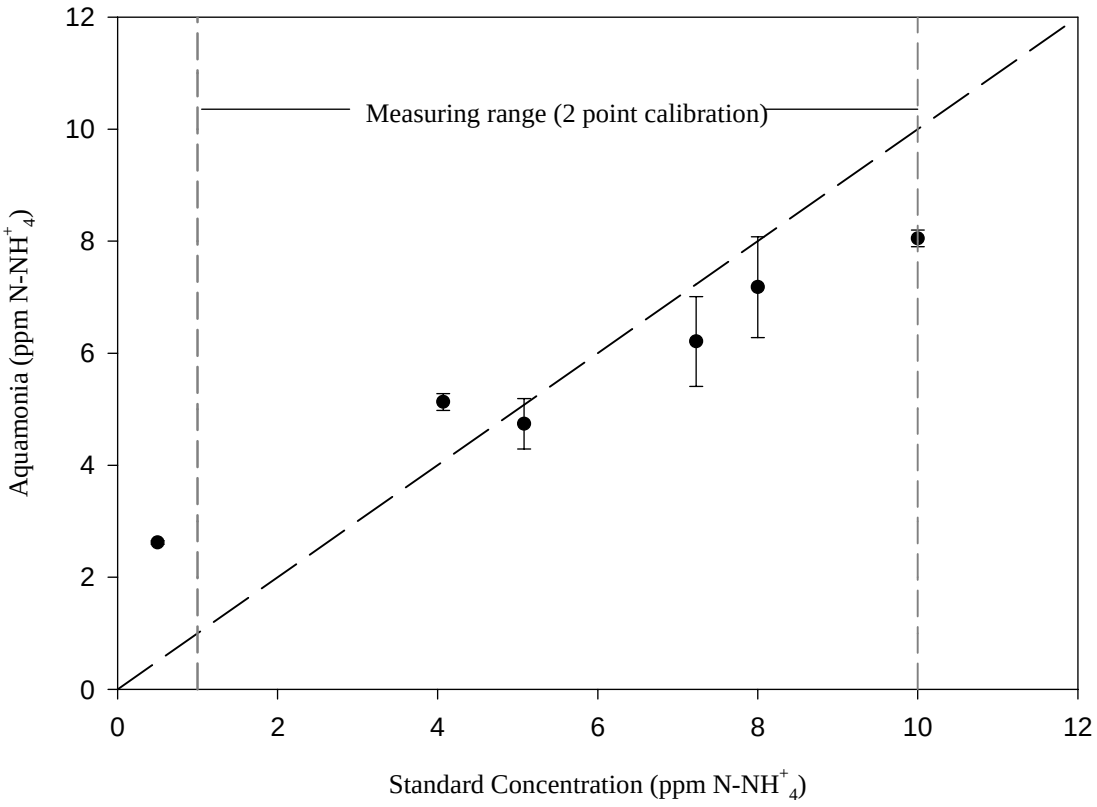

Figure 2.10.- Results from several experiments performed with different concentrations of ammonium. Average concentration measured by Aquamonia over each experiment (•).

A relative standard deviation of 2-12% is obtained, while the relative error between the real concentration and the value given by the analyser is below 14% when working within the range defined by the calibration standards.

The typical range of operation for ammonium in the outlet of compartment III is usually 0-10 ppm $N\text{-}NH_4^+$, and the first tests performed with ammonium standards have been performed within this concentration interval.

During some transitory states, however, some ammonium accumulation may be found and hence the analyser should be able to operate at higher concentrations as well.

2.16 Tests using fresh medium from the nitrifying column

Some tests were performed with samples consisting of different ammonium concentrations dissolved in fresh nitrifying medium.

These experiments were carried out within the range 10-50 ppm N-NH_4^+. Although it would have been convenient to have some points in a lower concentration range (0.1-10 ppm N-NH_4^+), from the observation of figure 2.11 it becomes clear that the precision and the repeatability are satisfactory even when working at the limits of the calibration interval.

Several experiments were carried out operating the analyser for a period of time around 2 hours for each of the samples. The results are presented in figure 2.11 in terms of average concentration and its standard deviation is also displayed.

In table 2.12 the number of replicates for each one of the samples that are represented in figure 2.11 are presented.

