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On-line Instrumentation for N balance

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1 INTRODUCTION

The nitrifying compartment of the MELISSA loop has been studied in order to characterise the operation of the used fixed bed reactors and the effect of the main variables on the biofilm nitrifying process as reported in previous technical notes.

To increase the knowledge on the biofilm nitrifying process and to improve the control of both the third compartment and the total MELISSA loop, a mathematical model is being developed by one of the MELISSA partners (LGCB of University Blaise Pascal, France).

After a first step of parameter optimisation of the proposed mathematical model, the next step will be to develop a nitrite predictor that will become a control law for compartment III of the MELISSA loop (ADERSA, France).

Tests with different levels of dissolved oxygen, to study the effect of this parameter at both transient and stationary states, were conducted in phase 4 observing how this parameter can affect on the ammonium conversion and nitrite and nitrate concentrations (WP 47.2).

Results showed that the nitrification process with immobilized cells is mainly controlled by mass transfer of oxygen, in particular between the gas and liquid phase. If enough oxygen is provided to the liquid phase, the nitrification conversion was always rather complete for the range of ammonium loads studied. This fact proved that the proposed bioreaction system is a good alternative to be implemented as the Compartment III in the MELISSA loop with high conversion and stability for long-term operation.

However, from the results obtained in the previous phase, there are at least two main reasons to assess the need to perform on-line measurements to face N-balance calculations with minimal guaranty and data consistency.

Firstly, following the WP47.2, experiments were carried out in such a way that more detailed information about the reactor and compartment performance could be obtained. In particular, additional tests with different levels of dissolved oxygen were conducted studying the effect for both transient and stationary states, observing how this parameter could affect the ammonium conversion and nitrite and nitrate concentrations.

Although the residual N-NO_2 % decreases when increasing the aeration (vvm), the conversion to nitrate does not increase in a large extent as expected in a reaction-in-series system (NH_4^+--> NO_2^- --> NO_3^-). Among other possible explanations for this fact, there is the different closure of the nitrogen balance and a possible denitrification process in the system. (Pérez, J. *et al.*, 2001)

Secondly, a control law to be applied in the nitrifying pilot reactor has been developed and presented by ADERSA in a previous phase (TN43.3). The control scheme is expected to be shortly implemented and to proceed to its validation numerous and consistent data are required. Thus, it is necessary to review, study and evaluate the specific instrumentation as well as general equipment necessary to perform an on line N-balance based on nitrogen compounds only present in the liquid phase, that is NH_4^+, NO_2^- and NO_3^-

It is very interesting to consider not only commercial instrumentation analysers already installed in the compartment but also potential improvements affecting hardware and quality of the analyses. A general study and evaluation of the present state of the art of the N-compound analysers will be carried out, and as conclusion, new analysers could be necessary to be purchased, to test their performance.

In order to evaluate the capabilities and general performance of the analysers, among others, the following key analysis parameters should be compared: analysis range, accuracy, response time, artefacts, possible drift and suitable calibration procedures. On line determinations will be compared with their corresponding off-line standard method, such as spectrophotometry or capillary electrophoresis, to check whether a good concordance between them is obtained.

Therefore, to achieve an automatic and autonomous operation of the nitrifying compartment, it is a need to provide the pilot reactor with a proper on-line instrumentation for monitoring the concentration of N-species in this compartment. Using this on-line instrumentation it will be possible to know the conversion attained in the reactor and to detect a possible nitrite accumulation in the reactor. The residual ammonium and nitrite concentrations must be minimised in the outlet flow of compartment III.

2 REVIEW, STUDY AND EVALUATION OF ON-LINE INSTRUMENTATION

2.1.- General considerations

On-line measuring techniques and detection systems

A wide variety of analysers for on-line measurements in flowing liquids have been developed in recent years based on different principles. Among these techniques, flow injection analysis (FIA) and continuous flow analysis (CFA) are the most common techniques in laboratory analysis.

All analysers studied in this report are based on the flow injection analysis technique, although the detection system for the analyte is different in each case. 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 measuring systems used by the analysers mentioned in this report are potentiometry and the measuring of absorbance at a certain wavelength. These are all properties that depend selectively on the measured amount of analyte in the sample, permitting determinations in which a separation step is not required.

Performance of on-line analysers

The translation of either absorbance or potential measurements performed by these analysers into concentration values is achieved by means of a calibration procedure previous to sample analysis.

