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EOLI
Efficient Operation of Urban Wastewater Treatment Plants

Work Package 1
First mid-term Report

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INTRODUCTION

The Work Package 1, WP1, is dedicated to the process experiments. It will be the guiding line of the project since there will be process experiments from the beginning of the project until its end. The discontinuous processes known as sequencing batch reactors, SBRs, will be utilized because of their flexibility over conventional continuous processes. SBRs present the following advantages : They are time-oriented, where flow, mixing, aeration and the reactor volume, are variables according to more or less predetermined periodical operational strategy. Thus the system presents a great flexibility allowing varying the operation duration to improve the removal of contaminants (Irvine et al, 1997). They allow the selection of micro-organisms capable to tolerate toxic compounds as well as variable environments (oxic, anoxic) that favour the nutrient removal, because of the environmental pressures applied to the microbial consortium. The total volume of the discontinuous reactor could be inferior compared to that of a continuous one.

The benefits of the SBRs have been shown for nutrient removal (Keller et al., 1997), the control of filamentous bacteria (Wanner, 1992) and the removal of specific organic compounds present in industrial wastewaters (Buitrón et al., 2001). In this project three types of urban industrial wastewaters will be considered: the first one coming from a dairy industry, contaminated with organic carbon components as well as nitrogen; the second one, typical for the chemical industry, containing toxic or recalcitrant compounds. For this case, a synthetic wastewater contaminated with a 4-chlorophenol will be used. The third one consists of actual domestic wastewater which will be occasionally spiked with pulses of toxic or organic compounds to simulate unexpected, often illegal, waste discharges which may overload the treatment plant.

Laboratory, pilot and field pilot scale reactors will be used. At the start of the project, process experiments will be performed to provide experimental data for parameter identification of the dynamical models of the SBRs. These will follow an experimental protocol defined in WP2 in order to optimize the information content of the data for parameter identification while accounting for the different process constraints. Then the experiments will be carried out to test the different results of the project, i.e. hardware sensors, software sensors, control laws, Fault Diagnosis and Isolation (FDI), supervision system. The processes will be instrumented with: O_2, NH_3, concentration, T, pH, OUR, and other off line measurements (e.g.: NO_2^- and NO_3^-, COD, microbiological counts and activities) in order to study more accurately the behaviour of the biomasses. However, the general idea is to design a system with the minimal number of sensors. Finally, validation of the integration of all the project results will be performed in order to test the global performance of the complete monitoring and control system in a real situation. This will be done for the three cases in a field scale pilot plant.

OBJECTIVES

Perform SBRs experiments at lab-scale for the development and the validation of the different algorithms to be developed within the framework of EOLI. Three particular cases will be studied, in three different reactors: toxic compounds, sludge level and purges automatization, carbon and nutrients removal.

Perform the laboratory experiments needed to validate and integrate software sensors and optimal control laws in pilot aerobic bioreactors for the treatment of toxic compounds, for the control of the sludge level and for carbon and nutrient removal.

Evaluate the long-term behaviour of the pilot plant and the improvement obtained in the process by the control and supervision strategy for the treatment of toxic compounds and the level of the sludge.

Perform field-scale pilot reactor experimentation and data collection, in order to test the controller in an industrial actual case.

INVOLVED PARTNERS

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Universidad Nacional Autónoma de México (6)
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This report contains the description of the experimental work effectuated between November 2002 and April 2003. Two tasks have been approached: the pilot plants implementation and the experiments for evaluation of the mass-balance model. Next is presented the advances sent to the project leader by each partner.

1. DESCRIPTION OF THE PILOT REACTORS AND METHODOLOGY

1.1 Carbon and Nutrients removal. Politecnico di Milano (partner 2)

The main objectives of Polimi for these first six months of experimentation were:
Design and construct a laboratory and pilot scale Sequencing Batch Reactor.
Instrument the SBR in order to measure, at least: temperature, DO, gas flow rate, redox, pH, NH_4^+
Inoculate and start up the SBR

With reference to the above objectives, Polimi could cope with the construction of an appropriately instrumented laboratory SBR which was stated from a sludge inocolum drawn from a full scale SBR. The SBR has been now operated for approximately 3 months with synthetic urban wastewater and the main parameters monitored. Steady state conditions couldn't be reached so far, likely because of the quality of the inoculated sludge, which was collected during a period of unstable operation of the full scale SBR.

However, during the SBR working period it was possible to check the proper operation of the on-line instrumentation. As far as the pilot scale reactor is concerned, we are at the moment considering the feasibility of experimenting on a pilot SBR which is going to be built and managed by ENEA in Bologna. The feasibility of having ENEA as a subcontractor needs to be negotiated.

1.1.1 Set up of a laboratory scale SBR

The SBR is a 30 L cylindrical tank with a working volume of 20 L (V_{MAX}). The SBR is equipped with: 1 peristaltic pump for feeding (PA); 1 peristaltic pump for effluent discharge (PS); 1 peristaltic pump for waste sludge discharge (PF); 1 aeration system (air compressor + stone diffuser) (SA); 1 variable speed mechanical mixing system (SM); 1 controlled heating system to maintain sludge temperature at 22 ^oC; 4 timers for activation/deactivation of PA, PS, SA e SM. This SBR operates according to 4 cycles per day. Each cycle lasts $t_C = 6h$.

Each cycle includes the following phases:

ANOXIC FILL (F) - (PA: ON; PS: OFF; SA: OFF; SM: ON), duration: $t_F = 13$ min;

ANOXIC REACT (ANR) - (PA: OFF; PS: OFF; SA: OFF; SM: ON), duration t_{ANR} = 150 min;
AEROBIC REACT (AER) - (PA: OFF; PS: OFF; SA: ON; SM: ON); duration t_{AER} = 150 min;
SETTLE (S) - (PA: OFF; PS: OFF; SA: OFF; SM: OFF), duration t_S = 37 min;
DRAW (D) - (PA: OFF; PS: ON; SA: OFF; SM: OFF); duration t_D = 23 min;

The SBR is fed with a synthetic influent (*OECD Guidelines for testing of chemicals*. (1993). 209 'Activated sludge inhibition Test', OECD Paris) with the following composition: peptone: 0.457 g/l; meat extract: 0.314 g/l; urea: 0.086 g/l; NaCl: 0.02 g/l $CaCl_2 \cdot 2H_2O$: 0.011 g/l $MgSO_2 \cdot 7H_2O$: 0.006 g/l K_2HPO_4: 0.08 g/l. According to analytical analyses, this influent is characterised by: COD = 770-800 mg/l, N = 130 mg N_{tot}/l, P = 11.4 mg P_{tot}/l.

At the beginning of each cycle, 4 L of the above described influent are loaded (ΔV). The resulting volumetric exchange ratio (VER), feeding rate (Q_{FEED}) and hydraulic retention time (HRT) are therefore: VER = ΔV / V_{MAX} = 4L/20 L = 0.2; Q_{FEED} = ΔV / t_C = 4 L / 6 h = 0.66 L/h; HRT = V_{MAX} / Q_{FEED} = 20 L / 0.66 L/h = 30 h. Every day 1 L of mixed liquor are discharged as waste sludge (Q_{WS} = 1 L/d), therefore the sludge retention time (SRT) is: SRT = V_{MAX} / Q_{WS} = 20 L / 1 L/d = 20 d
.
An on-line acquisition system has been set up by using MARTINA (Multiple Analyte Reprogrammable TItratioN Analyser) to monitor temperature, pH, ORP and DO signals.
The following probes are in use: ORP: Mettler Toledo (Greifensee, Switzerland), InLab 501; DO: Endress Hauser (Reinach, Switzerland), COS 3s, pH: Mettler Toledo (Greifensee, Switzerland), InLab 412; Temperature: IKD, (Varese, Italy), Pt100 TRM.

Acquisition frequency can be defined by the operator (max: 1 data per second). During this first part of the experimentation, the acquisition frequency has been set at the value of 1 data/min. The spare acquisition channels of the MARTINA instrument (1 ORP, 1 pH and 1 DO channels) are used to perform pH-DO-stat titration tests on sludge samples (0.5-1 L) manually collected from the SBR reactor.