Table 2.12: Description of the samples used for experiments in figure 2.11

Expected concentration (mg/L N-NO_3^-)	number of replicates Aquanitra
10.1	11
20.1	6
30.0	6
30.2	6
40.0	5
40.2	8
40.2	3
50.3	7

The reliability of the analyser in this concentration range is high and is in accordance with the technical specifications of the equipment. In figure 2.11 a high correlation between the real concentration values of the samples, represented by the diagonal of the graph, and the measurements performed by Aquanitra is observed. The repeatability of the measurements in terms of relative standard deviation has a maximum value of 3.5%, being the most common value around 2%, and the precision of the measurements is between 0.7% and 5.5%, excepting one of the points that has a 12% deviation from the real concentration value.

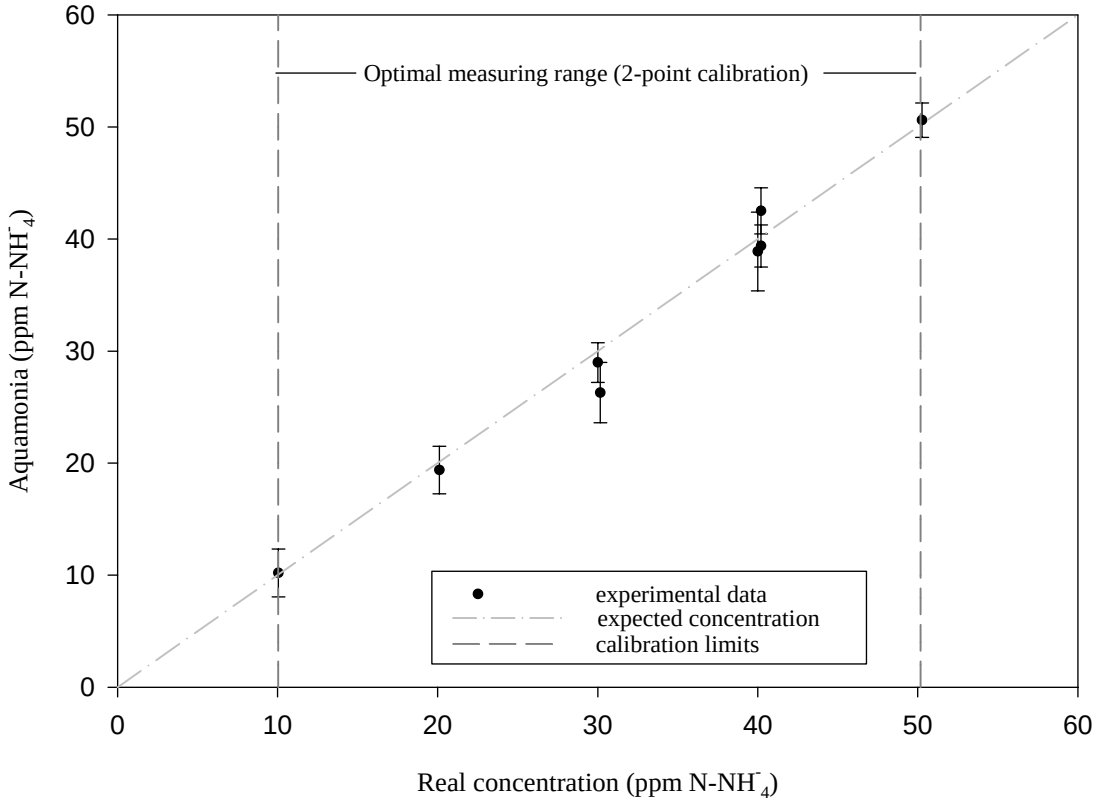

Figure 2.11.- Off-line experiments with Aquamonia® using fresh nitrifying medium as a solvent instead of deionized water. Samples containing different concentrations of nitrate were analysed (•) and the results obtained compared to their expected concentration (— · —).

2.17 Maintenance requirements of the ion selective electrodes

The nitrate selective electrode was used in variable operating conditions due to the fact that different experiments needed to be performed during the characterisation of the nitrate analyser. During this period, several changes were made in the concentration of the reagent modifier solution, the frequency of analysis and the injection time. This set of variable operating conditions causes difficulties to estimate an average or typical decrease of the electrode sensitivity. In spite of these difficulties, a protocol for the replacement of the electrode is described and discussed in the present section, to try to standardize the correct maintenance of the analyzer.