The main features of the performance of these analysers that need to be taken into account are described below:

- *Sensibility* is a parameter given by the slope of the calibration curve, thus, to ensure a high accuracy in the concentration values given by the analyser, measurements should always be performed in the linear range of the fit curve.

- *Reproducibility:* A high repeatability in subsequent analysis of the same sample is an indication of the correct performance of the analyser. The obtained concentration values are statistically treated and reproducibility is given in terms of relative standard deviation.
- The *low detection limit* is the concentration under which the analyte concentration cannot be distinguished from the concentration of interfering ions present in the sample.
- *Response time:* The time consumed until the measurement attains a stable value is described as the response time of the analyser.
- *pH range:* The optimal pH range of operation needs to be established for every analyser according to the composition of the sample. It is very important to keep the pH stable at an adequate value when working with complex samples with high potential ion interference.
- *Ion interference:* Interference from other ionic species present in the sample needs to be identified and operational conditions of the analyser have to be optimised to minimise the problem.
- *Electrode lifetime:* When working with potentiometry-based analysers a persistent decrease in the value of the slope of the calibration curve is a sign of a defective performance of the electrode.

2.2.- Nitrogen analysers trade-off

On a first approach, the nitrogen analysers that had been supplied with the MELISSA pilot plant were considered for use and their performance was evaluated, the advantages and drawbacks of each one of them being discussed in the present report.

2.2.1.- Ammonium Analyser (Amtax LYX720, Dr Lange, Germany):

The measuring method used by AMTAX LYX720 is based on the indophenol blue standard method (procedure DIN 38 406-E5-1 and ISO/DIS 7150), which consists in measuring the absorbance of the indophenol blue complex at a certain wavelength.

The analyser calibrates itself automatically by a two-point measurement. The reaction is linear all over the measuring range and thus only the zero solution (0 mg/L NH_4^+-N) and one standard solution (5 mg/L NH_4^+-N) are sufficient. The system switches automatically from the zero solution to the standard and, once the calibration has finished, to the sample, by means of a solenoid valve. Time between calibration cicles can be fixed by the user.

Measuring range	0-80 mg NH_4^+-N/ L
Accuracy	2% referred to the final value
Detection Limit	0.1 mg/ L NH_4^+-N
Response time	6 min
Time between analysis	6 min
Reagent consumption	Reagent supply: 5L / 21 days of operation Zero/Standard supply: 5L /year

Table 1: Technical data of Amtax LYX 720 on-line ammonium analyser

This analyser has a response time of approximately 12 minutes, which is considered sufficient as well as its precision. However, analysis of samples containing ammonium concentrations over 80 ppm NH_4^+-N would require the installation of a dilution chamber previous to the detection system, but at the present stage only ammonium concentration at the outlet of compartment III needs to be monitored and it is lower than 80 ppm NH_4^+-N.

2.2.2.- Nitrate analyser (NITRAX LPG192, Dr Lange, Germany) :

Despite its high accuracy and short response time, the current nitrate analyser has several drawbacks that make it inadequate for the purpose of monitoring the outlet stream of the nitrifying pilot reactor. In addition to the low detection range (concentrations higher than 50 ppm N-NO_3^- can not be consistently determined) the inability of this equipment to distinguish nitrite from nitrate would lead to high errors during calculations of the closure of the nitrogen balance, and possible nitrite accumulations in compartment III could not be possibly detected. Therefore, the use of this analyser for monitoring of nitrate in the MELISSA pilot plant has been rejected.

2.2.3.- Commercial alternatives to current analysers:

Due to the problems arisen from the performance and the technical characteristics of the current analysers, a search for suitable and more modern on-line analysis equipment was carried out.

Repeatability, accuracy and analysis range are some of the main parameters that have to be taken into account when selecting the most suitable analyser. The importance and the required values for these parameters in the monitoring of the outlet stream of compartment III are exposed below:

The measuring range is an important factor that needs to be taken into account. The selected analysers must have the capacity to measure concentrations in the usual range of operation of the reactor as well as during a temporary perturbation in the system.

N species	**Normal operation (ppm N)**	**Exceptional Values (ppm N)**
NH_4^+	Inlet: 300-600 Outlet: 0-10	Inlet: >700 Outlet: >100
NO_2^-	0-10	>50
NO_3^-	300-600	>700 or <200

Table 2: Concentration ranges of the different Nitrogen species in compartment III

A high accuracy and repeatability are also essential for all the monitored nitrogen compounds due to the fact that these values will be used to evaluate the closure of the nitrogen balance in the MELISSA loop and to determine nitrite concentration.

