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Efficient Operation of Urban Wastewater Treatment Plant

EVALUATIONS OF THE APPLICABILITY OF EXISTING SENSORS AND BIOSENSORS

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Name of responsible: Professor André Pauss

Abstract:
The objective was to evaluate the applicability of existing sensors (titration sensor and UV-spectrophotometric sensor) to monitor the performance of sequential batch reactor (SBR) for wastewater treatment. The two partners POLIMI and GRADIENT investigated lab-scale titration (DO-stat and pH-stat) sensors and the UV-spetrophotometric sensor, respectively. For POLIMI, a lab-scale SBR was constructed to provide the sludge required for testing the sensors. On one hand, by the experiments performed during the first year of the EOLI project, it can be stated that titration biosensors (pH-DO-stat technique) are suitable to monitor biological processes in SBRs. At this moment it is possible to 1) assess the rapidly biodegradable organic content of the SBR influent, 2) assess the end point of nitrification reaction of the SBR, and 3) measure the maximum nitrifying activity of the SBR. More investigation is needed to monitor the denitrification process with synthetic and real sewage, and to obtain more reliable DO-stat tests with the new titrator prototype MARTINA.On the other hand, the first results obtained from the UV spectral analysis are promising to monitor biomass, total organic carbon and the nitrogenous compounds (nitrates, nitrites and ammonia) during SBR cycles. Indeed, for each wastewater (OECD, LBE-Narbonne and UNAM effluents), these compounds absorb in the UV at wavelengths sufficiently different to ensure discrination between the corresponding spectrum peaks. This will permit in the next year to build models for the monitoring of these compounds.

Keyword List: SBR, wastewater treatment, titration sensors, DO-stat, pH-stat, UV-spectral analysis

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I – Introduction

Within the EOLI project main objectives of POLIMI experimentation for the first year is the evaluation of the performance and usefulness of existing lab-scale titration (DO-stat and pH-stat) sensors to monitor the performance of SBRs.

To cope with this objective, the first year of Polimi activity included the following phases.

First an accurate review of literature data on the application of pH-DOstat technique to monitor biological processes, allowed to design an experimental protocol to apply this technique to monitor those bio-reactions typically taking place in an SBR reactor processing municipal wastewaters. Specifically, experiments were performed aimed at:

- the assessment of the biodegradable organic content of the SBR influent;
- the feasibility to assess the end of the nitrification process during the aeration phase of the SBR cycle;
- the determination of the maximum nitrification activity of the SBR sludge;
- the assessment of the denitrification maximum activity of the SBR sludge.

Then, Polimi constructed and put into operation a lab-scale SBR to provide the sludge required for testing the pH-DO stat technique. Technical details on the SBR can be found in the report related to WP1.

Laboratory prototypes of pH-stat and DO-stat titrators were initially used to assess the applicability of these techniques to monitor SBRs performance. Preliminary titration tests on SBR sludge were performed with two existing instruments. The first was an existing pH-stat laboratory prototype, developed according to the specifications of the ANITA (Ammonium NITrification Analyser) instrument (Massone et al., 1998). The latter was designed to measure activity of ammonium oxidisers and ammonium concentration in activated sludge samples. The second instrument was a DO-stat titrator that was developed in accordance with Ficara et al., 2000. This titrator was designed to measure the respirometric activity of aerobic activated sludge samples.

The experience gathered during this experimental campaign allowed to identify some limits of the existing titrators, which have been overcome by designing a new combined titrator to be applied to SBRs monitoring. The new instrument was named MARTINA (Multiple Analysis Reprogrammable TItration Analyser), characterised by an increased software flexibility and by a new signals acquisition board developed to increase hardware reliability and to reduce components cost.

Then, this prototype was tested on SBR off-line monitoring. Details about this prototype are reported into the SPES-WP3 report.

In the first year of the EOLI project, GRADIENTwas charged to evaluate the applicability of the UV-spectral analysis to monitor biomass, total organic carbon and nitrogenous compounds (nitrates, nitrites and ammonia) during SBR cycles. The technique was applied on pure constituents and on the wastewaters used by the partners in the EOLI project. The possibility to discriminate the spectrum

peaks corresponding to the compounds absorbing in the UV was investigated to ensure the possibility to build models for the monitoring of these compounds. The results are presented in this report.

II – State of the Art of the hardware sensors for Sequential Biological Sensors

II.1 Review of the existing hardware sensors

An accurate literature review evaluated the existing hardware sensors for monitoring biological processes in SBR and the results are synthetically reported here below.

The following table and this paragraph constitute the contribution of POLIMI to the "Report on existing lab-scale hardware sensors to monitor SBR reactors", which is the first milestone requested within WP3 contract negotiation forms at T0+10.

The most promising hardware sensors in the field of upset early warning systems belong to the class of biosensors and bioassays and measure the interaction of pollutants with biological systems. They incorporate a biological component, which allows to obtain more complete information than conventional physico-chemical sensors, such as DO (dissolved oxygen), pH, ORP probes etc… and can be classified according to the measuring principle as reported in Table 1 (summarising Love and Both, 2000).

Table 1 Review on existing biosensors for biological process control

Name	Measuring principle	References
pH-stat titration	It exploits the ability of some microorganisms to convert a neutral substrate into an acid or basic product, or to consume an acid or basic substrate to make a neutral product. These systems are potentially applicable to any pH affecting reaction in aerobic, anoxic or anaerobic conditions	Ramadori et al., 1980; Ficara et al., 2003.
Respirometry	It is the most broadly applied technology in WWTP biosensing. It is based on the measurement of oxygen uptake rate as an indicator of the biomass activity	Spaniers et al., 1998.
Microcalorimetry	It allows to evaluate the rate of a biochemical reaction by the assessment of the heat exchanged with the reaction environment	Beaubien and Jolicoeur, 1985. Aulenta et al., 2002.
Whole cell assays and biosensors	These techniques allow for the assessment of the metabolic state of a viable microorganism by means of a physico-chemical tranducer. The latter is located close to the biomass (bioassays) or on the support on which the biomass is fixed (biosensors). The most commonly applied bioassay is the Microtox®, which detects, via a luminimeter, the metabolic state of a naturally bioluminescent marine bacterium. Other widely applied amperometric transducers are dissolved oxygen probes. The biomass may be immobilized on the polyethylene membrane of the DO probe, typically by entrapping it in a polymeric matrix, which is then held in place by another appropriate membrane	Roger and Gerlach, 1996, 1999.
Molecular based biosensors	In these devices the biological component is a specific molecule (e.g. an enzyme, an antibody or a nucleic acid) which reacts with a specific substrate. This technology, initially applied in clinical and food processing fields, is at present being extended to environmental applications. Because of their specificity, these devices are generally devoted to the detection of a defined class of analytes of environmental concern. Based on their recognition element, molecular biosensors can be subdivided into two main groups: biocatalytic (enzimes) biosensors and bioaffinity (antibodies, receptors, DNA) biosensors. Current applications for these biosensors include the detection of pesticides (such as 2,4-D, carbamates, triazines), organic compounds (such as alcohols, sugar acids, benzo-a-pyrene, BTEX, cyanide, formaldehyde, organonitriles, phenol, BCP), metals (mercury, cadmium and zinc), potential carcinogens and endocrine dirsruptors	Roger and Gerlach, 1996, 1999.

Several experiences are already available in the literature demonstrating the feasibility of using pH and DO-stat titration to monitor biological processes used in environmental applications as demonstrated in the following paragraph.

II.2 Ph-stat and DO-stat titration

Literature review on pH-stat and DO-stat titration

The principle of operation of these techniques is rather simple and hereafter described.
The activity of a microbial population in a batch reactor may be evaluated by monitoring and maintaining constant, by controlled addition (titration) of an appropriate titrant, the in-reactor concentration of one among the chemical species which is either consumed (reagent) or produced (product) by the assayed biomass (Rozzi and Ficara, 2001). Theoretically, this principle of operation is applicable to any reaction involving at least one reagent/product which is allowable to be measured and controlled at a constant (static) concentration. The biological activity of the sample is determined by measuring the titrant flow rate and by taking into account the stoichiometry of the reaction. The initial concentration of the consumed substrate is derived by measuring the total volume or the total mass of titrant added.
Up to now, this technique has been applied to reactions producing acidity or alkalinity and to reactions consuming dissolved oxygen. In the first case, protons concentration is measured by a pH probe and maintained constant by addition of an acidic or alkaline solution (pH-stat titration). In the second case, dissolved oxygen (DO) is measured by a DO-probe and maintained constant by the addition of an oxygen-enriched solution (DO-stat titration).