The nitrate and ammonium analysers coupled with the nitrifying reactor of compartment III of the MELISSA pilot plant are based on a potentiometric measuring method, as has been discussed in the present technical note and in previous reports (TN 52.21, TN 52.4). Optimal performance of an ion selective electrode is defined by several parameters, the most important of which are sensitivity, lowest detection limit, response time and reproducibility. The evolution of these parameters along the lifetime of the electrode can be used to define a protocol for the replacement of the electrodes. Because of the operation of the ammonium and nitrate analysers being based on the interpolation of potential measurements (mV) in a calibration curve, it becomes clear that the calibration procedure plays a very important role in the correct performance of the analyser.

As established by the Nickolskii-Eisemann equation (2.1), the slope of the calibration curve has a constant value that gradually decreases because of continuous operation of the analysers, 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 2.2 and 2.3 respectively.

The maintenance protocol proposed for the replacement of the electrodes is based on both the use time and on the decrease of the sensitivity shown by the slope variation of the calibration curve.

Electrodes should be replaced when the slope of the calibration curve reaches a certain value that is to be fixed according to the application they are given and to the recommendations given by the supplier. In the case of compartment III of the MELISSA pilot plant 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 is based on two constraints: the slope of the calibration curve that establishes the sensitivity of the concentration measurements, and the time of operation of the electrode. The values for these parameters were based on the recommendations given by the analyzer and were modified during the characterisation process in order to fulfil the requirements of operation in the MELISSA pilot plant (i.e. more conservative values were adopted to ensure accuracy of the results).

Constraints:

- Slope of the calibration curve:
    1. Nitrate analyzer: ($45<K_2<65$)
    2. Ammonium analyzer: ($40<K_2<80$)
- Maximum effective operation time of the electrode:
    1. Nitrate analyzer: 2 months
    2. Ammonium analyzer: 6 months

Actions to be taken:

- Replacement of the ion selective electrodes

The ion selective electrodes should be replaced when either the slope of the calibration curve or the maximum time established above for each electrode is surpassed. Constraint values have been fixed in accordance to the recommendations of the supplier and taking into account the information obtained after a first period of operation of the analyzers as well as the specific analytical requirements of compartment III of the MELISSA pilot plant.

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

3 ON-LINE INSTALLATION

The outlet of compartment III consists of a two-phase flow with an important fraction of gas, which cannot be pumped straight forward to the analysis circuit. Although the analysers include a vessel where potential gas bubbles are removed, the fraction of gas present in the main flow is too high not to have an effect on the correct performance of the analysers. Therefore, the sample cannot be directly fed to the analysers before the liquid flow is separated from the gas flow. A buffer tank is used as gas-liquid separator that has been installed between the outlet of compartment III and the analysers (as can be observed in figure 3.1).

On a first approach the possibility of working with a stirred buffer tank was discussed and its advantages and drawbacks were analysed, the main drawbacks encountered being:

- Cell attachment and development of biofilm on the surface of the stirrer, even in case a magnetic stirrer was used.

- Mixing would lead to samples not being representative of the reactor outflow due to a homogenizing effect.

  For these reasons the volume of the buffer tank was reduced to 25 mL and stirring was not used. In any case, the sampling loop set-up was developed only as a preliminary solution to test the performance of the analysers, and a better design will be provided to ESA, included in the redesign of compartment III. (WP 78.6, Phase 10: “Re-design of comparment III pilot reactor”)

The outlet stream from compartment III continuously enters the buffer tank and the analysers pump the required sample from the bottom of the tank and through a 0.22 μm liquid filter (Millipore) used as an interface between the reactor and analysis loop. The filtration step is required in order to guaranty axenicity of the co-culture of *Nitrosomonas* and *Nitrobacter* inside of the reactor, but its volume needs to be minimised because otherwise, the low sample flowrate that goes through it and to the analyser, would lead to important dead times. The

contamination between samples is avoided in this configuration by allowing a certain amount of sample to flow through the analysers before the actual analysis cycle starts.

Bio safety is guaranteed in the reactor because the buffer tank used as a liquid/gas separator is a closed, sterilised vessel that is separated from the analysis loop by a filtration step.