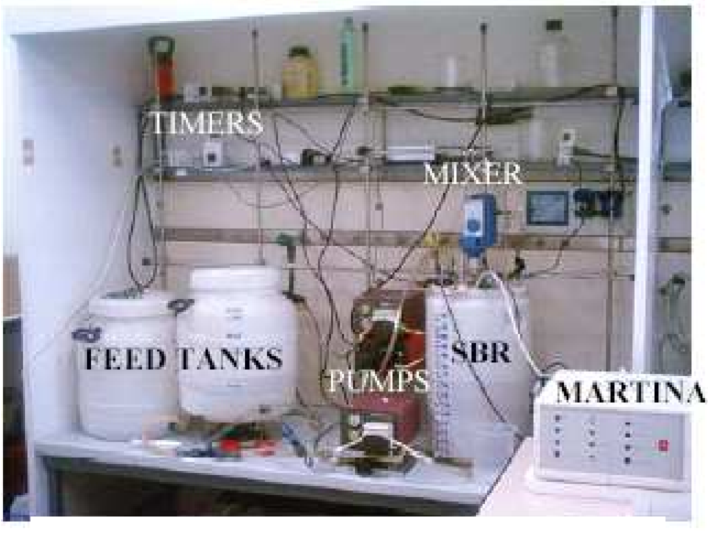


Figure 1.1.1 SBR and MARTINA unit

1.2 Sludge Level and purge automatization. LBE-INRA pilot (partner 4)

A 200 liter SBR filled with a semi-synthetic dairy wastewater is used. This kind of reactor is characterized by a series of process phases : fill, react, settle, and draw. All phases take place in the same tank as it is shown in figure 1.2.1. The influent is loaded in organic carbon and nitrogen. Carbon removal is realized by heterotrophic organisms under aerobic conditions. Nitrogen removal requires two biological processes: nitrification that requires aerobic conditions and denitrification under anoxic conditions.

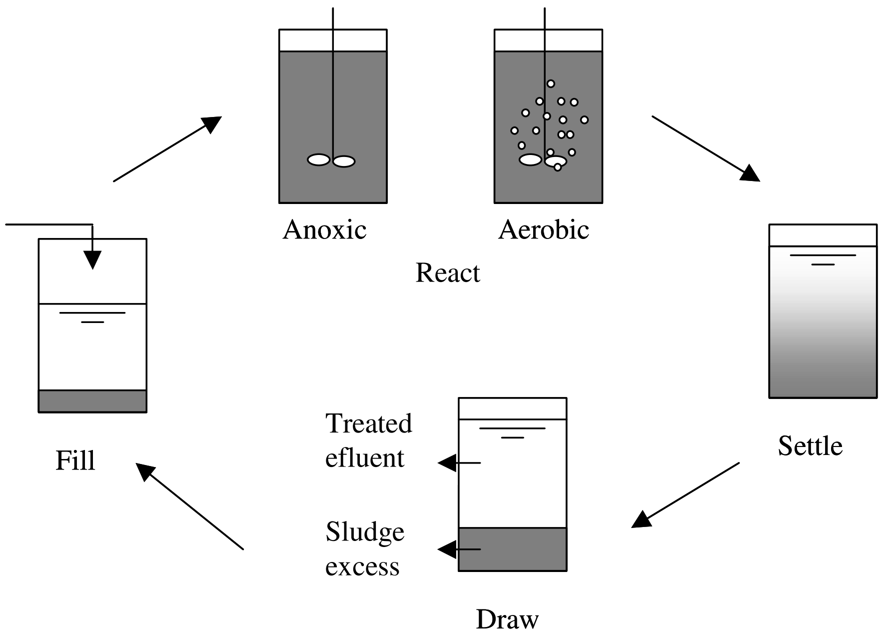


Figure 1.2.1 The operating principle of the SBR process

Nitrification is schematically described by :

$NH_4 \rightarrow NO_2 \rightarrow NO_3$

Nitrite (NO_2) is only an intermediate product: at the end of the aerobic cycle; nitrogen should completely be transformed into nitrate (NO_3). This reaction is realized by autotrophic organisms.

Denitrification is schematically described by :

$NO_3 \rightarrow NO_2 \rightarrow N_2$

The heterotrophic species present in activated sludge are able to denitrify. These organisms usually consume carbon. They use preferably DO as electron acceptor, but are able to switch to NOx if no DO is available. Thus, the denitrification process only takes place when denitrifiers meet organic carbon in an anoxic environment (presence of NOx, absence of DO). Under those conditions, denitrifiers consume organic matter using nitrate and nitrite as oxidizers.

1.2.1 Pilot plan and materials

1.2.1.1 Activated sludge
The sludge used in the LBE SBR comes from the urban wastewater treatment plant of COURSAN, France. This plant was chosen because both of carbon and nitrogen are treated in aerobic and anoxic conditions. The tank was filled with about 100 litters of this sludge. For two months, the sludge was simply aerated and filled with a low organic loading rate (<0.7 COD kg/m^3/day). At this day, the purification rate is greater than 98% and high sludge settleability is observed with almost no suspended solid in the effluent. The actual sludge concentration is about 9 g/l.

1.2.1.2 Influent wastewater
Semi-synthetic dairy wastewater is used as influent. A concentrated waste of 70g/l and 2 g/L of total kjedahl nitrogen (NTK) is collected in a cheese industry. It is diluted in order to obtain a Chemical Oxygen Demand of 5 g/l. In order to change the C/N ratio to get an easily biodegradable substrate, we add organic nitrogen as Urea (CH_4N_2O).

The initial total cycle duration has been fixed to 1 day. We use about 2 liters per day of the concentrated waste to prepare the influent. In standard conditions, the volume treated in one day is 30 l. Its average composition is : 5 g/l COD (2 liters of concentrated waste at 70 g/l = 140 g of COD. The COD of the influent is then 140 g in 30 liters ~ 5 g/l), 350 mg/l N-NTK, 10 g/l TSS and a pH equal to 6,23.

1.2.1.3 Reactor
The tank has the following characteristics : 50cm ∅ and 130cm height with total volume of 255L. Three independent controllable pumps are used to fill the reactor, draw the effluent and waste the sludge. Air is used for aeration and mixing during aerobic periods. Its flow rate is controlled by a volumetric flow meter. An immerged pump is used to guarantee mixing during anoxic phases.

In practice, the pilot plant was started up in February 2003. The reactor is operated at an ambient lab temperature of about 20°C. The cycle duration is 24 hours. At the beginning of a cycle, the reactor containing 170 liters of activated sludge is filled with 30 liters of effluent. It is then mixed and aerated for 22 hours followed by 2 hours of settling. Then, 30 liters are withdrawn and, if necessary, a portion of the sludge is removed. At that time, a specific denitrification phase was not necessary since, given the low nitrogen concentration, the denitrification took place immediately after the oxygen was stopped at t0+22 hours. Once the process will correctly perform nitrogen removal under the above mentioned conditions, the nitrogen concentration will be set higher and a specific denitrification phase will be added to the cycle.

1.2.1.4 On line measurements

The following parameters will be available on line from the beginning of May : pH, REDOX, temperature, dissolved oxygen in the liquid phase, input airflow rate, output gas analysis (O2 vs CO2) and sludge blanket. The probes used for measurements and the flow meters are connected to a PC. All measurements are captured by MSPC software and stored in a data base. The use of the "SPES integrated EOLI solution" should be discussed.

1.2.1.5 Off line measurements

According to the Narbonne meeting, the following off-line measurements will be available in the data the LBE will provide for model identification : VSS, TSS, TOC, COD (total and soluble), NTK, Nitrite, Nitrate and Ammonium. For all these measurements, standard analytic procedures are followed : samples are filtered by cellulose filter or centrifuged at 15000 round/min with 20 cm ∅ rotor for 15min. then the filtered sample is analyzed.