The final aim of monitoring nitrogen compounds on-line is to implement a control law that will base its response on the concentration values given by the analysers. Therefore, response time is a very important parameter to optimise.

The need for minimum maintenance requirements is also considered essential and thus sample and reagent consumption are important features to be taken into account in the final selection of analysers.

Among the commercial nitrogen analysers available, the search has been narrowed to those accomplishing the requirements described above. The main technical characteristics of these analysers are summarised in table 3 and table 4. The cells corresponding to the selected analysers have been shaded in grey.

EQUIPMENT	AMTAX LYX 720 Dr. Lange	AMTAX Compact Dr. Lange	AQUAMONIA AGBAR (Spain)	A101 AUTOCLEAN WTW (Germany)	AM200 (AWA)	AMMONIUM MODEL 255 EASI Tecnologies
Range (mg NH_4^+-N /L)	0.1-80 (selectable ranges)	0.5-1200 (Selectable ranges)	0.01-255 (adjustable to higher values)	0.1-1000 (3 possible ranges)	0-390	0.1-1000
Measuring principle	Spectrophotometry	Spectrophotometry	Potentiometry	Potentiometry	UV spectroscopy	Potentiometry
Measuring method	Indophenol blue method equivalent to DIN 38 405 E5	Indophenol blue method derived from DIN 38 406 E5	Ion Selective Electrode + Semipermeable membrane	Gas sensitive pH electrode	based on absorption spectrum of NH_3 released by addition of NaOH	Ion Selective Electrode + semipermeable membrane
Flow	1 L/hr	not available	not available	0.3 L/h	0.5 L/min	not available
Repeatability (CV %)	2 % Precision	2 % DIN 38 402	5 % Precision 3 % Exactitude	<5% measured value	1% measured value	≥ 2 %
Response time	t_{90} = 12 min	t_{90}= 10 min	≤ 4 min	T_{90}< 5 min	5 min	T_{90} = 5 min
Minimum recommended measurement interval	≥ 12 min	≥ 10 min	≥ 4 min	not available	≥ 5 min	≥ 5 min
Calibration	2-point automatic standard calibration	2-point automatic standard calibration Automatic cleaning	Automatic standard calibration Interference minimised	2-point automatic standard calibration	not available	Automatic standard calibration Interference minimised
Reagents	2 reagents	2 reagents	NaOH	NaOH, Na_2EDTA	10% NaOH	not available
Dimensions	1190x380x260 mm	350x640x220 mm	not available	1280x570x380 mm	650x450x250 mm	not available
Required modifications	Dilution (>80 ppm)	None expected	None expected	None expected	None expected	None expected

Table 3: Commercial Ammonium Analysers

EQUIPMENT	AQUANITRA® AGBAR (Spain)	UV-4100 / 6101 (ChemScan®)	N201 AUTOCLEAN WTW(Germany)	NT200 (AWA)	NITRATE MODEL 253 EASI Technologies
Range (mg NO_3^--N /L)	225-2250 ppm NO_3^--N (linear zone)	not available (parameter/site dependent)	0.5-50	0-50 (other ranges on request)	1.0 – 1000 ppm N-NO_3^-
Measuring principle	Potentiometry	UV absorption	Dual-wavelength photometric absorption method	UV spectroscopy	Potentiometry
Measuring method	Ion Selective Electrode	Multiple wavelength UV absorbance detection system	UV absorption	UV absorption	Ion Selective Electrode
Flow	< 1 L/hr (analysis frequency dependent)	0.5-5 L/min	not available	0.5 L/min (typical)	not available
Repeatability (CV %)	2.2 % (at 4 analysis/h)	2-5% of range	1-2% measured value	0.2-0.3%	≥ 2 %
Response time	3.7 min	3-5 min (analyte dependent)	T_{90} < 60s	not available	T_{90} = 10 min
Minimum recommended measurement interval	monitoring analysis time = 9 min		not available	not available	≥ 10 min
Calibration	3-point standard calibration	Pattern recognition of spectral data	Automatic zero correction only	automatic standard calibration	Automatic standard calibration
Reagents	No reagent (conditioning solution and calibration standards only)	Change every 2-4 weeks approximately	No reagent	details not available	details not available
Dimensions	1200x600x600 mm	1200x500x250	1280x570x380	650x450x250 mm	not available
Required modifications	ion interference optimisation	dilution	Dilution > 50 ppm NO_3^--N	none expected	Interferences compensation

Table 4: Commercial Nitrate Analysers

2.3.- Final decision

Taking into account the required measuring range as well as other technical characteristics such as accuracy, reproducibility or minimum maintenance requirements, the following analysers have been selected:

2.3.1.- Ammonium Analyser (AMTAX LYX720, Dr Lange, Germany)

At the present stage only measurements of ammonium in the effluent of compartment III are strictly necessary. Amtax LYX720 has all the required technical characteristics to monitor this stream and, in addition, is already part of the equipment of the pilot plant reactor. Therefore, it is selected as the ammonium analyser to be used.