This on-line analyser has been designed to work with stream water and waste water, and thus their filtration demands are not very important, however, it is necessary to introduce some improvements on the design of this interface between the reactor and the analyser so that the delay between sampling and measurement becomes acceptable for control purposes. These improvements will be considered in the new design of the new pilot reactor for compartment III. The tank has been designed to contain a constant liquid volume of 75 mL so that sample requirements of the analysis loop are satisfied, while all the liquid in excess will be pumped to a 50 L buffer tank situated between compartments III and IVa.

A general scheme of the set-up installation of both on-line analysers is also presented in *figure 11*.

The flow rates of the different streams will be determined in order to estimate the total dead time elapsed between the exit of the sample from the reactor and the instant its concentration is finally displayed.

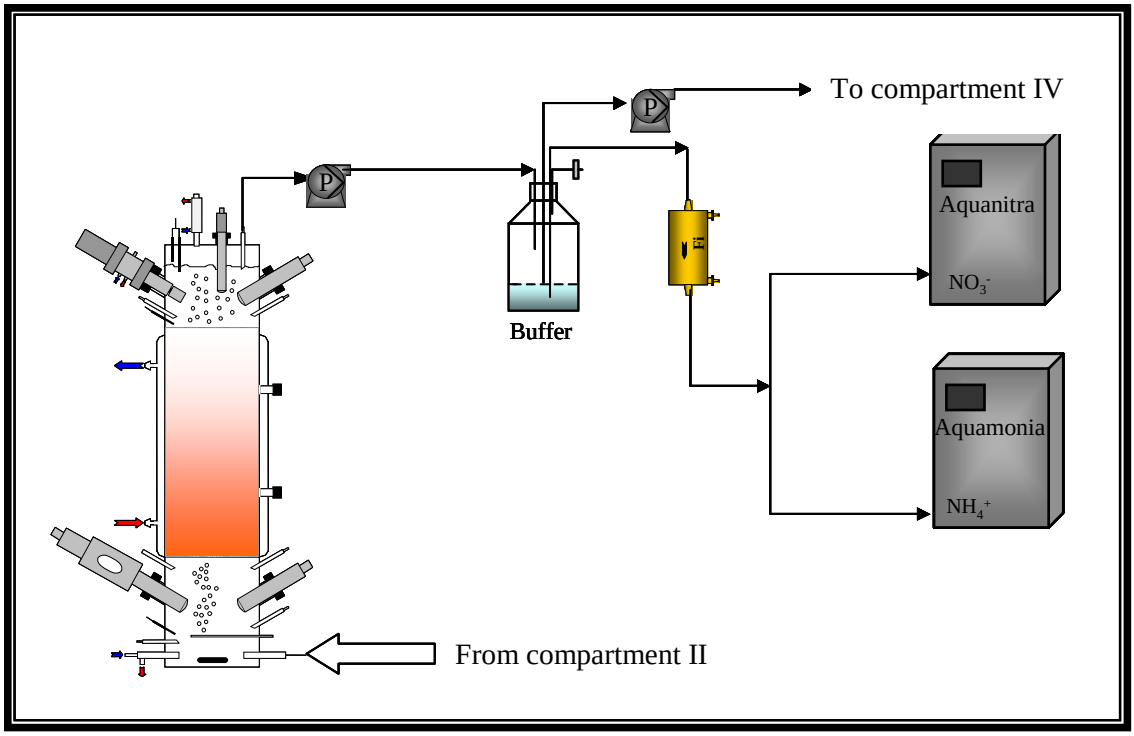

Figure 3.1.- Sample extraction system diagram. *Fi*: liquid filter (0.22 μm).

The analysis cycle carried out by Aquanitra® and Aquamonia® is composed of several steps that can be adjusted in order to achieve the best possible performance of the analysers. The different steps are mainly the same in both analysers, and are defined herein:

- *Initial time*: During this period of time previous to the analyses itself, sample is injected through the system in order to replace the remaining sample from the previous analysis cycle. During this elapse of time the sample does not reach the electrode.

- *Stabilising time*: deionised water is flushed to the analyser in order to stabilise the electrode before each analysis. In this way it is also ensured that there is not any reagent modifier or sample left when the new analysis cycle begins and that the baseline, to which all measurements will be related, is correctly set-up.