The pH-stat titration technique

As stated above, pH-stat titration is applicable to processes which modify the suspension pH. Within processes applied in the field of wastewater treatment, the following have been considered for assessment by pH-stat titration:

- nitrification activity (Ramadori et al, 1980; Massone et al., 1998; Gernaey et al., 1998; Ficara et al., 2000a, 2001, Ficara and Rozzi 2001);
- denitrification activity (Massone et al., 1996; Bogaert et al., 1997; Foxon et al., in press)
- acetoclastic methanogenesis (Rozzi et al., 2002)

aerobic oxidation of organic substrates (Ficara and Rozzi, 2002)

Moreover, pH-stat titration has been used to characterise wastewaters, specifically it was applied for:

- determination of the nitrifiable nitrogen content of a wastewater (Massone et al., 1995, Yuan & Bogaert, 2001),

- determination of the readily biodegradable organic load of a wastewater in term of BND (Biological Nitrate Demand) (Rozzi et al., 1997a) ,
- determination of the presence of volatile fatty acid in wastewaters (Rozzi et al., 1997b),
- determination of the organic content of a wastewater in term of carbon dioxide production (Ficara and Rozzi, 2002).

Those applications which are considered of interest for sequencing batch reactors are here below reviewed in more details.

Nitrification monitoring

The application of pH-stat titration to nitrification activity assessment was first proposed by Ramadori et al. (1980). This idea was then extensively developed and results on the application of this system to the estimation of nitrification rate and to ammonia concentration can be found in Massone et al. (1998). The proposed methodology is here briefly reviewed. A fixed volume of activated sludge containing ammonium is poured into a thermostated vessel where it is kept aerated and mixed. The pH-stat titration unit keeps the pH of the mixed liquor within the interval: pHstat ± ΔpH. The value of pHstat corresponds to the equilibrium pH of the mixed liquor aerated under endogenous conditions. The tolerance ΔpH is fixed at 0.02 pH units and it is required to stabilise the titration process.
A typical titration curve is reported in Figure 1. At the beginning, concentrated base is required to increase the sludge pH to the set point value. Once this pH has been reached, diluted base is dosed to compensate for the acidity produced by nitrification. Finally, when ammonium has been completely consumed, base addition stops and the cumulative base addition curve reaches an horizontal asymptote. The nitrification rate is estimated from the slope of the titration curve calculated from titration data collected for 4-5 minutes after the pHstat equilibrium has been reached. The ammonium concentration originally present in the sludge sample is proportional, via the nitrification stoichiometry, to the amount of base added during the test. However, since at the beginning of the test, base is also added to increase pH to the pHstat value, the cumulative titration curve is corrected by taking into account that the nitrification rate during these first minutes is practically the same as the previously determined nitrification rate. Thus, B0 (in Figure 1) represents the amount of base added to correct the pH, while the volume of titrant between B2 and B0 is proportional to the ammonium oxidised during the test.

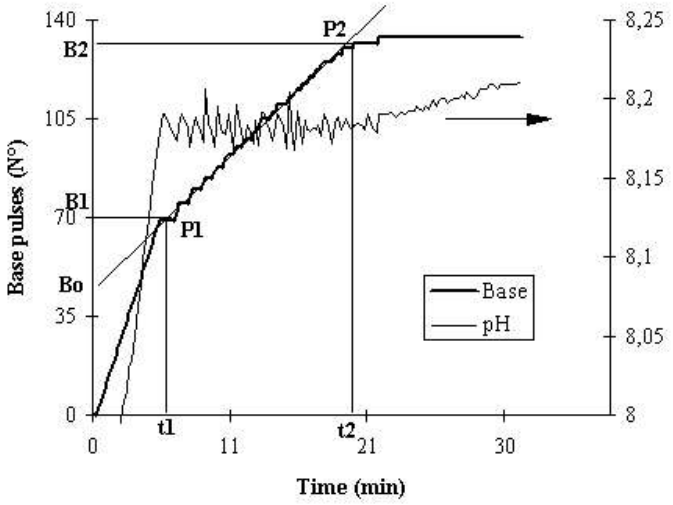


Figure 1 Evaluation of nitrification rate and initial ammonium concentration from the cumulative base volume curve (from: Massone et al., 1998).

The instrument used in this experimentation was named ANITA (Ammonia and NITrification Analyser) and the method proposed to measure ammonium concentration was later successfully patented (Rozzi et al., 1999).

The titrimetric technique for assessment of nitrification kinetics was also coupled to a respirometric technique (open respirometry) by providing ANITA with an oxygen electrode. This experimental set-up allowed the simultaneous collection of titrimetric and respirometric data, and was used to compare the two techniques. Kinetic parameters of ammonia oxidisers estimated using these two techniques appeared to compare very favourably (Ficara et al., 2000). Furthermore, titrimetric and respirometric data allowed for the simultaneous estimation of the kinetic constants for both ammonium and nitrite oxidisers. In fact, although oxygen is consumed by both ammonium and nitrite oxidisers, pH is affected only by the former bacteria. This means that titration data enable subtraction of the contribution of ammonia oxidisers to the total oxygen uptake rate (OUR), evidencing OUR due to nitrite oxidisers (Ficara et al., 2000). This procedure enables the complete characterisation of the nitrifying biomass kinetics by means of simple, short (1÷1.5 h) tests without the need for analytical substrate determination.

An eco-toxicological application of pH-stat titration is potentially very interesting since nitrifiers are among the bacteria most sensitive to a great variety of toxic substances. Therefore, they can be used to provide a realistic estimate of the potential toxicity of new chemicals, especially xenobiotics. Two preliminary investigations were carried out in 1997 (Gernaey, 1997; Massone and Rozzi, 1997). Later a standard titration procedure was proposed by Ficara and Rozzi (2001) as an alternative to the ISO 9509 standard. The latter requires a considerable number of time-consuming and expensive chemical analyses (seven determinations for each of the following parameters: NH_4, NO_2 and NO_3), whereas the

proposed methodology allows the EC_{50} to be obtained in less than four hours with no analytical determinations. A nitrification inhibition test based on the titration procedure is plotted in Figure 2a. It was performed on a sample of nitrifying activated sludge drawn from a domestic wastewater treatment plant. First, ammonium was spiked at such concentration that nitrification could be considered as a zero order reaction during the whole test. The maximum nitrification rate is considered as the 'blank' activity. Starting from t = 20 min, aniline was spiked several times (as indicated by the arrows in the diagram), in order to obtain progressive increasing concentrations. The reaction rate after each addition is compared (as a ratio) to the blank value and plotted against the corresponding inhibitor concentration (the dose-response curve, Figure 2b). The latter graph allows the estimation of the EC_{50} concentration (50% effect concentration) for the tested toxic substance.

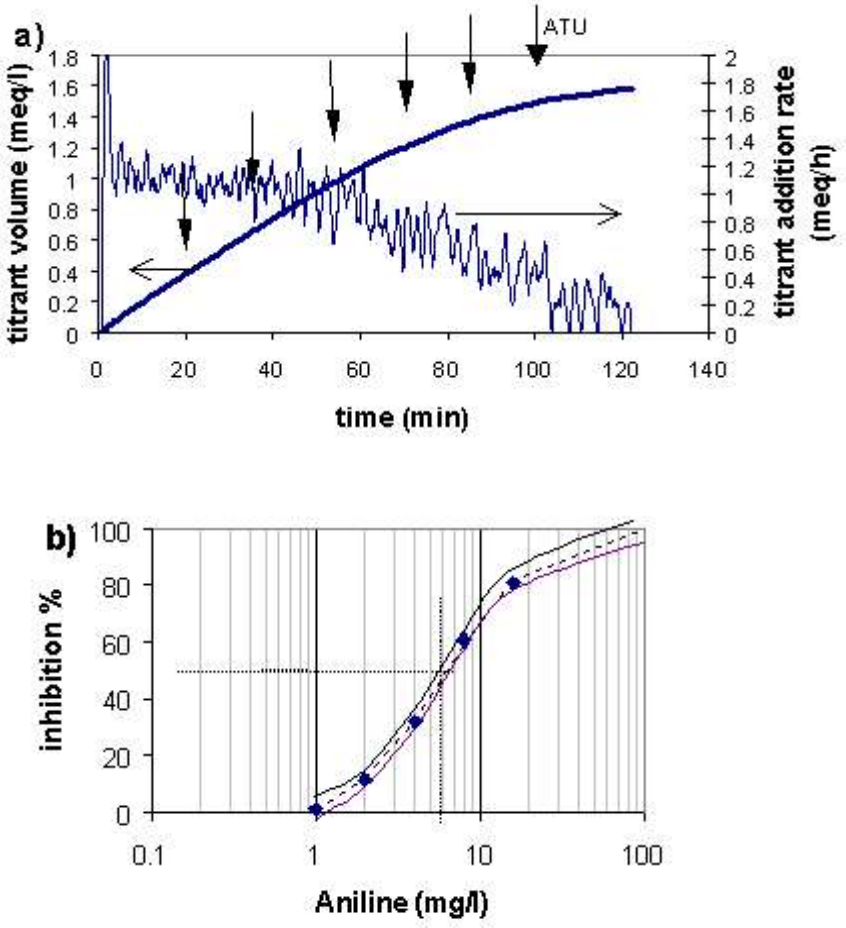


Figure 2 Example of inhibition test with aniline: a) cumulative titrant volume and titrant addition rate, (the first 5 arrows indicate aniline spikes, the last one indicates ATU addition). b) concentration – inhibition curve (from: Ficara and Rozzi, 2001).

pH-stat titration was finally applied for monitoring nitrification activity and ammonia concentration in full scale wastewater treatment plants. Gernaey et al. (1998b) coupled a pH-stat titrator to an on-line

ammonium analyser to check for the reliability of the estimation of ammonium concentration at the outlet of a nutrient removing pilot plant. The titrator sampled the sludge from the last one of three successive aeration tanks. A satisfying correspondence was found between the two measuring systems (Figure 3).