During preliminary testing of this analyser an error was detected that prevented the analyser from completing the calibration procedure. Due to complications during the identification of the problem, the testing of the analyser has been postponed and the results can not be included in the present report. In the meanwhile alternative commercial analysers are already being considered.

For further steps in the integration of the pilot plant, particularly in the connection of compartments II and III, a higher ammonium concentration will have to be measured, and thus the purchase of a new analyser will be eventually required.

In case the purchase of a new ammonium analyser was eventually necessary, Aquamonia® (Aquatec, Spain), which has been developed by the same company as the selected nitrate analyser, is considered a good alternative. The main characteristics of this analyser are presented in table 3.

2.3.2.- Nitrate Analyser AQUANITRA®, Aquatec, Spain)

After an exhaustive evaluation of the technical characteristics of the above-presented commercial alternatives available for nitrate analysis at the moment, Aquanitra® (AQUATEC, Spain) was considered to be the most appropriate choice. This analyser has all the required technical characteristics concerning measuring range, minimum maintenance costs and a good precision. In addition to the technical features,

the fact that this analyser has been developed at the Department of Chemistry at UAB provided the possibility to assay its performance with real samples from the nitrifying reactor before purchasing it. It is also considered an additional advantage that this equipment can be re-adjusted or up-dated in order to adapt it to future needs (changes in range, accuracy, avoiding interference, etc.), due to the proximity and cooperation existing with the group that developed this analyser.

A more accurate description of the selected nitrate analyser is presented below in the next section of this report.

2.3.3.- Nitrite Analyser:

An exhaustive study of the technical data of the different nitrate and ammonium on-line analysers available, has lead to the conclusion that the monitoring of these two parameters will not be sufficient to evaluate the closure of the nitrogen balance with the required high reliability. On-line analysers have been developed in such a way that they are suitable for long periods of continuous operation with a minimum need for maintenance. However, these analysers have not been developed to provide as high an analytical precision as off-line techniques such as capillary electrophoresis.

This observation is applicable not only to Aquanitra but also to the other analysers presented in this report and leads to the consideration of nitrite analysers as well as nitrate and ammonium analysers in order to monitor the effluent of compartment III in a way that the data can be used for the implementation of a control law in the reactor.

On-line nitrite analysers have not been as widely developed as nitrate and ammonium analysers. One of the alternatives commercially available is presented in table 5:

Equipment	***WTW N501 Autoclean***
Measuring method	Spectrophotometric method (Azo Dye Method)
Measuring range	0.005-0.6 mg/L NO_2^-N
Repeatability	<1% (Relative Standard Deviation)
Response time	t_{90}<5 min (measuring interval selectable)
Calibration	Automatic 2-point calibration

Table 5: Technical characteristics of a commercial nitrite analyser

2.4.- Description of Aquanitra®

2.4.1.- Measuring method:

The measuring technique used by AQUANITRA® is based on potentiometry. The analyser measures nitrate by means of an ion selective electrode (ISE) inserted in the sample flow, which is submitted to previous treatment in order to obtain accurate measurements. Possible interference from other ions needs to be identified and their effect on the correct performance of the analyser has to be studied individually.

The initial signal measured by the analyser, which constitutes the base potential line, is due to a flow containing the following components:

- *Carrier solution:* As a flow injection analysis based system, Aquanitra needs a carrier flow in which sample will be injected. Aquanitra uses distilled water as a carrier.
- *Reagent modifier:* A solution containing 0.5M Na_2SO_4 and 10^{-3}M NO_3^-, with a pH of 2.3, is flowing continuously combined with the carrier providing the required conditions for the measurement of nitrate. The use of this solution not only makes it possible to obtain a linear relation between the measured potential and the concentration of the analyte but its low pH prevents species such as carbonate from interfering in nitrate measurements.