- *Injection time*: Sample is injected to 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.

3.1 Sample and reagent requirements of the analysers

The total sample requirements of both the ammonium and the nitrate analyser and the different times involved in the analysis cycle presented in table 3.1 will make it possible to estimate the total dead time.

Table 3.1.- Main parameters and reagent consumption of the ammonium and nitrate analysers

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	11s	**Injection time**	100s
Time between samples	4 min	**Time between samples**	4 min
Total Sample/analysis	6 mL/analysis	**Total Sample/analysis**	12 mL/analysis
Na_2SO_4	7mL/analysis 21 mL/calibration cycle	**Tris**	4 mL/analysis 16 mL/calibration cycle
Water	7mL/analysis 21 mL/calibration cycle	**Water**	8mL/analysis 32mL/calibration cycle
Nitrate Standards	0.5 mL/calibration cycle	**NaOH**	2mL/analysis 8mL/calibration cycle
		Ammonium Standards	7.5 mL/calibration cycle

The volume of the buffer tank installed after the nitrifying reactor and the volume of liquid contained in the liquid filter used in these preliminary on-line tests are both of approximately 75 mL. Taking into account these two dead volumes a preliminary estimation of the dead time can be made. However, this estimation does not take into account the exact length and diameter of all the tubing and should only be used as a first approach to the calculation of the actual dead time.

All the different flow rates involved as well as the amount of sample consumed in each analysis cycle have been measured. The sample volume consumed by the analysers in each analysis cycle is presented in table 3.1. To solve the mass balances in the filter, the total amount of sample required by the analysis loop, i.e. the ammonium and the nitrate analysers, was calculated. The volumes in table 3.1 were measured in the laboratory after the optimal configuration of the analysers had been set up. The total volume of sample consumed in these conditions by the nitrate analyser was of 6 mL/analysis cycle, while the requirements of the ammonium analyser were higher, with a volume of 12 mL/analysis cycle used by Aquamonia and leading to a total volume of 18 mL.

By solving the mass balances in the reactor, the buffer tank and the filter unit, the response time of the analysis system to a perturbation on the steady state of the reactor has been estimated.

3.2 Estimation of the dead time in the buffer tank

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 liquid retention time in compartment III according to equation 3.1:

$$\tau_{buffer} = \frac{V_{buffer}}{V_{reactor}} \cdot \tau_{reactor} \quad (3.1)$$

This ratio between the two retention times is obtained from the combination of equations 3.2 and 3.3.

$$t_{dead\,buffer} \equiv \tau_{buffer} = \frac{V_{buffer}}{Q_{out\,CIII}} = \frac{V_{buffer}}{Q_{C\,IVa} + Q_{filter}} \quad (3.2)$$

$$\tau_{reactor} = \frac{V_{reactor}}{Q_{out\,CIII}} \quad (3.3)$$

Taking into account the normal range of flowrate in the reactor (0.15 L/h-0.6 L/h) and the volume of the buffer tank, its residence time can be calculated using the equations presented above. The dead time in the gas/liquid separation step is between 30 minutes (with a flowrate of 0.15L/h) and 7.5 minutes (with a flowrate of 0.60L/h) when a 75 mL buffer tank is used. The dead time due to phase separation can be estimated as 2% of the liquid residence time in the reactor.

In order to reduce the delay caused by the buffer tank, its volume can be significantly reduced. To this effect, the design of a new sampling system is foreseen and will be considered when the hardware of compartment III is updated.

3.3 Estimation of the dead time in the filter

The main contribution to the total delay in the nitrate and ammonium measurements comes from the use of a filter with an important dead volume. The flow rate that enters the analysis loop during injection is of 3mL/min, but it needs to flow through the dead volume of the filter before it reaches the analysers, and thus the delay is highly dependent on the characteristics of the filter. Initially a liquid filter (Opticap®, Millipore) with a pore size of 0.22μm was used but

its dead volume lead us to look for an alternative filter. In the end it was replaced with a 0.45μm filter that had a much lower dead volume of only 5 mL approximately.