Chemical Oxygen Demand COD. The COD is the oxygen quantity which is necessary to oxidize one liter of effluent. To measure COD (total or soluble), we use the potassium dichromate method (AFNOR T90-100, 1969). For total COD we use rough effluent without centrifugation. A volume of 10 ml of the sample is oxidized at 150°C for two hours with sulfuric acid (H_2SO_4) and silver sulfate ($AgS0_4$). The sample is then titrated with Mohr salt 0,25N. COD is given by mg COD /l and depends on the Mohr salt volume used for titration.

Total Organic Carbon TOC. TOC is measured by Dohmann DC-80 analyzer. First, the sample is acidified and oxygenated during 5 minutes in order to eliminate the inorganic carbon. Then, organic carbon is mineralized in CO_2 form. It is oxidized in a solution of potassium persulfate under low temperature and UV light. Finally, double IR light analyzer detects the carbon dioxide (CO_2) formed.

Ions measurements. Nitrite, Nitrate and Ammonium are measured by chromatographic analyzer (Dionex DX-100). Anions and cations are analyzed in two different columns.
Ions are separated in distillation column. This column is flooded by suspension membrane where ions will be detected. Dionex DX 100 is equipped of an automatic injector and a software which provides the result of the analysis.

Total kjedahl nitrogen mesearments. With this method, the organic nitrogen and ammonium are measured. First, samples are heated with kjedahl katalyst (H2So4, Na2So4 and CuSo4). During this step, organic nitrogen is mineralized. Then, it is distilled by Buchi 320 in adding NAOH (300g/l). Ammonium vapor is captured in borique acid solution. This last one is titrated by HCL 0.02N.

Total and Volatile Suspended Solid (TSS, VSS). Either centrifugation or filtration methods can be used to measure TSS. It depends on the quantity of suspended solids present in the sample. The solid obtained after centrifugation (27 000 g) or after filtration is dried at 105°C for 24h. Then it is weighted. To measure VSS, the solids obtained after centrifugation are heated at 500°C for 2 hour.

1.3 Toxic Compounds. Universidad Nacional Autónoma de México (partner 6)

This report presents the activities of the partner 6 (UNAM) concerning the WP1 of the EOLI project realized during the period November 2002-April 2003. During this period the experiments were designed, analytical methods were implemented and the reactor was assembled. The first experiments of were conducted.

1.3.1 Experimental Strategy

During the Narbonne meeting it was discussed the number of experiments to be conducted. 20 experiments were fixed. In our case, some particular conditions will be studied in relationship with acclimatization/deacclimatization. Thus, additional experiments are planned, giving a total number of 40 experiments:

Experiment 1. Variation of the relation oxygen/substrate: 3 experiments
Experiment 2. Variation of the relation carbon/biomass: 4 experiments
Experiment 3. Standard conditions Kinetics: 1 experiment
Experiment 1 Aclimatization/Deaclimatization: 16 experiments

- Acclimatization of the microorganism to degrade 50 mg4CP/L :at least 5 experiments
- Deaclimatization with periods of starvation (8,12 and 24 h): 3 experiments
- Acclimatization of the microorganism to degrade 200 mg4CP/L: at least 5 experiments
- Deaclimatization with periods of starvation (8,12 and 24 h): 3 experiments

Experiment 2 Aclimatization/Deaclimatization B: 16 experiments

- Acclimatization of the microorganism to degrade 100 mg4CP/L
- Deaclimatization with periods of starvation (8,12 and 24 h)
- Acclimatization of the microorganism to degrade 200 mg4CP/L
- Deaclimatization with periods of starvation (8,12 and 24 h)

1.3.2 Calendarization

Table 13.1 presents the experimental planning for the project. At the moment of the elaboration of the report (April '03) activities 1, 2, 7 and 7.1 were conducted.

Table 1. Experimental planning

	Activity	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul
1	Reactor design and set-up								
2	Analytical implementation								
3	Standard conditions								
4	Variation of the relation oxygen/substrate								
5	Variation of the relation carbon/biomass								
6	Standard conditions Kinetics								
7	**A**. Acclimatization 50 mg4CP/L								
7.1	starvation (8,12 and 24 h)								
7.2	Acclimatization 200 mg4CP/L								
7.3	starvation (8,12 and 24 h)								
8	**B**. Acclimatization 100 mg4CP/L								
8.1	starvation (8,12 and 24 h)								
8.2	Acclimatization 200 mg4CP/L								
8.3	starvation (8,12 and 24 h)								

1.3.3 Bioreactor Characteristics

A pilot reactor of 7L of working volume with was used; the reactor has a conical form to favor the settling of the sludge (Figure 1). The system of control was implemented using a personal computer and a data acquisition card (National instruments 6025E data acquisition card and the Labview package). An automatic temperature controller based on a recirculation water pump was used to maintain a constant temperature of 20^{o}C inside the reactor. The input and output water flows were controlled by the computed system using two peristaltic pumps (Cole-Parmer model 7523, Masterflex series), respectively. The computer controls a mixer and a gas mass flow controller (Cole-Parmer E-33116-20) that turned on the aerator from the bottom of the reactor. The aeration flow was 1.5 L/min. The synthetic wastewater contained 4-chlorophenol (4-CP) as carbon source and complemented with nutrients with the next composition per liter of water: KH_2PO_4 25.5 mg, K_2HPO_4 32.5 mg, $NaHPO_4$-$7H_2O$ 75 mg, $(NH_4)_2SO_4$ 7.5 mg, $MgSO_4$-$7H_2O$ 42.5 mg, $CaSO_4$-$2H_2O$ 36.4 mg, $Fe_2(SO_4)_3$-$6H_2O$ 0.25 mg, $MnSO_4$-$2H_2O$ 0.003 mg, H_3BO_3 0.0057 mg, $ZnSO_4$-$7H_2O$ 0.0042 mg, $(NH_4)_6Mo_7O_{24}$ 0.0035, EDTA 0.0055 and $Fe_2(SO_4)$-$6H_2O$ 0.0044 mg.

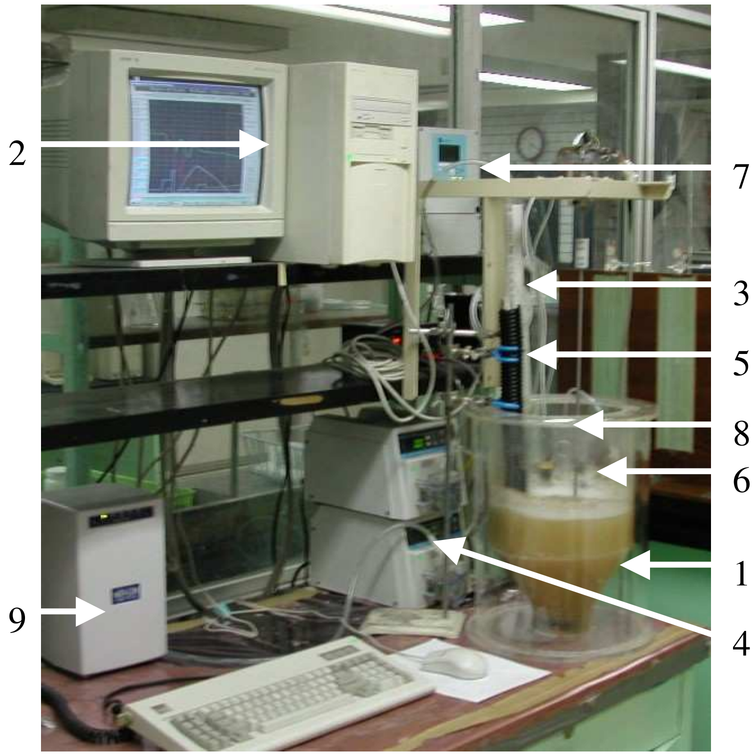


Figure 1.3.1 Reactor automatized for the toxic compounds degradation 1:Reactor with double wall and water recirculation to preserve constant temperature of 20°C, 2: PC, 3:Acquisition card, 4: peristaltic pumps (Master flex), 5:Temperature and Dissolved Oxygen Sensor, 6: pH Sensor, 7: Temperature and Dissolved Oxygen sensor, 8: Level Sensor, 9: No break

1.3.4 Phases of the SBR Control

1.3.4.1 Star-up and inoculation (acclimatization experiments)
The reactor was inoculated with activated sludge from a municipal wastewater treatment plant containing 2000 mgVSS/L. The acclimatization was conducted operating the reactor with the following strategy: pre-aeration time (15 min) fill time (5 min), reaction time (variable depending on the necessary time to reach a removal efficiency of 4-CP of 99%), settle time (12 min) and draw time (1 min).