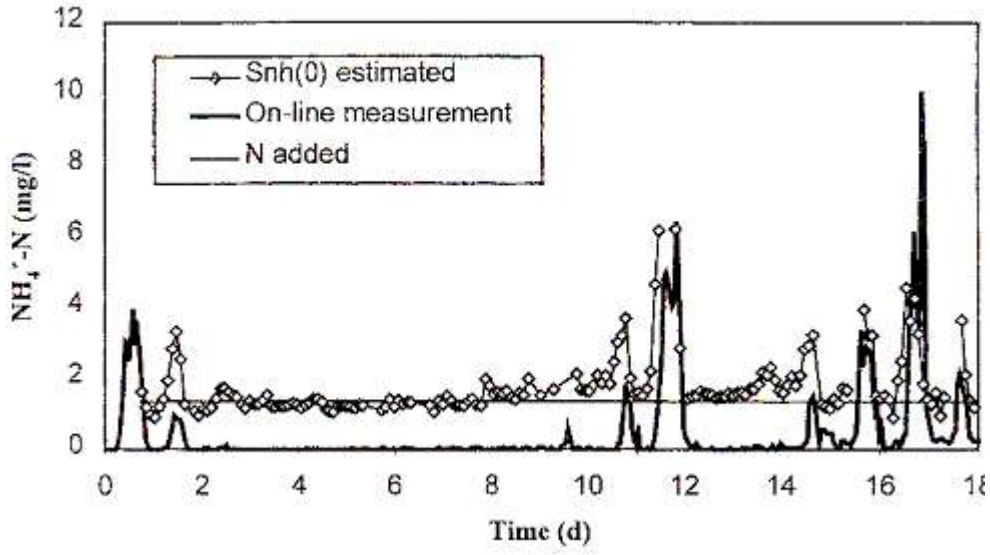


Figure 3 Comparison between ammonium estimation from the pH-stat titration unit and the on-line ammonium analyser (from: Gernaey et al., 1998b).

Another experimental campaign on the applicability of pH-stat titration to the monitoring of nitrification was conducted on a pilot scale activated sludge treatment plant, installed side-stream to a full scale wastewater treatment plant treating textile (70% of the influent load) and urban wastewater. The pilot plant consisted in 3 completely stirred tanks in series, simulating the hydraulic behaviour of the full scale aerobic basin. Titration tests were carried out by sampling sludge from the 3 tanks at increasing oxidation level. Figure 4 reports a typical trend for titration rates obtained. In the first phase, the titration rate is at its maximum to increase the sludge pH to the setpoint value. In a second phase, it decreases to a value which is proportional to the nitrification rate of the activated sludge sample. Once the nitrifiable-nitrogen is fully oxidised, it reaches a lower rate, proportional to the heterotrophic carbon dioxide production rate (third phase). Nitrification rate was estimated by subtracting the titration rate related to the third phase to the rate measured during the second phase. Furthermore, the area under the titration rate (i.e. the titrant volume) was used to estimate the nitrifiable-nitrogen content (Ficara et al., 2001). To validate the titration procedure for the estimation of the nitrifiable-nitrogen, a known amount of ammonium chloride was spiked in sludge samples in endogenous conditions (i.e. drawn from the third tank of the pilot plant). When compared to the spiked concentration, estimates of ammonium concentration were found to be satisfactory as one can

observed from Figure 5 (maximum difference of about 20% on 40 determinations). Moreover, the maximum nitrification rate for the 3 tanks were found to correlate with the ammonium concentration in the influent to the plant (Figure 6, unpublished data).

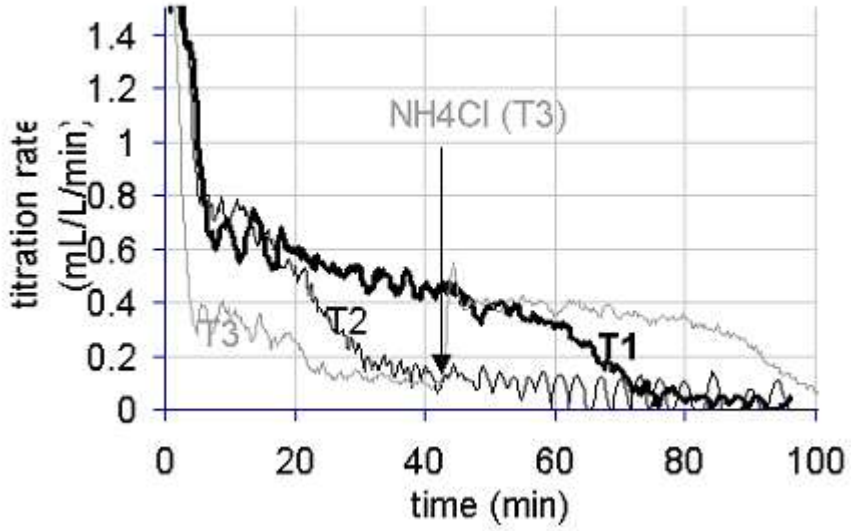


Figure 4 Titration curves obtained from activated sludge samples drawn at increasing oxidation levels (from T1 to T3).

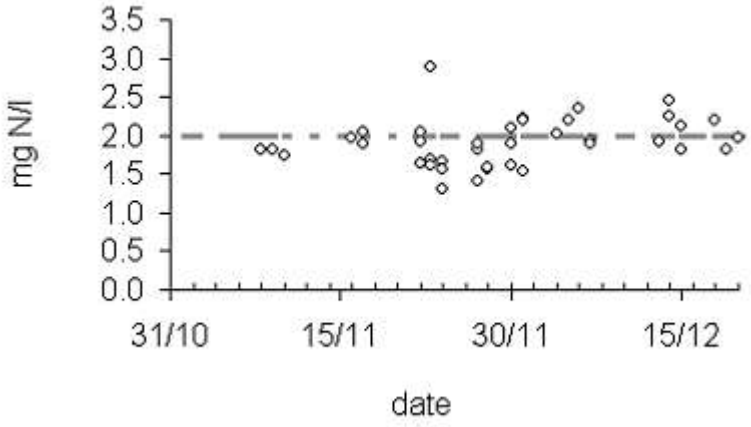


Figure 5 Comparison between actual ammonium concentration and that one estimated by pH-stat titration.

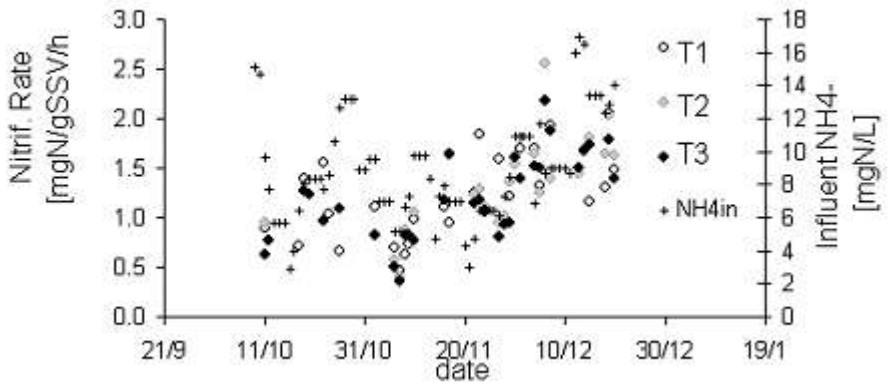


Figure 6 Correlation between nitrification rates estimated by pH-stat titration and ammonium concentration in the influent.