Similarly to the estimation of the dead time in the buffer tank, the delay due to the filter was calculated with equation 3.4.

$$t_{dead\ filter} \equiv \tau_{filter} = \frac{V_{filter}}{Q_{analyser}} \qquad (3.4)$$

The dead time in the filter is determined by the sample requirements of the analysers (see table 3.1). It was assumed that the analysis cycles of both analysers are synchronized, and the estimated dead time referred to the total volume of the filter was estimated to be 0.89 min/mL, which stands for a dead time of 65 minutes with the 75 mL filter we used during the first tests, and a dead time of less than 5 minutes with the 0.22 μm filter with the filter holder. The total amount of sample consumed in each analysis cycle by each one of the analysers can be found in table 3.1. Taking into account that every cycle lasts for 16 minutes, an average flow rate of 1 mL/min going through the filter during analysis is obtained.

Table 3.2: Description of the main elements of the connexion between the pilot reactor of compartment III and the nitrogen analysis loop.

Liquid volume reactor	3.8 L
Residence time reactor	6 - 25 h
Gas-liquid phase separator	75 mL (first tests) – 25 mL
Filter	5 mL
Average flow rate $Q_{Aquanitra}$	0.4 mL/min
Average flow rate $Q_{Aquanomia}$	0.75 mL/min
Dead time in phase separator (25 mL buffer)	2.5 – 10 min (depending on residence time)
Dead time in filter	5 min
Dead time analysis loop and tubing	0.5 min
Total dead time	8-15.5 min

In order to reduce the total dead time, the initial time during which the sample is flowing through the system could be increased, although the frequency of analysis would eventually be reduced and there would not be a significant improvement on the response.

Foreseen improvements should include significant reduction in the dead volumes involved in the sampling extraction, phase separation and filtration. Homogenisation of the liquid sample after phase-separation in the buffer tank would also lead to more accurate results.

4 PRELIMINARY TESTS WITH ON-LINE ANALYSERS

As a final validation, a series of experiments was performed after connecting the nitrate and ammonium analysers on-line.

4.1 Tests performed with Aquanitra

The results obtained during two periods of time within the same steady state are presented in figure 4.1 as a function of time. The black horizontal line symbolises the expected nitrate concentration, while data provided by the analyser over the time are represented by the black circles.

With the results shown in table 4.1 the correct performance of Aquanitra® connected on-line to the nitrifying reactor is verified.