1.3.4.2 Usual operation
When the reactor was acclimatized, the usual control strategy was used with this policy time: preaeration time (15 min) fill time (5 min), reaction time (240 min), settle time (12 min) and draw time (1 min).

1.3.5 Control System

The system was developed to introduce the preselection times of the strategy of operation for each of the realizing experiments.

1.3.5.1 Control window

In the manual control window of BIOREC (figure 1), the user have the total control of the reactor functions, i.e. can determine the fill flow (1) and effluent flow (2), the settle time and draw time, mixer by a stirrer (3) and a valve that turned on the aerator (4) . The window shows the reactor volume (5) and the dissolved oxygen concentration (6).

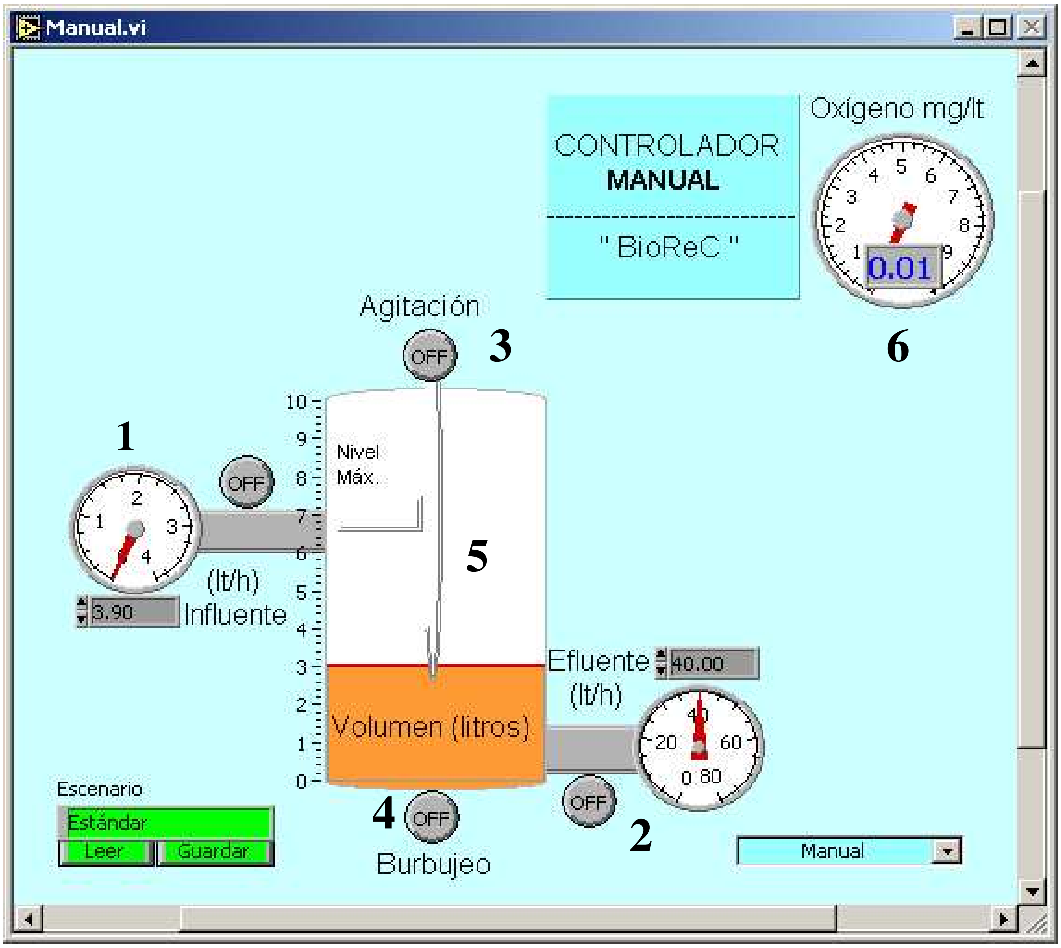


Figure 1. Control BIOREC window system

1.3.5.2 Process register window

The process register window show the dissolved oxygen concentration (1), the reactor volume (2), the phase and the time elapsed of the phase (3), the file name (4), pH (5), temperature (6), estimated substrate (7), movements in the control register (8) and another parameters that are used in another control strategies.

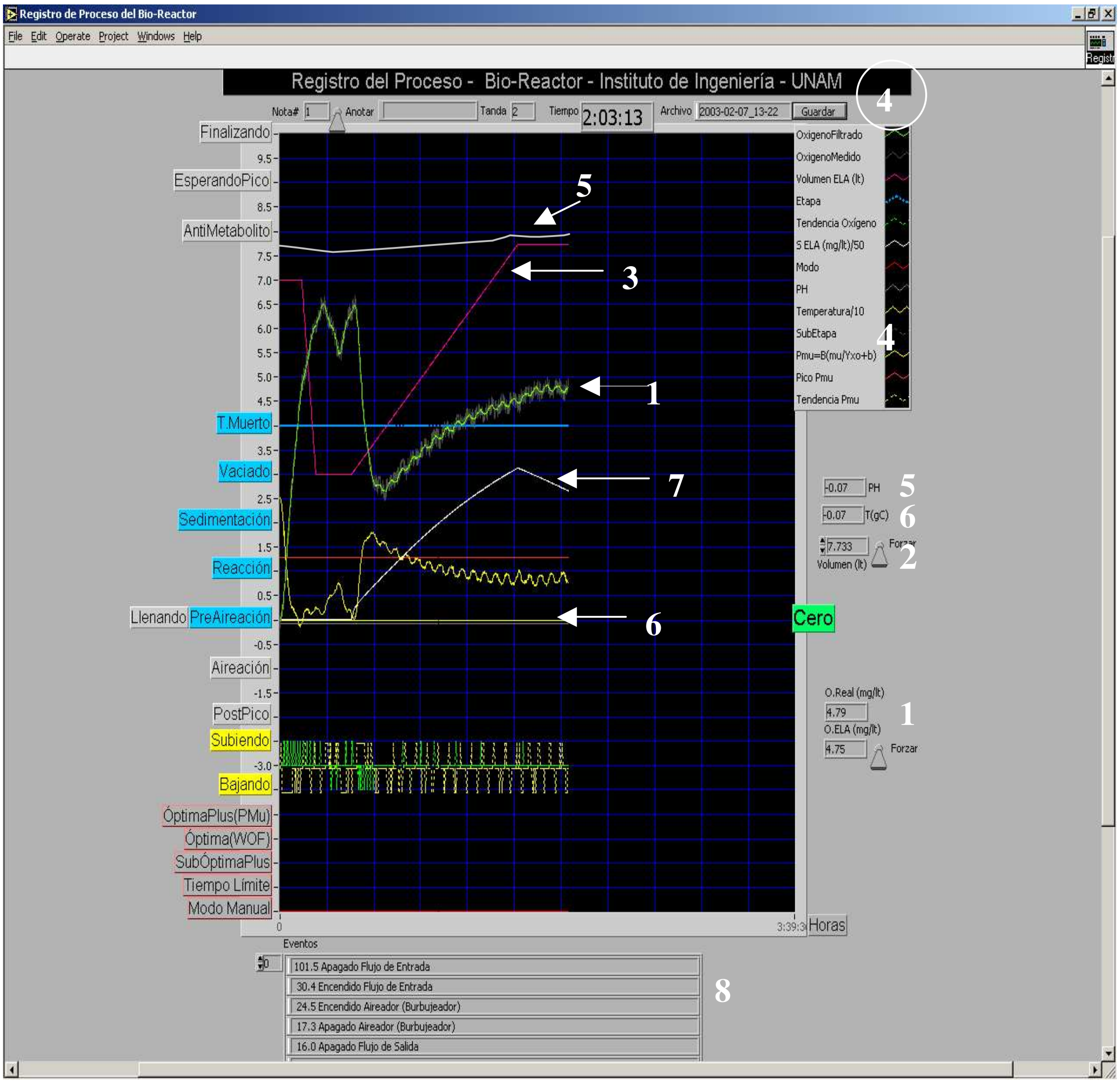


Figure 2. Process graphic register window

1.3.6 On Line Measurements

The on line measurements were obtained with the next devices:

- Dissolved Oxygen and Temperature sensor, Hendress+Hauser (Liquisys-M)
- pHmeter ORION 720A
- Alimentation flow: Peristaltic pump Cole-Parmer model 7523- 50 (0-100 rpm), Masterflex series.
- Draw flow: Peristaltic pump Cole-Parmer model 7523- 40 (0-600 rpm), Masterflex series (The reactor volume evaluated integrating input ant output flows in time).