Denitrification monitoring

It is well known that denitrifiers are alkalising bacteria. The DENICON (DENition CONtroller), is the first application of pH-stat titration to an alkalising biomass (Massone et al., 1996). A wastewater containing nitrates is added to a vessel with denitrifying activated sludge and readily biodegradable organic carbon (rbCOD) well in excess with respect to denitrification requirements. Denitrifying activity and nitrate concentrations are respectively measured by monitoring the addition rate and the total volume of acid required to neutralise the alkalinity until the reaction is completed. Titration profiles and nitrate recovery factors obtained using the DENICON are similar to those obtained by ANITA in nitrification. Details of DENICON's operation and on data processing can be found in Massone et al. (1996) and Rozzi et al. (1999b).

The above methodology, originally designed to measure nitrates, was modified to measure rbCOD. The procedure is as follows: a sample of wastewater containing readily-biodegradable organic carbon (rbCOD) is added to a denitrifying biomass sample in the presence of non-limiting nitrate concentration. As in the previous applications, denitrification produces alkalinity, which is neutralised by the addition of an acid titrant. The biodegradation of rbCOD is indirectly determined by the reduction of the nitrate fraction needed to oxidise those organics (Rozzi et al., 1997a; Rozzi et al., 1998). The main problem with this application is the variable stoichiometric ratio of COD/N-NO_3, which depends on the composition of the organics to be biodegraded.
The amount of nitrates needed to biologically oxidise a given mass of organics in anoxic conditions has been defined as the Biochemical Nitrate Demand (BND), and this could be used to estimate the denitrification potential of a wastewater or a given chemical product (Rozzi et al., 1997a).

An automated titrator was used to monitor the influent concentration in a pilot scale fluidised bed anaerobic reactor, as explained above. In Figure 7, the COD measured by an on line instrument is plotted (Rozzi et al., 1998).

The titrimetric procedure was also applied to estimate nitrate concentration in the influent to a wastewater treatment plant in Zalm (B). This estimated concentration was then used to control the carbon source addition to enhance denitrification, leading to a significant reduction in the effluent nitrate concentration (Bogaet et al., 1997; Yuan et al., 1997).

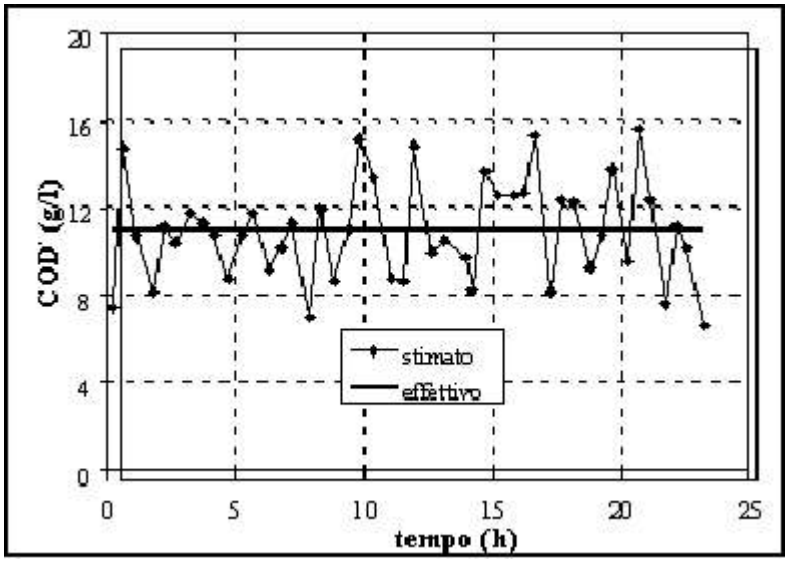


Figure 7 rbCOD content estimated by pH-stat titration compared to the actual value (Rozzi et al., 1998).

The DO-stat technique

The DO-stat technique consists in maintaining constant the DO concentration by titrating with a solution which contains oxygen (Rozzi et al., 2003). As a rule, in DO-stat determinations the appropriate titrant should be an oxygen oversaturated solution. To overcome technical limitations related to the use of such solutions, which do not allow to exceed concentrations over 40 mg/l, an adequately diluted hydrogen peroxide solution was selected as titrant. Compared to classical open and closed respirometers, i.e. gas flowing and gas static systems (Spanjers et al., 1998), this instrument offers the following advantages (Rozzi et al., 2003):

It allows to work at a constant DO level which can be freely chosen by the operator, the only limitation being the necessity to avoid significant oxygen transfer between liquid and gas phases. Since tests are performed at a constant DO level which can be selected well above the semisaturation constant for DO, there is no interference due to the kinetic limitations by DO depletion during the test.

As DO can be freely chosen, tests can be performed at the most appropriate DO concentration, e.g. at the DO level at which the biomass was grown or the DO level maintained in the aeration tank. Moreover, by performing several respiration test at various DO level, the semisaturation constant for oxygen can be easily and accurately assessed.

there is no need to estimate the gas transfer coefficient (K_La), differently from gas flowing systems, since oxygen is provided by a liquid flow;

no re-aeration cycles are required, thus continuous measurements can be performed, differently from gas static systems, since oxygen is supplied whenever it is consumed;

accurate calibration of the DO probe, required for both gas flowing and gas static systems, is unnecessary, since the probe is only employed to sense oxygen variations within a given tolerance range. Moreover, the response dynamic of DO probes does not significantly affect respirometric estimations since the probe dynamics only influences the maximum range of DO oscillations around the set point value.

Experimental data processing is very simple since H_2O_2 addition rate is directly proportional to the oxygen uptake rate and the volume of H_2O_2 required to keep DO at the set point value is directly proportional to the substrate oxidation rate (Figure 8).

Apart from the above advantages, this procedure may lead to underestimation of the biomass respiration during long duration tests when low biomass concentration (< 2÷3 gTSS/L) is used. This toxic effect could limit the applicability of the DO-stat for respiration experiments on diluted biomass. However, because of its simplicity, this procedure is suitable for on-line process control. In such an application H_2O_2 toxicity effects are unlikely to occur since a new biomass sample can be drawn from the aeration basin for each respirometric determination.

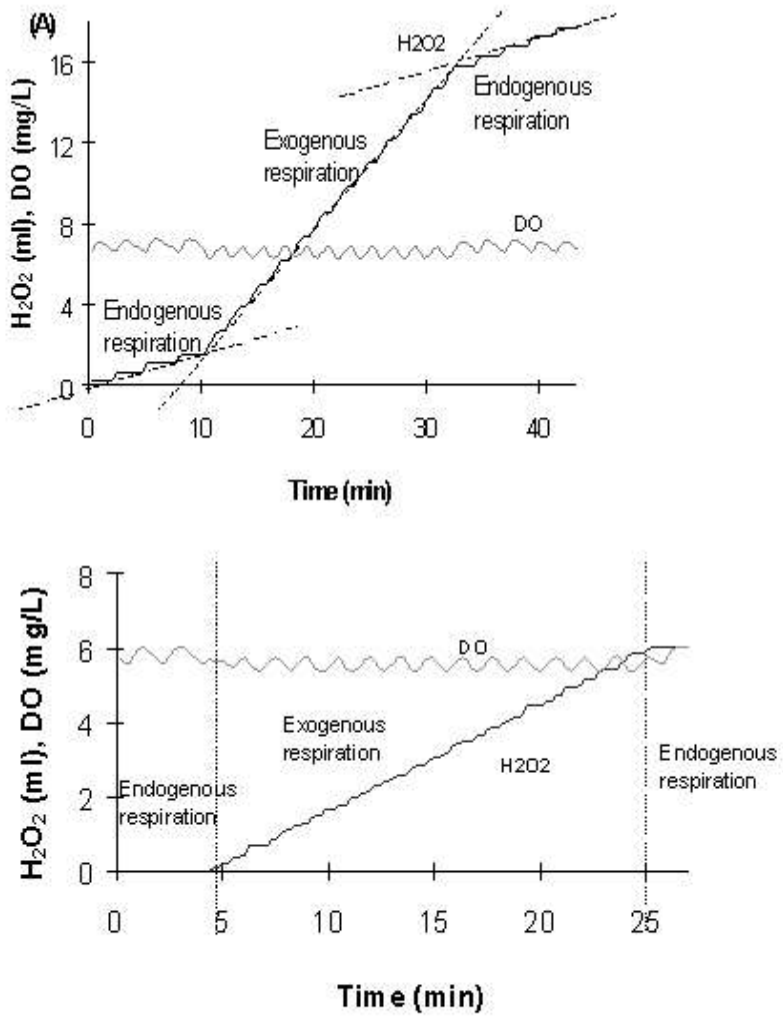


Figure 8 DO-stat titration curves. The slope of the titration curve is proportional to the oxygen uptake rate (Rozzi et al., 2003).