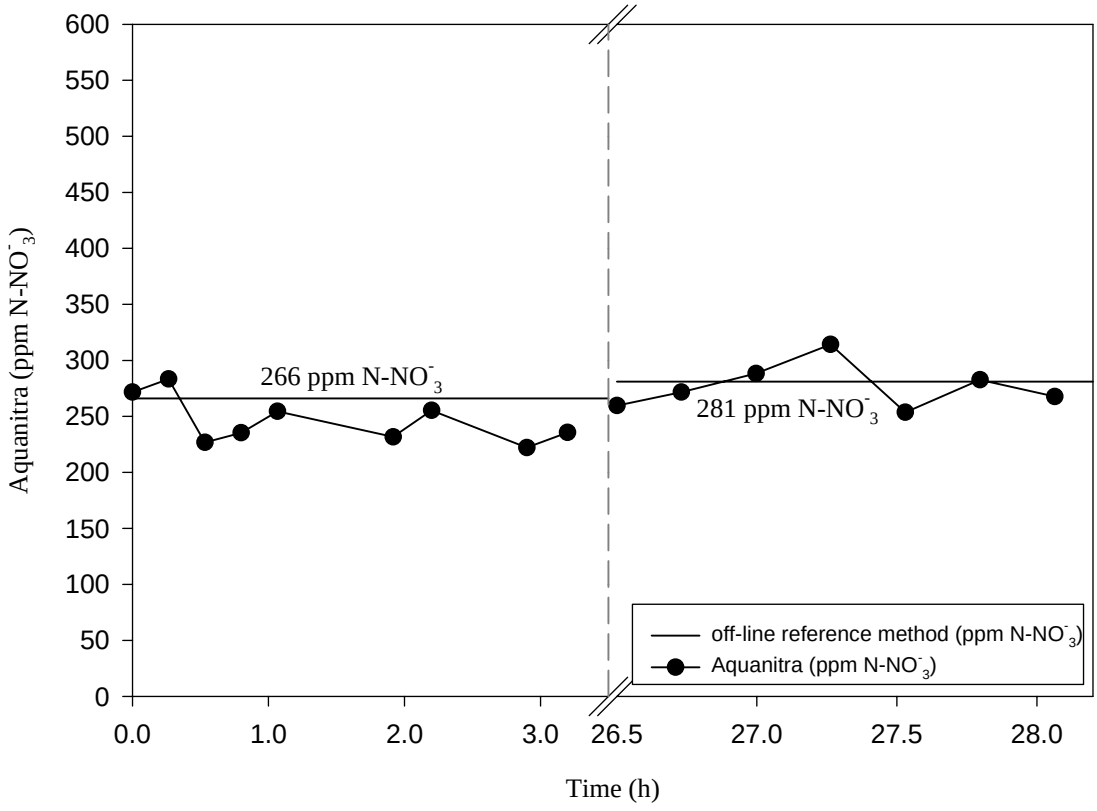

Figure 4.1.- On-line tests performed with Aquanitra over two periods of time.

The same procedure was followed with the ammonium analyser and the results will be presented herein. It must be taken into account that, by the time these preliminary experiments were taking place the nitrifying reactor was operated at a very low flow rate, and thus the conversion to nitrate was almost complete. This led to very low concentration of ammonium, which were not adequate to check the correct performance of the analyser in a more usual, and higher, concentration range.

Table 4.1.- Deviation and repeatability summary for the experiments shown in figure 12

Real (ppm N-NO_3^-)	**Aquanitra (ppm N-NO_3^-)**	**Standard Deviation (ppm N-NO_3^-)**	**Relative SD (%)**	**Relative Error (%)**
266	246.3	21.14	8.6	-7.4
281	276.8	23.82	8.6	-1.5

4.2 Tests performed with Aquamonia

The high differences between the results obtained off-line with the reference method (1.36 mg/L N-NH_4^+) and the results obtained with the on-line analyser, which can be found in figure 4.2, can be reduced by selecting two standard solutions for calibration in a lower concentration range. During these preliminary experiments, degradation of ammonium in the nitrifying reactor was almost complete and thus the standards of 10-50 ppm were not adequate and lead to important errors as can be seen in figure 4.2.

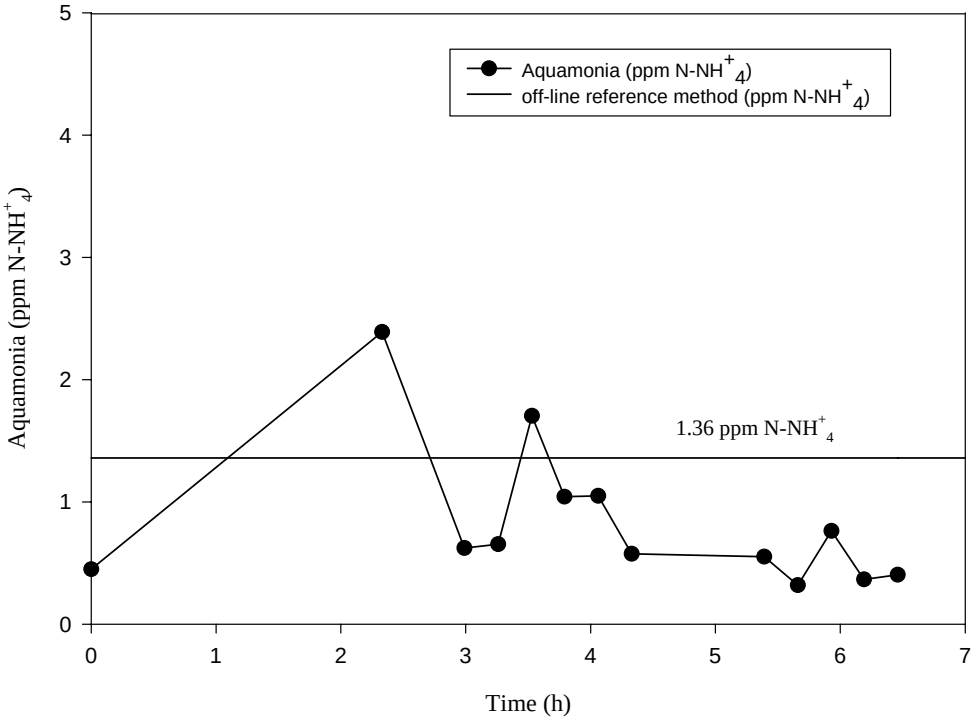

Figure 4.2.- On-line tests performed with AQUAMONIA over two periods of time.