1.3.7 Off Line Measurements

1.3.7.1 Total phenols (4-CF)
The phenols determination was made by the 4-aminoantipyrine (colorimetric method), the phenolic compound have a reaction with the 4-aminoantipyrine to pH 7.9 +/- 0.1 in presence of alkaline medium for form a aminoantipyrine complex with color, this is read at 500 nm.

1.3.7.2 Chemical Oxygen Demand (COD)
The method used is according to HACH (method 420 COD Low Range). The sample was filtered with a glass microfibre GFA (Whatman).

1.3.7.3 Dissolved Organic Carbon (DOC)
The DOC is used in order to detect the degradation of 4-CP (organic compound) during the degradation kinetics. The sample was filtered with a glass microfibre filter (Whatman), and the analysis was efectuated by means of a Carbon Organic Total Analyzer (Shimatzu 5050)

1.3.7.4 Metabolite
The Metabolite 5-chloro-2hydroxy-muconique acid semialdehyde (Coomandeur and Persons, 1990) is a compound formed by an alternate degradation route of 4-CP by the microorganisms, this can be inhibitory for the microorganisms. The compound is detected by spectrophotometric at 380 nm measured in a HACH spectrophotometer.

1.3.7.5 Volatile Solid Suspended (VSS) and Total Solid Suspended (TSS)
The VSS and TSS were done according to the S*tandard Methods* (APHA, 1992).

1.3.7.6 Activity of microorganisms
The activity of the microorganisms was obtained by a respirometric method, following the next protocol: A biological oxygen monitor (YSI Model 5300) with a 5357 micro oxygen probe was used. A solution of water saturated with oxygen was prepared during, at least, 2 hours before realizing the calibration. The calibration value was corrected by the effects of pressure (585 mmHg at Mexico City level) and temperature of the experiments (20 ºC). It was verified that the membrane of the oxymeter electrode was in good condition, in correct position and without any bubble of air in there.

A reactor of a volume of 60 mL was used and placed in a container with water maintained at a constant temperature of 20°C. The nutrients solution was saturated with oxygen for a period of time of around one hour. The reactor was filled with the aerated nutrients solution and a magnetic bar, taking care to not produce any bubble of air. The electrode was introduced very slowly until the stopper of rubber closes. Then, a sample of 10 mL of activated sludge (2000 mgVSS/L), previously harvested from the reactor at the end of the reaction period, was introduced to the mini-reactor with a syringe. The oxygen consumption as function of time (approximately 20 min) was registered. The reactor was mixed continuously. This experiment produces the endogenous respiration. The same procedure was conducted to obtain the activity against acetate (200 mg COD/L) and 4-CP (50 mg/L). For these cases, acetate and 4-CP solutions and nutrients were filled to the reactor.The VSS concentration was determine in the mini-reactor according to *Standard Methods* (APHA,1992). Maximal slope of the curve obtained from the plot of oxygen concentration vs time was divided by the VSS giving the specific activity.

1.4 Dairy Processing Industry wastewater. Universidad de la República Oriental del Uruguay (7)

The main activities were related to: personnel selection, personnel training, process and setup design (including reactors design, instrumentation, etc), setup acquisitions and construction, techniques and literature review, and sludge acclimatation. They are described in the next sections.

1.4.1 Personnel

- María Viñas (IIQ), Rafael Canetti (IIE), Soledad Gutiérrez (CEI), Lucía Muxi (MB) and Claudia Etchebehere (MB) were assigned to this Project.
- The local responsible of WP1 was María Viñas (†). Now the responsible is Soledad Gutiérrez.
- After a public call, 2 Chemical Engineers : Adrián Ferrari and Alejandra Benítez were selected to cover 2 laboratory assistant positions. The personnel selection was performed on 19 candidates.
- Angela Cabezas (Assistant) since 1/1/2003. Angela will perform nitrifying and denitrifying specific activities, enumeration of nitrifiers by FISH and Most Probable Number. She will isolate and characterize nitrifiers and denitrifiers.
- An assistant from Analysis Department of the CEI will perform HPLC analysis and will offer support with the discussion of analytical techniques.
- Ing. Melga Galisteo, from the CEI will contribute with respirometric techniques and parameter determination experimental set-up.

1.4.2 Funds

The funds for the project were received on Dec. 31th, 2002.

1.4.3 Experimental set- up

1.4.3.1 Reactors Design

Physical design of the (2) reactors and on-line measurements devices were carried out. Reactors will be identical. Several aspects were considered for design purposes:

1.- Reactor volume was selected taking into account: the minimum required sample volume for programmed analysis routine, and the laboratory stock capacity, considering that wastewater must be supplied by the factory, once a week..

2.- Possibility of removing sensors easily was carefully considered, as soon as implementing a cleaning routine for them.
3.-The possibility of measuring gas flow rate and composition was considered, by means of sealed reactors design.
4.- A control level device will be employed to adjust minimum and maximum volumes.
5.- Selection of aeration system: flow rates, mass transfer efficiency. (still under evaluation)
6.- Mixing selection: an evaluation of several possibilities of mixing systems was carried out. Mechanical agitation was agreed.

Figure 1.4.1 shows a reactor scheme. Reactors are actually under construction. The diagram shows:

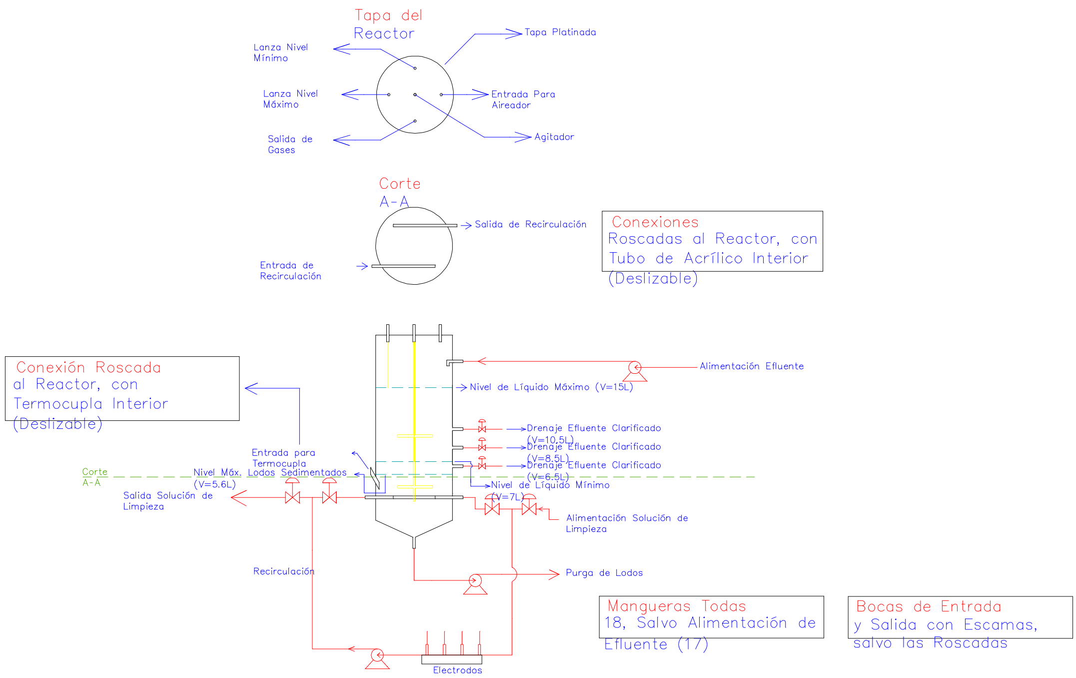


Figure 1.4.1 reactor scheme

Input and output ports, including those for recycle flow, input and output of sensor cleaning solution, sludge purge output, more than one port to effluent drainage, sampling port, gas output, etc.