The carrier, the standard solutions and the sample are switched to a peristaltic pump by means of five solenoid valves, while the reagent modifier is continuously pumped to a 50 cm mixing coil and the flow subsequently goes through a de-bubbling system before detection. Detection of the analyte is carried out by means of a nitrate selective electrode, in series with a conventional Ag/AgCl reference electrode.

2.4.2.- Ion Interference:

Aquanitra is provided with a method for ion interference compensation. As previously mentioned, a conditining solution is added to the main flow (sample + carrier solution) which maintains the ionic strength as well as the pH of the media at a constant

value. By keeping the ionic strength constant this buffer solution makes it possible to measure concentration instead of activity of the samples and its constant pH value is a treatment against ion interference.

From previous studies carried out at the department of chemistry at UAB, several conclusions had already been attained on which is the most suitable pH to minimise different ion interference. It was concluded that at a pH of 2.3 the effect of HCO_3^- is minimised, whereas at a pH of 4.0 the concentration of HNO_2 is decreased and thus the effect of nitrite interference is minimised. Therefore, the pH of the conditioning solution will be selected according to the characteristics of the stream to be monitored.

The characterisation of the ion interference of carbonate in the effluent of compartment III will be extensively discussed in this report. Carbonate is not only used as one of the components of the culture medium but it is also used for pH control purposes in the nitrifying reactor, and thus its concentration in the reactor is usually much higher than the initial carbonate concentration provided in the culture medium.

2.4.3.- Repeatability and calibration procedure:

From studies performed with Aquanitra® at the department of chemistry at Universitat Autònoma de Barcelona by the research group that developed the analyser, it was concluded that, by increasing the frequency of analysis from 1 analysis/hour (n=27 analysis) to 4 analysis/hour (n=97 analysis) the repeatability of the detector signal improves from an initial relative standard deviation of 3% to 2,2%. (Massana, M. *et al.*, 2001)

The analyser is calibrated by means of three standard solutions with different concentrations of nitrate. The three standards will be selected in such a way that their concentration range comprises the expected nitrate concentration range of the samples in order to always operate in the linear zone of the calibration curve.

It is important to take into account that interpolation in the calibration curve will lead to lower repeatabilities in the final measurement of concentration, and that the relative standard deviation will be higher the lower the frequency of analysis is, as previously reported.

Besides, the influence of the calibration on the final concentration value has been widely studied in this analyser, and some conclusions have been attained on how frequency of analysis can compensate, to some extent, this error. By increasing the frequency of analysis in the same way described above, the relative standard deviation of concentration measurements can be decreased from a value as high as 18.7% to around 10%. Thus, it has been demonstrated that although the interpolation in the calibration source is an important source of error, this error can be controlled by selecting a higher frequency of analysis. A high number of analysis leads to better characterisation of the sample and thus random error is minimised. (Massana, M. *et al.*, 2001).

This interpolation error also depends on the frequency of calibration fixed by the user, in a way that concentrations interpolated using the same calibration curve result on a better repeatability. However, the periodical calibration of the analyser is needed for a correct use of the electrode and it can not be avoided. Both the frequency of calibration cycles and the frequency of analysis can be selected by the user.

Before any further discussion is done on the advantages and possible drawbacks of this analyser, it is important to state that the error provided in the commercial brochures, which is typically lower than 5%, does not include the interpolation error either, and thus the repeatability attained with *Aquanitra* is in the same order as that of the other analysers presented in table 4. Besides, the collaboration of the department of chemistry at UAB provided us with the unique opportunity to test the analyser with real samples from the pilot reactor. Also the experience of the research group that developed the considered analyser is an advantage when it comes to identifying possible interference and adjusting the analyser for an optimal performance.

2.4.4.- Technical data:

Measuring method	Ion Selective Electrode (Potentiometry)
Measuring range	225-2250 mg NO_3^--N / L
Repeatability	2,2-3% (frequency dependent)
Response time	3.7 min (total monitoring analysis time = 9 min)
Flow	< 1 L/hr

Table 6: Technical data of *Aquanitra®* N-102 on-line nitrate analyser

3 STUDY PERFORMED WITH AQUANITRA® ANALYSER

3.1.- Evaluation and quantification of ion interference

On a first approach to the considered analyser several samples from the effluent of compartment III were analysed with *Aquanitra* and the obtained results compared to capillary electrophoresis and Dr Lange test kits.