The potential of the DOstat technique has been verified on a reactor where an accurately controlled addition of a reducing chemical (sulfite) was titrated by hydrogen peroxide and compared with open and closed respirometric procedures. An excellent correspondence was found among oxidation rates estimated by open respirometry, closed respirometry and DOstat titration (Figure 9). This technique was also used to monitor the activity of both ammonia and nitrite oxidizers as described in Ficara et al. (2000) and to evaluate the metabolism of heterotrophic biomass (Rozzi et al. 2003).

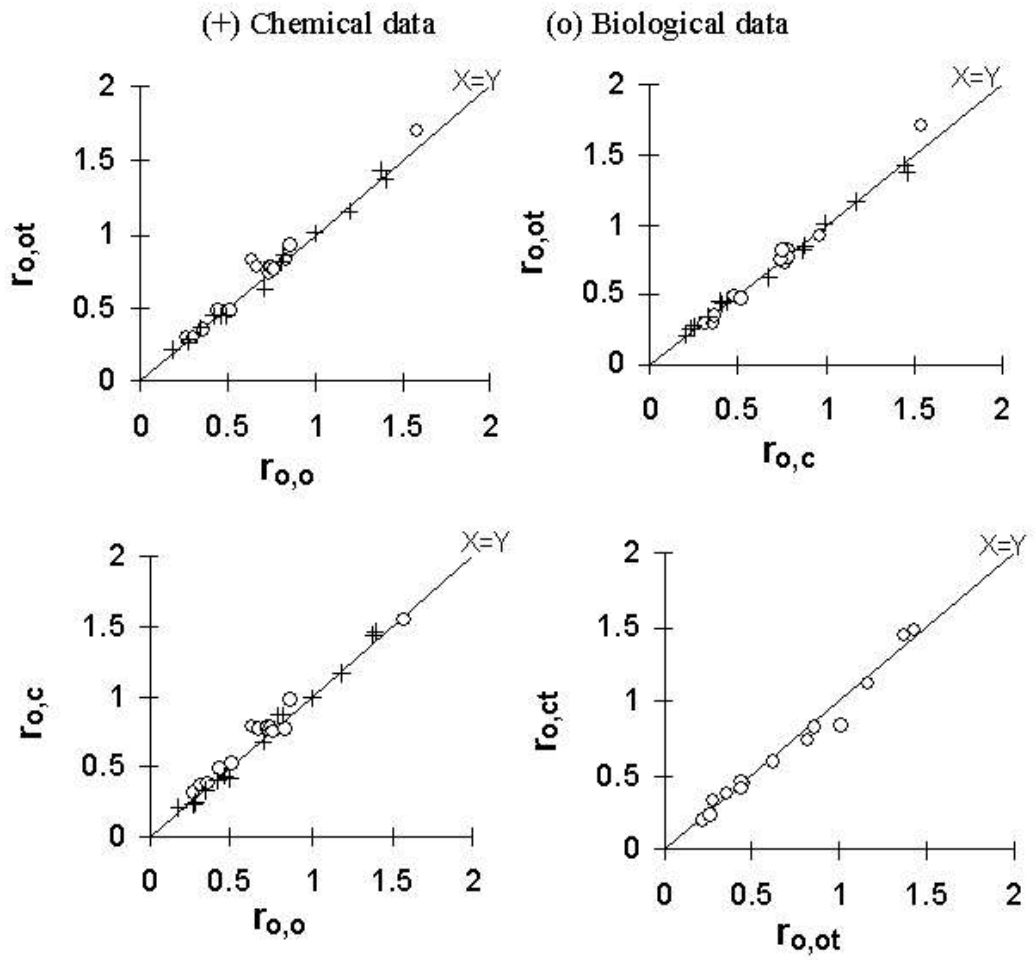


Figure 9 Comparison among oxygen uptake rate estimates (r_o) obtained by DO-stat titration ($r_{o,ot}$, $r_{o,ct}$), open respirometry ($r_{o,o}$) and closed respirometry ($r_{o,c}$) (Rozzi et al., 2003).

Combination of pH-stat and DO-stat techniques

DO-stat and pH-stat techniques were simultaneously applied to assess ammonium oxidisers kinetics and combined to enable the simultaneous assessment of both ammonium and nitrite oxidations kinetics leading to very reproducible estimates of both ammonium and nitrite oxidisers kinetic parameters (Ficara et al., 2000).

The pH-stat and DO-stat techniques can also be coupled to assess organic substrates oxidation by DO-stat titration of the DO consumed and pH-stat titration of the evolving CO_2. Biodegradation tests on simple substrates (Ficara and Rozzi, 2002) indicated that by coupling pH-stat and DO-stat titration, more information can be obtained about the biodegradation pathway of a substrate since the process is monitored by substrate (oxygen) disappearance and by product (CO_2) formation.

II.3 The UV-spectral analysis

II.3.1 Quantification of compounds in water using UV measurement

Two methods are used, the direct spectral analysis, with deconvolution, and the spectral analysis, with deconvolution, after one photochemical oxidation step.

– Direct spectral analysis (with deconvolution)

Let us consider that the experimental UV-spectrum to be analysed is a linear combination of elemental spectra (O. Thomas and co-workers Ecole des Mines d'Alès):

$$S = \sum_{i=1}^{p} a_i \cdot REF_i \pm r$$

where
S is the sample spectrum
a_i is the contribution coefficient of the ith reference spectrum REF_i
p is the number of reference spectra
r is the quadratic error
The development of the spectral UV analysis is based on the comparison between UV spectra of effluents and reference UV spectra of pure constituents (e.g. nitrate, detergent, and colloid) and/or measured global indicators (e.g. COD, TOC, BOD, TSS for WW). Typical UV spectra of some constituents or indicators are shown in Figure 10. Depending of the constituent or the global indicator, the development of a robust model may require the processing of more than 50 UV spectra. For wastewaters and because of their complex composition, it may be necessary to develop a specific model for each of them.

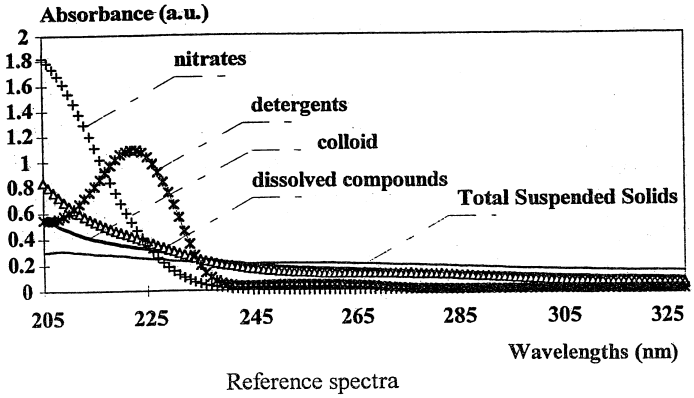


Figure 10 Typical UV spectra of some constituents and indicators.

- Spectral analysis (with deconvolution) after photochemical oxidation

As seen before, the spectral analysis can estimate the wastewater composition on the base of reference UV spectra. However some compounds do not exhibit UV signal, or the signal cannot be used efficiently. This is the case of nitrogenous organic compounds. One solution consists of oxidizing the molecules into other ones that exhibits UV signals. For example, total nitrogenous organic compounds can be quantified by oxidizing them into nitrates. Oxidation is performed using photochemical oxidation. In this project, photooxidation is performed using a Hg-lamp and the chemical oxidizing agent is sodium persulphate (Sigma Aldrich), at a concentration equal to 25 mg /L in presence of a sodium tetraborate buffer at pH 9.0 (Sigma Aldrich).
The principle of the spectral analysis (direct and with photochemical oxidation) is illustrated in 11.

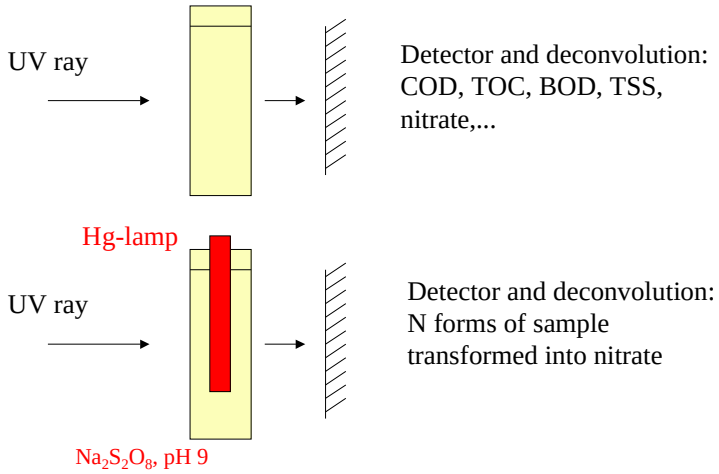


Figure 11 Schematic representation of the spectral analysis (direct and with photochemical oxidation).