1.4.3.2 On-line measurement.

- After evaluation of commercial sensors able to measure: pH, nitrate, nitrite, ammonia and dissolved oxygen, we decided to employ all these probes excepting the nitrite electrode. It was discarded taking into consideration: low concentration of nitrite, cost evaluation and described interferences. The instruments have been ordered.

1.4.3.3 Off-line measurement.

- A summary containing analytical techniques and a tune up of some of them for Volatile and Fixed suspended solids, COD, BOD, Organic Nitrogen, Ammonia Nitrogen, nitrate and nitrite concentration by HPLC was performed.

1.4.3.4 Operation parameters

A new evaluation of operational parameters is being carried out, considering:

-the reactors should operate in a stable way, that is, if it is possible, to avoid aperiodic dead times.
-COD concentration in the reactor should remain above 50 mg/l (detection limit of COD method) during a broad period in order to obtain reliable results for modeling
-Mean Biomass concentration can reach more than 5 gSSV/l, instead of 2.5 g/l originally stated.

Two alternatives of operation are being considered. For raw wastewater
Influent Flow rate: Q=4 l/d
Substrate Concentration in the influent S_o = 3000 mgDQO/l
Mean Biomass concentration: X = 4200- 4500 mgSSV/l
Cicles/day: 1 ó 2
Duration of a cycle: 24 ó 12hs.
θ_c^E= 20 days

For pretreated effluent a flow rate of 7l/d should be employed, with 2 cycles per day and other parameters similar to raw experiment.

1.4.3.5 Sludge Acclimatation
Sludge was cultivated employing diluted milk as substrate, in a batch-mode operation, of θ_c = 20 days , with the following objectives

a.- Ensure avaibility of biologic sludge to carry out setteability assays and experiences to select mixing system
b.- To generate a sludge adecuately acclimated to the wastewater, to encourage growing of nirtificating bacteria, in order to minimize transitory periods during the SBR experiences
c.- Allowing to get practice with laboratory techniques such as VCO, COD, BOD, SSF, SSV, nitrate y nitrite.

The batch is actually operated under the following mode:

Constant liquid Volume : 30 ±5 l
Dissolved oxygen concentration: mínimum 4 mgO_2/l
Substrate addition (whole milk): 250 ml/d, resulting in S_o 1730 mg COD /l
Daily purge of completely mixed reactor content: 1,5 l/d
Starting date of acclimatation: 01/abr/03 with 5l volume and y 20/04/03 con 30 l.

1.4.3.6 Dairy factory contacts

We came to an agreement with authorities of CONAPROLE (Factory 7), that will supply 70 l/week of pre-treated (anaerobic lagoon) effluent.

1.4.3.7 Effluent characterization

a.-We decided to simulate a raw effluent of dairy processing industry, by diluting whole milk, in accordance with the mean values concentrations measured in several dairy plants in Uruguay. pH of the raw effluent is nearly 7 due to that cleaning routine includes nitric acid cleaning and sodium hidroxide cleaning, and these allow a pH compensation. Pre-treated effluent correspond to the measured values of the output of an anaerobic lagoon of a dairy processing plant. (Planta nº7, Conaprole)

Wastewater characteristics

Wastewater	COD (mg/l)	pH	NH_4^+ (mgN-NH_4^+/l)
Raw	3000	7	100
Pre-treated	1500-2000	6.5-7.5	150

1.4.3.8 Bibliography

We reviewed publications about the following topics:

- SBR funamentals and operation
- Respirometry techniques
- Kinetic modelling of aerated systems
- Design of aeration systems, mixing theory, mixing design, mass transfer evaluation, etc.

1.4.3.9 Microbiological characterization activities

Literature revision on FISH technique. This led to the list of available probes for nitrifiers, known denitrifiers and anaerobic ammonia oxidisers, and to the definition of the probes to be purchased to complete the existing set (Nit3 comp, Nmv-cy3, Nsv443-Cy3; Pla46-6FAM; Amx820-Cy3, Thauera1458-Cy3, PMOA-a189gc; Pla46rc; Amx820). The order for these probes has already been placed.

1.4.3.10 Techniques

a) Ammonia measurement (Nessler).

This technique was adjusted and is now available for determination of ammonia depletion during nitrification.

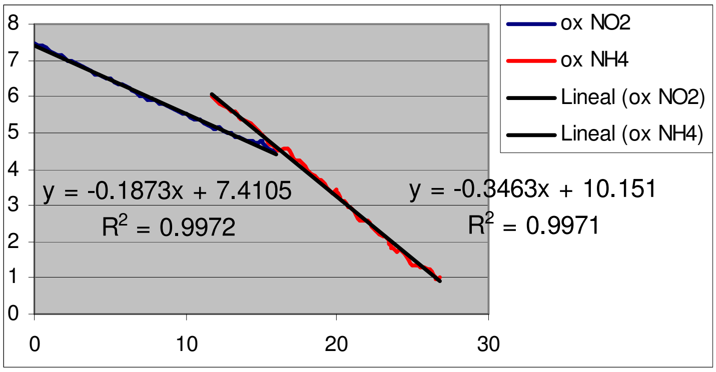
The method was shown to be adequate for ammonium determination in the presence of the expected maximum amounts of nitrate and nitrite according to the added ammonium, but peptone interferes with the determination.

b) Nitrifying activity.

In order to have a constant source of nitrifying biomass to adjust the techniques, a lab scale (2 l) nitrifying reactor was started. The inoculum was biomass from another lab-scale nitryfing reactor. The reactor is fed daily (10% of the volume) with a mineral medium as described by Campos et al. (1999).

The nitrifying activity of the biomass in the reactor was determined using the recommended technique, with the following conditions:
pH = 7.0 at room temperature (25ªC)

The following are representative plots of concentration of dissolved oxygen (mg O_2 /l) vs. time (minutes) for nitrite and ammonium as substrates.



More assays are going to be performed in order to verify linearity of oxygen consumption rates with respect to volatile suspended solids concentrations.

The equipment and software to complete the fluorescent microscope for FISH determination, and a HPLC column for nitrate determination have been ordered.

2. RESULTS AND DISCUSSION

2.1 Carbon and Nutrients Removal. Politecnico di Milano (partner 2)

2.1.1 SBR Start up

The SBR operation was started the 15th January 2003 by inoculating activated sludge drawn from a full scale SBR (Funo, Bologna, Italy) treating urban wastewaters and serving about 7000 PE.

Initially no waste sludge was discharged. The following analytical determinations were periodically performed on the effluent in order to monitor the SBR performance: suspended total and volatile solids (TSS, VSS) - Figure 2.1.1, effluent COD - Figure 2.1.2, effluent ammonium and nitrates - Figure 2.1.3.

During the first 3 weeks the feed was that one described above. During this period, COD removal was always satisfying while the SBR nitrification capacity was insufficient to oxidise the ammonium fed and the latter kept on accumulating in solution. Since high ammonium concentrations are known to have an inhibitory effect on ammonium oxidisers, starting from week 4 the SBR load was decreased by influent dilution and, during weeks 4 and 5, the SBR supernatant was replaced by tap water until an acceptable ammonium concentration was reached. Moreover, at day 29 sludge from a well nitrifying WWTP was inoculated (10% w/w of the SS present in the SBR) to enhance nitrification activity.