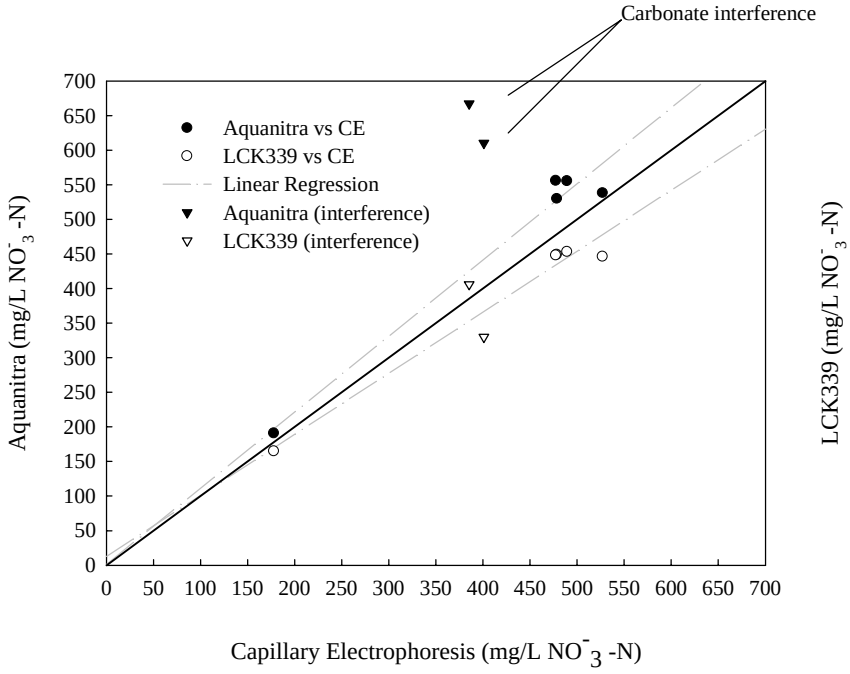

Figure 1: Preliminary experiments performed with *Aquanitra*

In figure 1 it can be observed how in some samples there was a very important deviation of the concentrations measured by the tested analyser *Aquanitra®* from the results obtained by using capillary electrophoresis or spectrophotometric detection with ready-to-use kits (Dr Lange, LCK 339).

During this first set of experiments the trend was for the concentrations obtained with the capillary electrophoresis analyser to be lower than those given by Aquanitra, whereas in further experiments this trend changes. This fact is due to some adjustments made in order to improve the performance of the analyser, but this first analysis is a good example to observe the effect of ion interference on the ion selective electrode used by *Aquanitra* for nitrate detection.

The fact that this problem was not found in all the samples suggested the presence of some kind of ion interference, most likely derived from the carbonate used for pH control purposes in the nitrifying reactor. The shape and height of the potential peaks given by the analyser were carefully analysed and compared to previous analysis performed with the analyser and registered at the department of chemistry at UAB, and it was concluded that the interference was indeed due to carbonate.

The registers of the state of the reactor during that period of sampling were revised and it was observed that samples whose nitrate concentration was erroneously determined by *Aquanitra* had been obtained while important changes in the reactor pH had been occurring. An incorrect performance of the pH sensor had derived in a high amount of carbonate solution being added to the reactor by the control system. A pH as high as 9.3 was attained in the nitrifying reactor and, although it was not possible to quantify the carbonate concentration in the sample, it was high enough to interfere with the correct performance of the nitrate selective electrode used by *Aquanitra*.

The problem was only detected after an important addition of Na_2CO_3 due to pH control, a situation which is unlikely to happen during normal and stable performance of the reactor, while the results obtained during stable operation of the nitrifying reactor, with normal levels of carbonate, were satisfying.

As previously reported, *Aquanitra* is provided with a buffer solution that can avoid carbonate interference at a pH of 2.3. However, when carbonate concentration exceeds certain values the buffer is unable to keep this pH and HCO_3^- is no longer prevented from interfering with the nitrate measurement. At this point HCO_3^- interferes on the potential signal given by the nitrate selective electrode.

Once the interfering species has been identified, an accurate study needs to be carried out in order to establish the maximum allowable concentration of carbonate in

the sample to assess a correct performance of the sensor and to evaluate the possibility to modify the analyser to adequate its performance to the operating conditions of the nitrifying pilot reactor.

Before any further analysis were made with real samples from compartment III, the analyser was optimised and the maximum allowable HCO_3^- concentration was established to be around 1500 mg/L. Concentrations higher than this value, although they may rarely occur during stable operation of the reactor, would interfere with the nitrate sensor and, as a result, on the reliability of the analyser.