II.3.2 The spectrophotometer used

The spectrophotometric sensor is an Anthelie UV-visible spectrophotometer (Secomam) controlled by UV-Pro software (see Figure 12). The pathlength of the Suprasil quartz cell is 2 mm and the scan speed is 1800 nm/min. Spectra were recorded from 190 to 800 nm. This sensor is equipped with a PenRay 22 cm Hg-oxidation lamp for the photoxidation process.

Figure 12 The Anthelie spectrophotometer (Seconam).

III – Evaluation on the applicability of lab scale titration sensors to monitor the performance of a SBR

III.1 Assessment of the rapidly biodegradable organic content of the SBR influent

DO-stat tests were performed to determine the ratio between Rapidly Biodegradable COD (rbCOD) and total COD of the SBR influent. These tests were performed by adding a known volume of influent (30 ml) to a sample of washed sludge from the SBR. Titrations were performed until endogenous respiration was reached. Nitrification was inhibited by adding allylthiourea (ATU) in order to measure only the oxygen demand related to the heterotrophic degradation of the organic matter. The amount of oxygen furnished by H_2O_2 addition was used to estimate the amount of rbCOD. The tests were performed on synthetic wastewater (modified from OECD Guidelines for testing of chemicals. 1993 - 209 'Activated sludge inhibition Test', OECD Paris, details can be found in "ANNEX WP1 – POLIMI – 1st year), and allowed to asses that the ratio rbCOD/totCOD for this influent can vary between 50 and 60%.

However, more investigation is required to ascertain the role of intracellular storage of organic bio-polymers (such as polyhydroxyalkanoates, PHA)

III.2 Determination of nitrification endpoint during the aeration phase of the SBR cycle

This set of tests were aimed to verify the feasibility to perform combined pH-DO-stat titration tests on samples of SBR sludge to determine the end of the nitrification process in the SBR reactor. Since, during the start up period, nitrification was never completed within the scheduled time of the aeration cycle, the duration of this phase was prolonged until the 'ammonia valley point' could be observed in the pH trend.

Experiments were performed as follows: a sludge sample was drawn from the SBR at the beginning of the aerobic phase and placed in the pH-DO-stat titration unit. Titration curves were then collected during several hours in order to observe the end of the nitrification process indicated by the bending points in the titration curves. In parallel, SBR was monitored by collecting pH and ORP data.

Results of one of such experiments is reported in Figure 13. On the left hand side, titration curves and titration rates for both pH-stat and DO-stat titrations are plotted, while on right hand side, pH and ORP data measured in the SBR are depicted.

In the titration curve, the end of the nitrification process is indicated by the sharp decrease in the titration rate and by the corresponding knee on the curve reporting the total volume of titrant added. According to titration data, nitrification rate started decreasing significantly after 400 min, indicating substrate-limiting conditions and was negligible after approximately 480 min from the beginning of the aeration phase.

By looking at the pH trend in the SBR (graph on the right), a minimum can be observed after 400 min ('ammonia valley'), then, pH increases and an inflection point is observed at time t=480 min. Moreover, a maximum is observed in the ORP profile at this time. Therefore, an excellent correspondence was found in this experiment between titration data and on-line pH and ORP determinations.

This result allows concluding that differences in pH and DO working values between the two reactors (constant versus variable values) did not cause significant differences in the rate of the nitrification process.

In conclusion, results of these experiments indicate that the fact that pH and DO keep constant in the pH-DO-stat titration unit (pH=8.3, DO=8.2), while they vary (pH = 7.6-8.0; DO=2-5 mg/l) within the optimal range for nitrifiers in the SBR, does not cause significant differences in the nitrification rate in the two reactors. Attention should be paid to maintain the two reactors at the same working temperature.

Although pH-DO-stat titration gives similar indications as pH and ORP monitoring in the SBR reactor, the first technique allows to assess the nitrification rate (proportional to the titration rate) and the influent load (proportional to the volume of titrant added), as well as the end of the nitrification process.

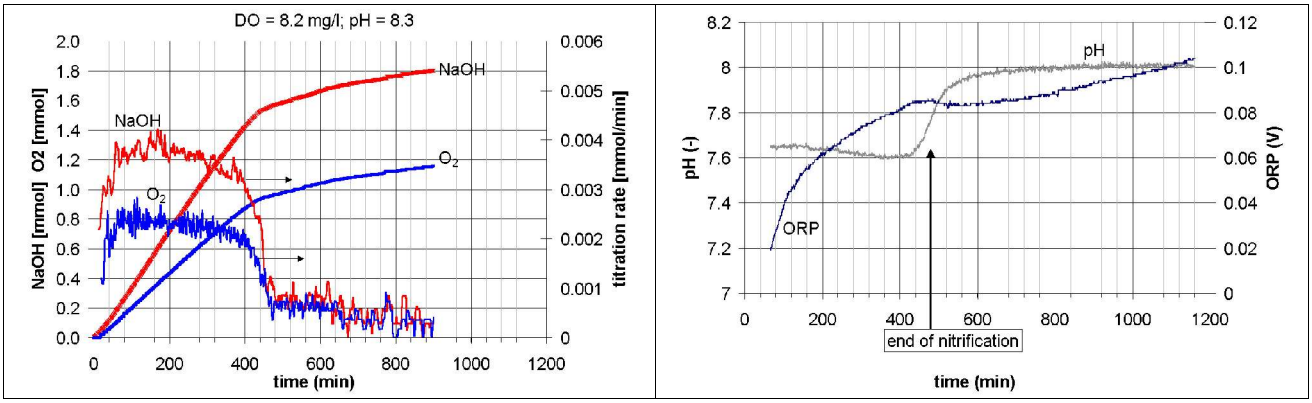


Figure 13 Comparing pH-DO-stat titration curves with ORP and pH trends in the SBR reactor.

III.3 Determination of the maximum nitrification activity of the SBR sludge

The MARTINA prototype was applied to monitor nitrification activity and ammonium concentration during the aerobic phase of the SBR at various loading conditions. For this purpose, a sludge sample was drawn from the SBR at the beginning of the aerobic phase and transferred into the biomass beaker of MARTINA. A titration test was then started within few minutes from sludge withdrawal. The cumulated volume of NaOH dosed was used to estimate the nitrification rate and the ammonium concentration, according to the procedure previously described (paragraph "pH-stat theory").

DO-stat titrations were not performed since reliable DO readings by MARTINA could not yet be obtained.

In order to validate the estimates of ammonium concentrations by pH-stat titration, these were compared with analytical determinations and with pH, DO and ORP trends determined in the SBR.

It has to be reminded that titration curves refer to the MARTINA biomass beaker, while the concentration values are measured in samples taken from the SBR tank.

Figure 14 reports results of a test performed under standard SBR loading conditions (i.e. COD = 600 mg/L; N = 75 mg Ntot/L as stated in the WP1 report). A good correlation can be observed between titration and analytical results, although few data are available for comparison.

By observing trends of DO, pH and ORP signals in the SBR reactor, break points described in the literature (DO break-point, pH ammonia valley, ORP bending point) can be detected. All three break points take place almost simultaneously and approximately 20 min before the end of the nitrification phase, as determined from both analytical determinations and titration profile.

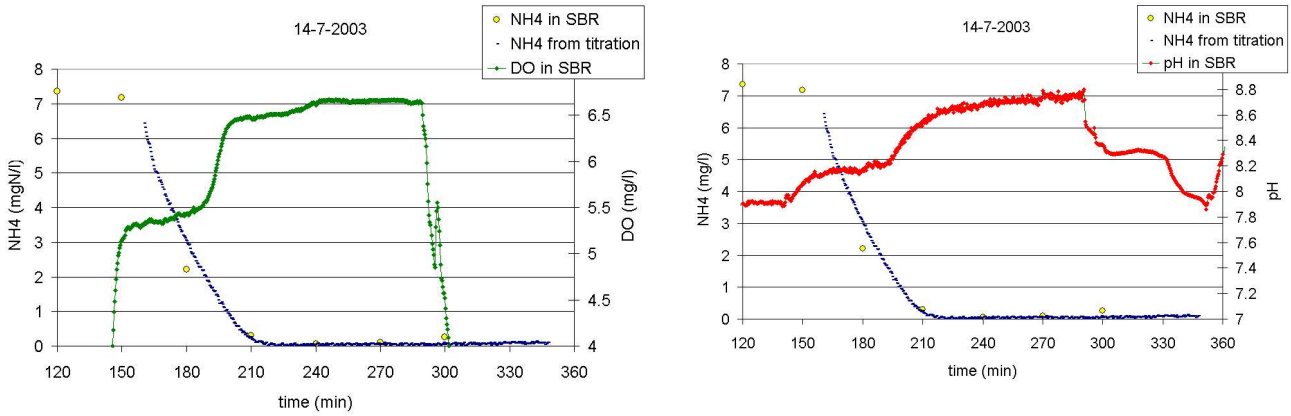


Figure 14 Results of the test performed under standard loading conditions

The ammonium concentration profile obtained by pH-stat titration can be fitted to obtain the maximum specific nitrification rate, considered as the nitrification rate observed under non limiting

substrate concentration (NH4-N > 1-2 mg/l). Results related to different loading conditions were collected.