During the following weeks, the SBR feed was kept diluted 1:2 with respect to the initial one.
Moreover, from the 46th day, sludge waste has been started. Since then, solids concentration (both TSS and VSS) started decreasing, and reached, within the following 2 weeks, values lower than 0.5 g/l.
No steady state conditions have still been reached.

During this start up period, some pH, DO and ORP profiles were collected to verify the efficiency of the MARTINA system. As an example Figure 2.1.4a and 2.1.4b reports data recorded during 5 cycles on days 62 and 63.

It can be observed that DO and ORP data compare well during the *aeration phase* (AER), both increasing with time. In the same phase pH decreases probably because of the acidity produced by nitrification. No ammonia valley could be detected due to the fact that nitrification was not complete as suggested by the significant ammonium concentration measured in the SBR effluent (10-20 mg N/l). During the *anoxic phase* (ANR), ORP decreases sharply within the first minutes, during which oxygen is rapidly used up, and then keeps on decreasing during the whole anoxic phase, while nitrates are consumed; pH initially increases, due to the alkalising effect of denitrification and then decreases probably because of carbon dioxide production.

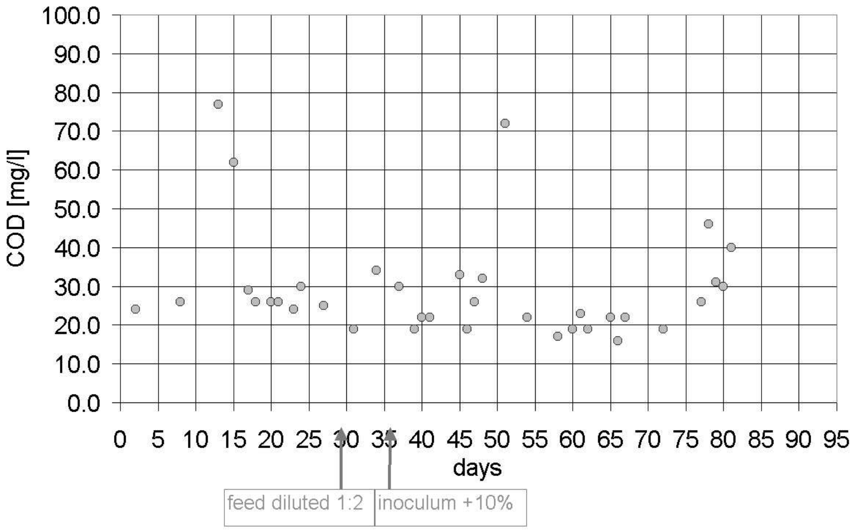


Figure 2.1.1: SBR effluent COD

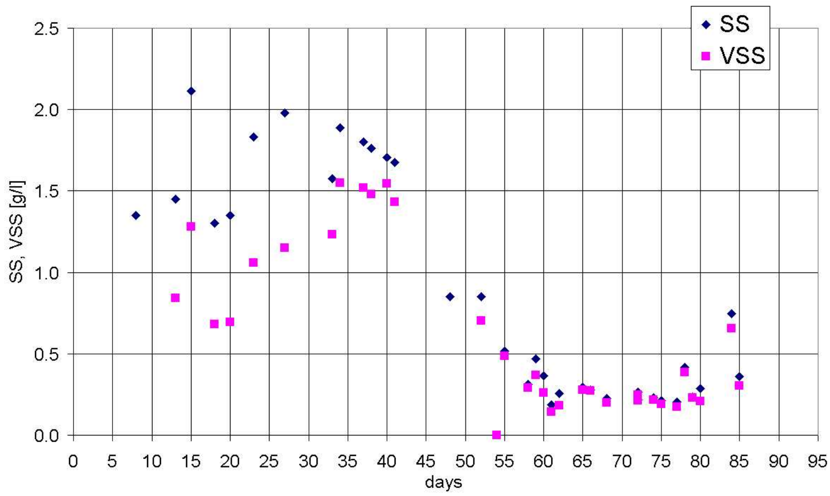


Figure 2.1.2: SBR TSS and VSS

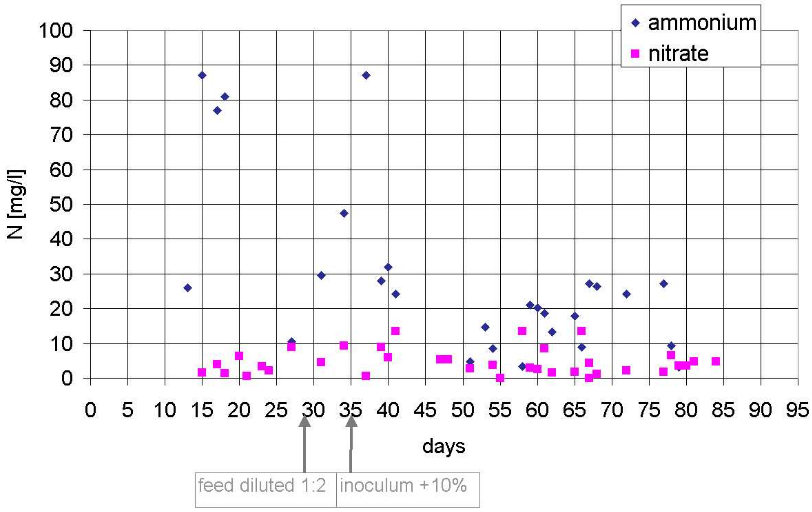


Figure 2.1.3: SBR effluent ammonium and nitrate

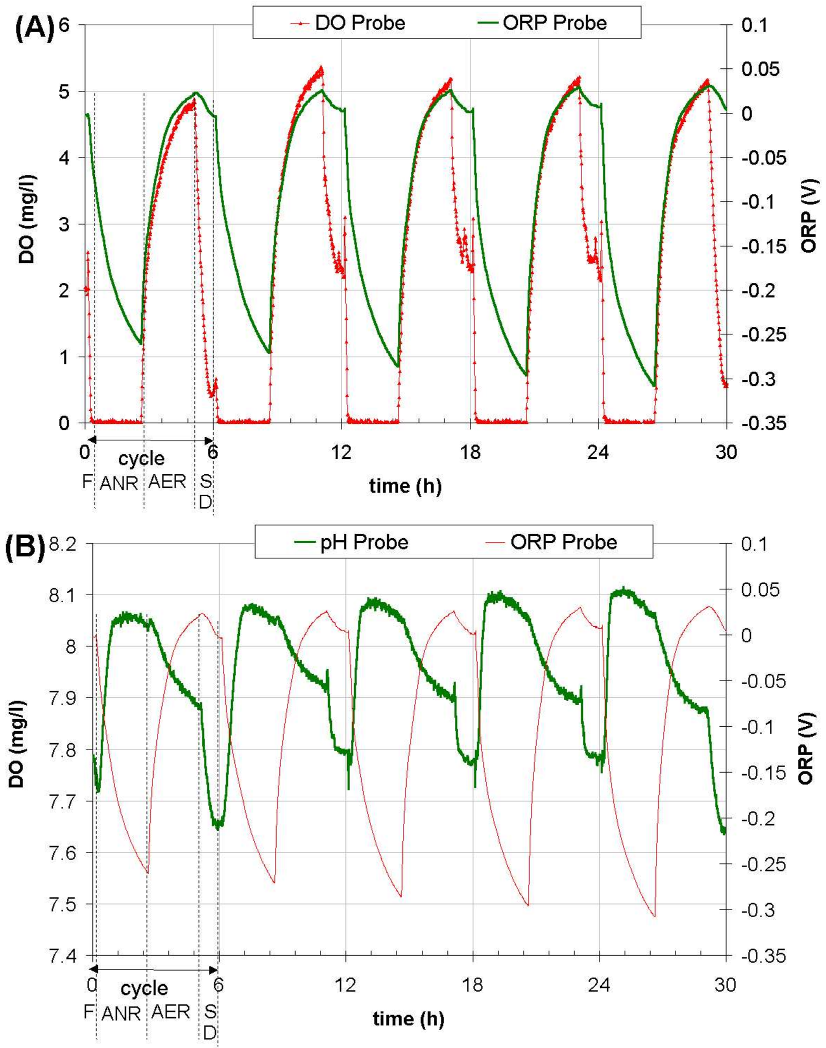


Figure 2.1.4: On-line data for 5 SBR cycles (days 62-63): DO, ORP and pH values.