3.2.- Selection of an off-line reference method

Samples obtained from the effluent of the nitrifying reactor as well as standard solutions of nitrate dissolved in the culture medium used in compartment III were analysed off-line using different methods in order to select a reference method to which the results obtained with the tested analyser could be compared for evaluation.

The two off-line methods considered in this study are capillary electrophoresis (Waters Ion Analyser), which has been selected for its high precision and repeatability, and the method currently used for off-line analysis of NO_3^- in the MELISSA pilot plant (Dr Lange, LCK339), which is based on the measurement of absorbance of ready-to-use probes containing the necessary reagents.

In order to select the most appropriate off-line reference method, both capillary electrophoresis and the photometric detection kits were tested by analysing a number of standard solutions prepared by dissolving a known concentration of $NaNO_3$ in a solution of culture medium and subsequently diluting it with this same medium.

The standard solutions, with concentrations between 0 and 25 mg/L NO_3^- -N were prepared in such a way that all ion species present in the sample had a concentration as similar as possible to that of the diluted real samples.

The dilution usually applied to the real samples so that they are comprised in the range of analysis of these off-line methods (0-20 mg NO_3^- -N) is 1:25. A solution of 20 mg/L NO_3^- -N was prepared by dissolving the necessary amount of $NaNO_3$ in diluted (1:25) culture growth medium. This solution, at its time, was subsequently diluted in the

same culture medium solution to obtain several standards in the desired nitrate concentration range. By using diluted culture medium as the solvent to prepare the standards an ionic strength similar to that of the real samples is attained.

These standards were analysed using both capillary electrophoresis and Dr Lange LCK339 ready-to-use probes. The results are presented in figure 2.

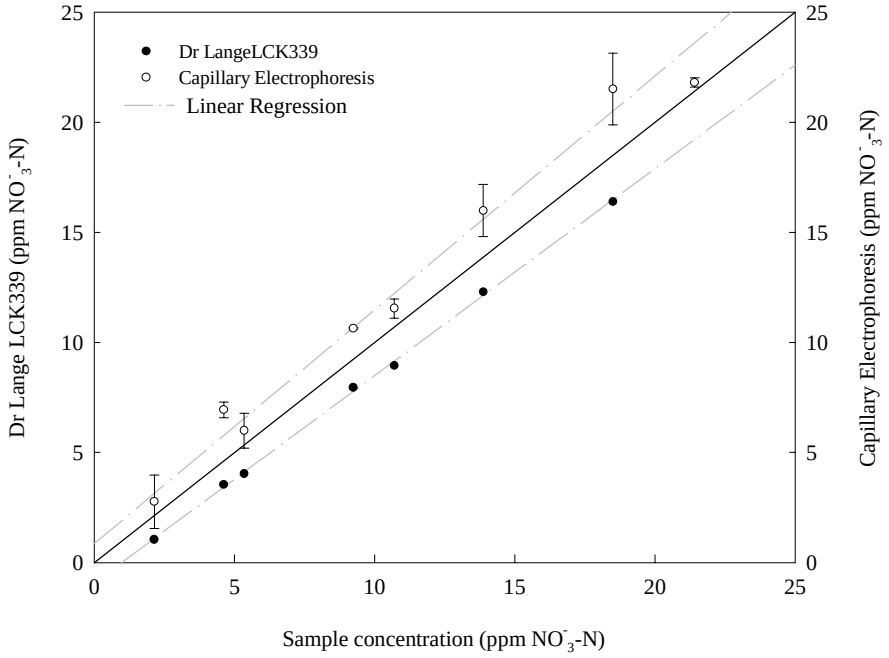

Figure 2: Evaluation of capillary electrophoresis and Dr Lange kits as reference methods

The deviation from the real concentration of the samples with capillary electrophoresis has a typical value between 7-15% and the repeatability of the measurements, evaluated in terms of relative standard deviation of three analysis, has a typical value of 0.5-11%.

The deviation of the spectrophotometry-based method from the real concentration of the sample has a value between –11 and –24% , which is higher than that of capillary electrophoresis.

Besides having a slightly higher precision, capillary electrophoresis has been selected as the reference method because it is a more versatile method and provides further information on the composition of the sample that can be useful to identify other possible sub-products present in the reactor that could affect the correct performance of the tested analyser.

3.3.- Analysis of real samples from the effluent of compartment III

Although the most adequate off-line method has been considered to be capillary electrophoresis, the samples have been analysed using the detection kits as well for further information.