In the two tests performed under standard loading conditions, very similar nitrification activities were estimated (difference< 2%). This value falls within the usual range for activated sludge fed on urban wastewater (e.g.: Barnes and Bliss, 1983). A relevant increase in the sludge activity was observed under increased ammonium loading. This fact can be explained by the increased available substrate for ammonium oxidisers.

It is noteworthy the almost perfect correspondence between the results given by the titration unit and those that have been measured in the SBR tank.

III.4 Denitrification activity tests with pH-stat titration model

A series of tests were performed to establish the denitrifying potential of the sludge from the lab-scale SBR by means of the pH-stat technique. Sodium acetate (CH_3COONa) was used as carbon source.

In order to balance the alkalinity produced by the denitrification reaction, nitric acid was used; by doing so, COD, not nitrate, was limiting the reaction.

Experiments were performed without any gas sparging to avoid liquid/gas mass transfer and simulate a closed system.

For each experiment, a sludge sample (0.7-0.8 L volume) was withdrawn from the SBR and kept under aeration for some hours to reach endogenous conditions. Moreover, at the beginning of the experiment, nitrate was dosed (as KNO_3) and 60 $mgNO_3^-/L$ was the initial concentration. Tests were performed at different pH constant values: the pH-stat ranged from 7.4 to 7.9 in the various tests, according to Ficara *et al*., 2002.

Figure 15 shows how the denitrification rate is calculated for the test performed. After a transient period, which is necessary to reach the pH-stat value, the system measures the endogenous activity as mgN/(gVSS*h). Then, carbon is added to the mixed liquor, and the slope of the volume of titrant dosed over time increases sharply. Once COD has been consumed, the system turns again to endogenous conditions. Denitrifying activity is therefore calculated as the difference between the slopes that have been observed under exogenous and endogenous conditions. In Figure 7, a test with four runs (four subsequent dosages of NaAc) is represented: tests appear to be repeatable and reliable.

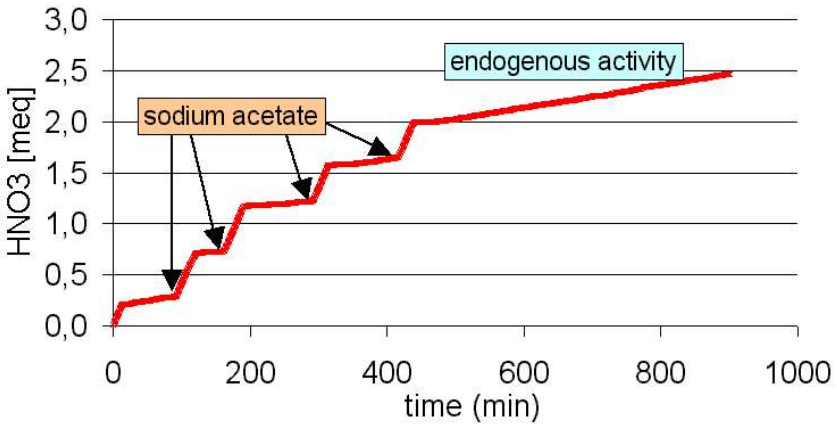


Figure 15 Denitrification test with sodium acetate as carbon source (4 runs)

Denitrification rates have been calculated during nine runs, they were varying between 12.68 and 20.16 mgN-NO_3/(gVSS*h). These rates compare well with those found in literature, as 20 mgN-NO_3/(gVSS*h) is the maximum value reported by Henze, 1991. The endogenous denitrification rate varied between 1.12 and 1.94 mgN-NO_3/(gVSS*h), comparable with values by Henze (0.2 - 0.5 mgN-NO_3/(gVSS*h); Henze, 1991) and Carucci (2.9 mgN-NO_3/(gVSS*h); Carucci *et al.*, 1996). Denitrifying activity of SBR sludge increased with time, as confirmed by analytical trials.

At present, a new series of tests is in progress to measure the ratio between titration rate and reaction rate when the carbon source is synthetic sewage. Therefore in this case it is not possible to calculate the actual COD oxidised, as it was done for NaAc, because is not known the elementary composition of the influent, as a consequence, and the stoichiometric ratio of the theoretical oxygen demand (ThOD) over NO_3-N is not known and has to be measured experimentally.
Once this is known, it will be possible to establish the denitrifying potential of the feed and to monitor the denitrifying activity of the SBR by running a pH-stat test.
The same obviously apply for real sewage, but the experimental determination of the COD/NO_3-N ratio has to be periodically checked.

IV - Evaluation on the applicability of le lab scale UV-spectrophotometric sensor

IV.1 On pure constituents

The UV sensor was first tested with pure molecules. As the SBR process involves the treatment of organic and nitrogenous compounds, both types of compounds were tested. Aminoacids, urea, ammonium chloride, and nucleic compounds were oxidised into nitrates as described before. The UV spectra were then obtained and deconvoluated.
Results show that most aminoacids are correctly oxidised and can be accurately quantified in the range of 1 to 9 mg /L of nitrate-equivalent (glycine, NH_4Cl, urea). For other molecules (like lysine), the UV spectral analysis is less efficient. Thymin, guanin, and cytosine were also analyzed for a unique concentration of 25 mg/l. These three nucleic acids were estimated at 93.6 %, 25.7 % and 71.7 % of the theoretical values, respectively.
These results show some discrepancies with the literature data with respect to the efficiency of the UV spectral analysis for constituent quantification in wastewater. They indicate conversely that, according to the author's experience, specific models are needed to achieve correct deconvolutions of nitrogenous compounds, starting that wastewaters can contain various different chemical species. For real reactors, building UV-models based on global parameters measured on the real wastewaters (COD, BOD, TSS, COT, etc.) would be better than considering single chemical species.

IV.2 On wastewaters

IV.2.1 LBE- Narbonne

The wastewater for the LBE-Narbonne (France) is a synthetic dairy wastewater. In Figure 16, the upper curve represents the UV-visible spectrum of the reactor influent, the lower the effluent. On one hand, significant differences between both spectra provide the adequate conditions to build further an accurate model. To build the final model, the lab-scale SBR will be fed with this wastewater during the second year of the project.

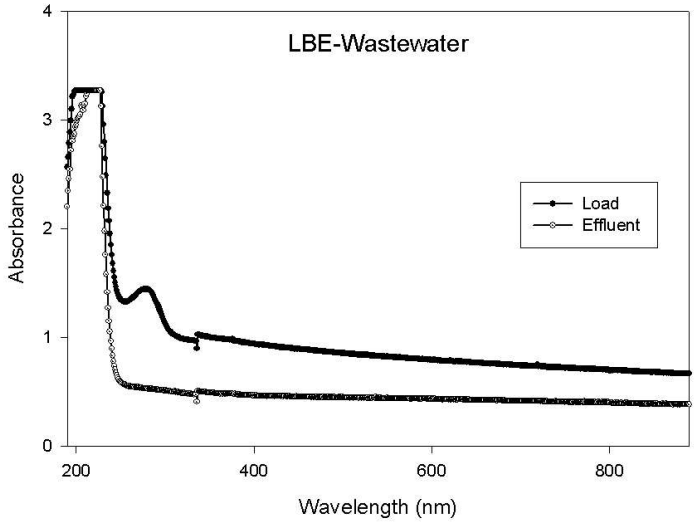


Figure 16 Load and effluent UV spectra for the LBE-wastewater.

On the other hand, the influent and the effluent spectra were obtained without separation of the suspended solids, which explains the almost constant absorbance in the visible range (350-900 nm). This linear absorbance can be used to estimate the suspended biomass (see subsection d).
In a second step, the influent was photooxidised with UV in presence of persulphate. Results are presented in 17. The oxidant exhibits an important UV spectrum that explains why its amount must be

carefully chosen. In too little amount, the compound is not oxidized enough and the concentrations of nitrogen compounds are consequently underestimated. In too much amount, a unique peak, corresponding to the oxidant one, is only visible, masking the peak of the oxidized nitrogenous compounds. It can also be seen that the borax buffer presents a small UV absorbance, and will not interfere with target compounds spectra.