Although the analyser has a detection limit of 0.02 ppm $N\text{-}NH_4^+$, its reliability in low ranges of concentrations strongly depends on the correct selection of the standard solutions used for calibration.

Therefore, in order to improve the performance of the analyser in future experiments special attention has been put in selecting the ammonium standards used for calibration.

5 CONCLUSIONS

Both the ammonium and the nitrate analysers have proved to have a good reliability either off-line or when connected on-line to compartment III. A set of experiments was performed with the main objective to select the best analytical variables for each analyser. Standard solutions, fresh medium with different concentrations of ammonium and nitrate, and real samples were used to perform these tests. Once the analysers had been tested over different steady states, and their reliability been proved in different operational conditions of the nitrifying reactor, the response of the analysers and the sampling system in an event of a disturbance has to be tested. The results from these new experiments will be presented in technical note 52.4.

The experimental set-up used to perform these preliminary experiments with the analyzers connected on-line to the pilot reactor of compartment III showed some problems, the most important being the fact that some conversion may be attained outside the bioreactor due to the biomass accumulation in the buffer tank and the tubing used to connect the outlet of the reactor and the analysers, which would make up the real levels of ammonium and nitrate inside the nitrifying reactor. To avoid this problem the buffer tank will be replaced periodically and the distance between the reactor and the analysers will be kept as short as possible in order to avoid this problem to the maximum extent.

The main interferences have been identified for the ammonium and the nitrate selective electrodes, and the optimal configuration to avoid the effect of interference has been discussed in this document.

6 REFERENCES

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7 COMMENTS ON TN 52.22

- **Section 2.17, page 38/51**
  - **Could you please precise where do the slopes figures are coming from?**
  - **Maximum effective operation time: are there recommendations from the supplier?**

The slopes and maximum effective operation times presented in this section of the TN are those the characterization process of the analyzers proved adequate.

Less conservative values for both parameters were indeed suggested by the supplier, but they were not suitable for the purpose these analyzers are intended for in the MELISSA pilot plant. The figures provided in the manual by the supplier are the following:

***Slope of the calibration curve**

1. ammonium analyzer: 40mV/dec<K_2<80mV/dec
2. nitrate analyzer: 35mV/dec<K_2<65mV/dec

***Maximum effective operation time of the Ion Selective Electrodes**:

1. ammonium analyser: 3 years
2. nitrate analyzer:3 years

The reason why the recommendations given by the supplier had to be modified can be mainly attributed to the following factors:

- The **frequency of analysis** required is much higher than that required by the application these analyzers were originally developed to perform, which leads to lower lifetime of the electrodes.

- The **complexity of the samples** to be analyzed. The first prototypes of these analyzers were conceived to monitor NO_3^- in much simpler media such as river water. The frequency of the replacements is notably increased in the nitrate analyzer due to the high concentration of interfering ions such as HCO_3^- and NO_2^-, whose high concentration not only was found to reduce the lifetime of the electrode itself, but it also made it necessary to use a reagent modifier with a high ionic strength to avoid interference, which also contributes to reducing the lifetime of the electrode.

- The **high accuracy required** by the application: the tolerance of the slope value (K_2) is related to the accuracy required by the application. Because the main function of these analyzers in the MELISSA pilot plant is to provide reliable data that can be used by a control law, a high accuracy is required and thus the interval of acceptable values for K_2 was reduced in respect to the interval suggested by the supplier.

- **Are these recommendations followed?**

Recommendations from the supplier were followed at the beginning of the validation process of the analyzers. However, in the course of this characterization process some adjustments had to be made to the maintenance protocol suggested by the suppliers so that the measurements obtained comply with the standards required by the application these analyzers are to be used for. This has been clarified in the TN text (see page 38).

The figures presented in TN 52.22 are more conservative and according to the experiments performed online following this protocol, they guarantee the proper operation of the analyzers.

- **What is the cost of the membrane replacement, of the electrode replacement?**

Membrane (ammonium analyzer): 5 €
Ammonium ISE: 250€
Nitrate ISE: 150 €