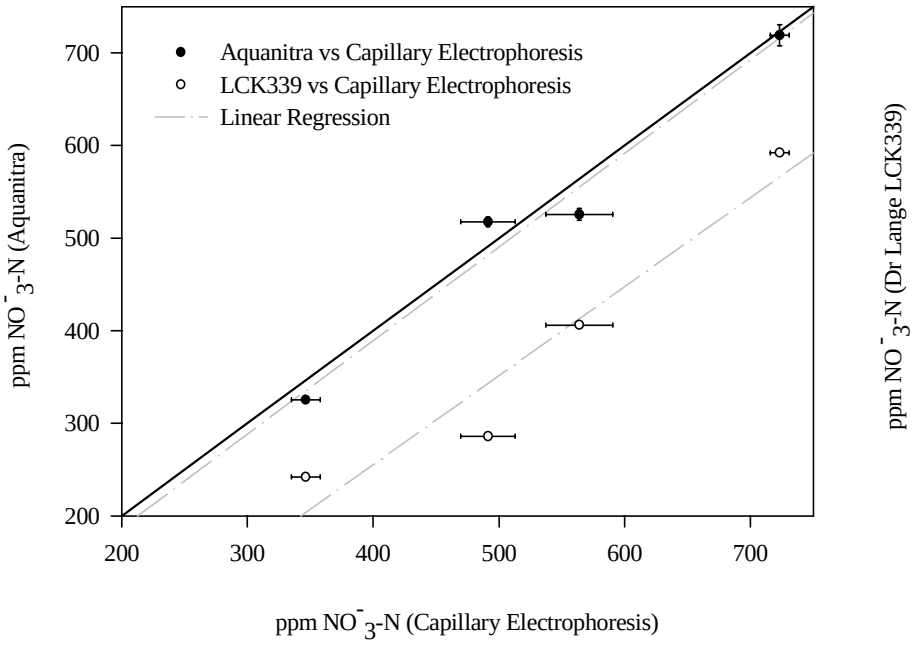

Figure 3: Analysis of real samples and comparison to the reference method (Capillary Electrophoresis)

In figure 3 it can be observed how the trend observed during analysis with standard solutions prepared using nitrifying medium (figure 2) is the same as the trend of the real samples.

Capillary electrophoresis is used as the reference method to which the results obtained with *Aquanitra* will be compared. The concentration given by *Aquanitra* has a deviation from the reference method of –5 to –7%, which, taking into account that capillary electrophoresis was found to have a deviation of 7 to 15% from the real concentration, leads to the conclusion that *Aquanitra* has a high accuracy and is adequate to be used in the MELISSA pilot plant for nitrate monitoring purposes.

4 CONCLUSIONS

Although testing of the current ammonium analyser (*AmtaxLYX720, Dr Lange*).available in the MELISSA pilot plant has been postponed due to a problem during the calibration procedure, its characteristics are considered adequate for the purpose of monitoring the effluent of compartment III, provided that the problem can be identified and fixed by qualified technicians in a reasonably short time.

In the meanwhile, other alternatives are being considered that have been presented in this report. Among the commercially available analysers, *Aquamonia®* is considered a good alternative and further information has been requested as it would be necessary to purchase a new analyser either now, if the problems with the current analyser can not be fixed, or in the future, when the ammonium concentration in the inlet of compartment III has to be monitored as well.

On a first approach it was proposed to monitor nitrate and ammonium concentrations in the outlet of the nitrifying reactor and calculate nitrite concentration by taking into account the closure of the nitrogen balance. However, from the studies carried out with *Aquanitra* and the information obtained of other commercial on-line analysers it has been concluded that precision and repeatability of on-line analysers are not sufficient for this purpose and thus it is necessary to consider the purchase of a nitrite on-line analyser.

Although a search for commercial nitrite analysers has been carried out, it is important to take into account that nitrite is not as common a monitored parameter as nitrate and ammonium are and thus equipment has not been as widely developed and is more difficult to find. The technical data of a nitrite analyser that could fulfill the requirements of range and accuracy are included in this report. Further research will be carried out in the near future concerning on-line nitrite instrumentation.

After carefully studying the technical characteristics of several commercial analysers for on-line monitoring of nitrate, *Aquanitra®* has been considered as the most suitable analyser to be installed in compartment III.

The performance of this analyser has been widely studied, and based on the positive results presented in this report the purchase of *Aquanitra®* as the analyser to be used to monitor nitrate concentration in the outlet of compartment III of the MELISSA pilot plant is submitted for approval.

5 REFERENCES

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