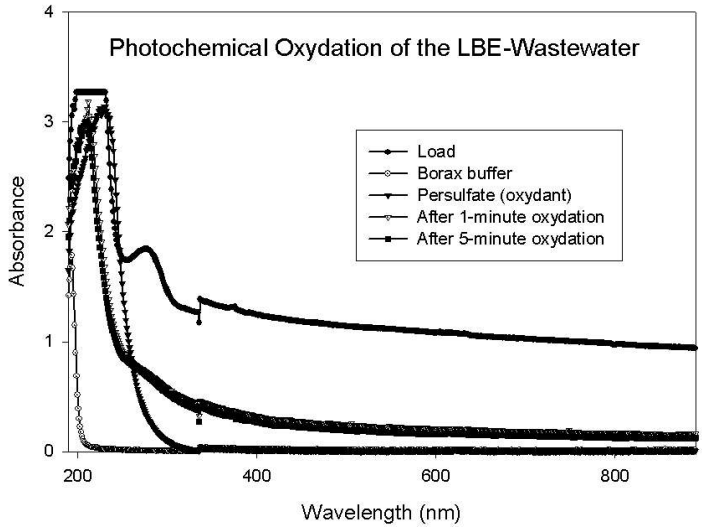
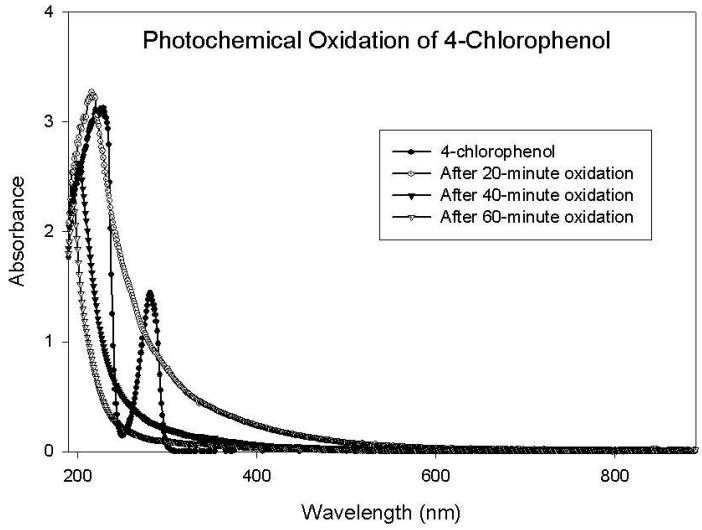


Figure 17 UV spectra after photochemical oxidation for the LBE-wastewater.

For the LBE-wastewater, the added oxidant amount seems to be adequate. After one minute, the influent is fully oxidized and the UV-spectra remain constant for longer time of oxidation. The UV spectra after one and five minutes are strongly different than those of the load and of the oxidant. The aspect of the peak suggests the presence of nitrate, coming from the nitrogen compounds of the load, and of other compounds. These results indicate that the UV-sensor can be successfully applied to the LBE-wastewater. However the oxidation experience shall be repeated to obtain the best oxidation conditions.

IV.2.2UNAM effluent

The UNAM effluent mainly contents 4-chlorophenol with an average concentration of 365 mg COD/L (266 mg/L). At this concentration, 4-chlorophenol exhibits a remarkable UV-spectrum with a double peak (see Figure 18). After 40 to 60 minutes the only signal came from the residual oxidant; this



configuration augurs well for further specific UV-model providing that oxidation conditions will be optimized to shorten the oxidation time.

Figure 18 UV spectra after photochemical oxidation for the 4-chlorophenol.

IV.2.3 OECD-effluent

The peptone and the meat extract present important UV absorbance, as shown in Figure 19. Peaks around 230 nm can be attributed to the aromatic amino-acids. These peaks could be confused with the nitrate and the oxidant peaks that are about the same wavelength. Careful models should be built to distinguish between the incoming material and the nitrate, one of the most important molecule during the SBR running. However, the small peak around 270 nm, that do not exist with the nitrate nor the oxidant, should help us. The SBR monitoring with this wastewater is described below.

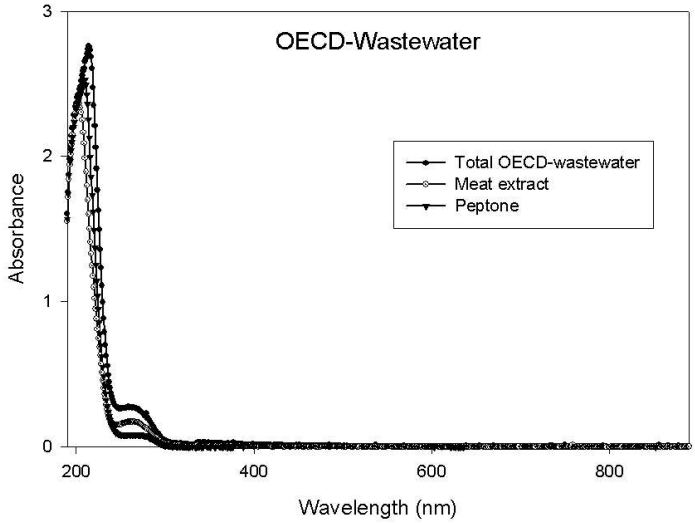


Figure 19 UV spectra of the OECD-wastewater and its principal constituents.

IV.2.4 Suspended solids

The UV sensor can also estimate the suspended solids of the wastewaters. Obviously, no information can be obtained on-line about the bacterial content of the suspended solids, but it is a practical way to have an on-line estimation of the bacterial content. The crude SBR effluent (OECD-effluent) was separated into two parts whose one was filtrated (0.22 μm). Both parts were diluted three times and the UV-visible spectra were recorded. Correlation between optic density measurements and concentrations of suspended solids is illustrated in Figure 20. The largest difference between the optic density of the crude and the filtrated parts is obtained for wavelengths ranged from 500 and 700 nm. At these wavelengths, a linear relation between the optic density and the mass of suspended solids can be obtained (that is for suspended solids from 0.38 to 3.1 g/L).

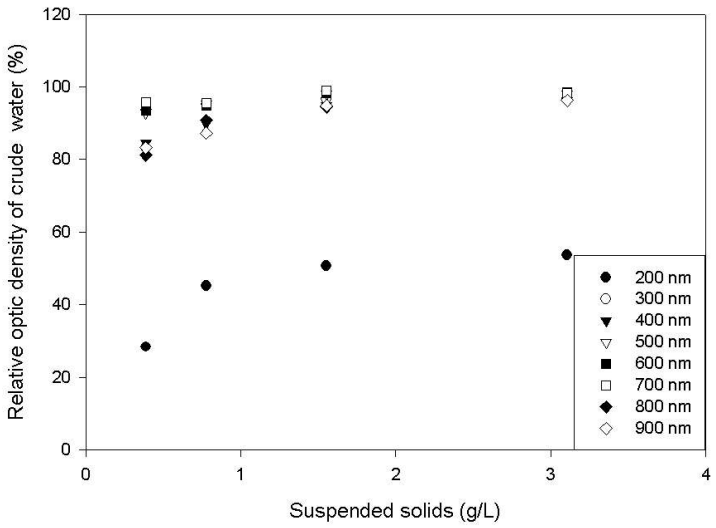


Figure 20 Correlations between optic density and suspended solid concentration.

IV – Conclusions

On one hand, by the experiments performed during the first year of the EOLI project, it can be stated that titration biosensors (pH-DO-stat technique) are suitable to monitor biological processes in SBRs. At this moment with these devices is possible to:

- assess the rapidly biodegradable organic content of the SBR influent;
- assess the end point of nitrification reaction of the SBR;
- measure the maximum nitrifying activity of the SBR.

More investigation is needed to monitor the denitrification process with synthetic and real sewage, and to obtain more reliable DO-stat tests with the new titrator prototype MARTINA.

On the other hand, the first results obtained from the UV spectral analysis are promising to monitor biomass, total organic carbon and nitrogenous compounds (nitrates, nitrites and ammonia) during SBR cycles. Indeed, for each wastewater (OECD, LBE-Narbonne and UNAM effluents), these compounds absorb in the UV at wavelengths sufficiently different to ensure discrination between the corresponding spectrum peaks. This will permit to build models for the monitoring of these compounds. Then, in the next year, models based on the polynomial deconvolution will be built using the commercial software developed by Secomam.

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