2.2 *Sludge Level and purge automatization. LBE-INRA pilot (partner 4)*

2.2.1 Start-up of the reactor

The sludge was acclimated in the tank for two month following the procedure described hereafter :

- the volumetric load is about 0,7 kg COD/m^3/day (5 * 30 = 150 g in 200 liters = 750 g in 1 m^3);
- the cycle duration is 24 hours : 22 hours in aerated conditions and 2 hours of settling;
- The airflow rate during aerobic periods is constant at about 0,15 VVM.

The following data were obtained over the last 3 first months of operation.

2.2.2 Carbon removal

As stated above, the average input COD is 5000 mg/l. The filling volume is 30 liters and the total reactor volume is 200 l. After dilution, the average initial COD is 750mg/l.

Figure 2.2.1 presents the evolution of total and soluble output COD (at the end of a cycle) compared with input COD concentration. Figure 2.2.2 presents the purification rate. Under standard conditions, notice that it took one month to obtain steady state from an input-output point of view. At the beginning, the output COD was 280 mg/l and the purification rate was 94,5 %. During a period of 30 days, output COD concentration decreased to finally reach a stable point at about 35 mg/l with a purification rate of 99,2 %.

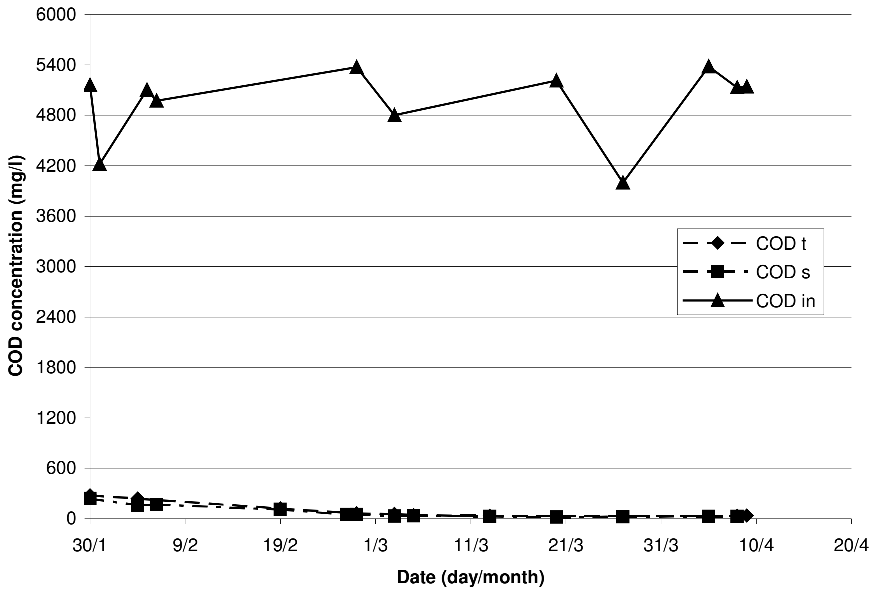


***Figure 2.2.1**: Evolution of the input COD, total and soluble output COD*

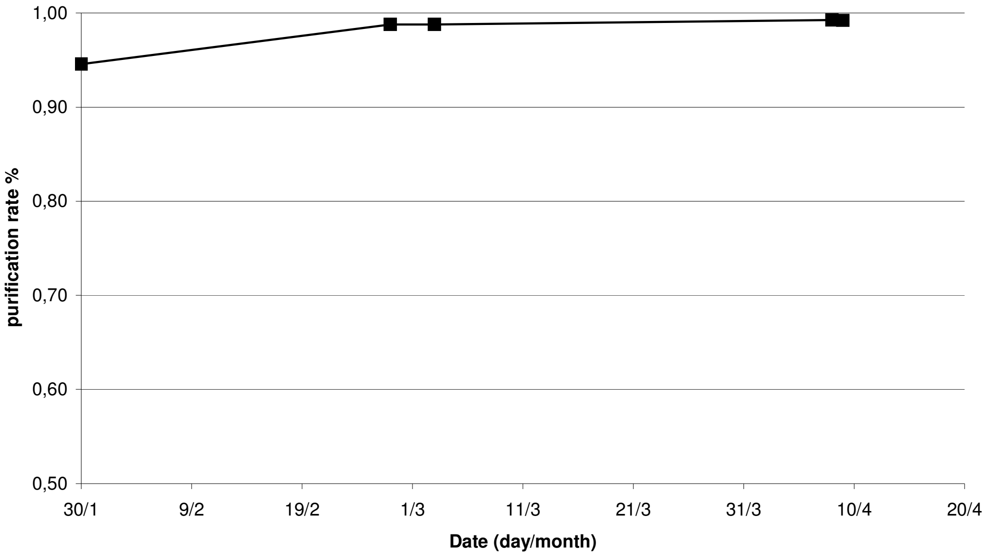


Figure 2.2.2: *Evolution of the purification rate of total COD*

2.2.3 Nitrogen removal

The average input NTK is about 350 mg/l. After filling, the average initial concentration in the reactor is 52 mg/l. At the date of 10/3 the reactor was accidentally filled with high NTK concentration : this explains the peak observed in the Figure 2.2.3. The objective of the starting period with respect to nitrogen removal is attained since the nitrification is now correctly performed. Today, the output NTK concentration is about 3 mg/l.

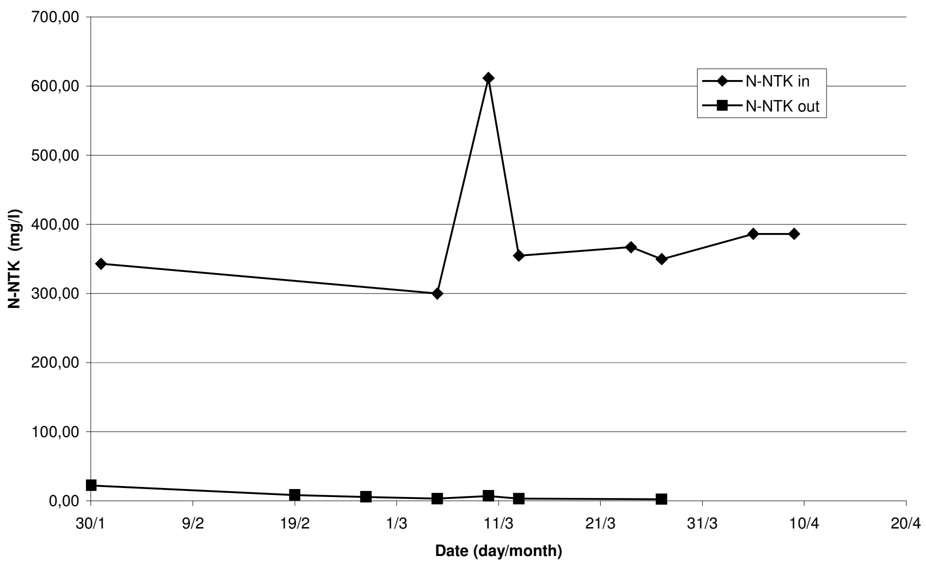


Figure 2.2.3: *Evolution of the input and output NTK*

2.2.4 Sludge growth

At the beginning, the concentration of total suspended solid was 4,43 g/l. the sludge was not adapted and the concentration decreased until the 12th day. After this date, TSS started to increase. We did not withdraw any sludge to maintain a high residence time which is necessary for nitrifiers. The sludge growth is quite constant rate as it can be seen in Figure 2.2.4. The growth production is 0,21 g of biomass /g of COD.

The evolution of the sludge level, at the end of a cycle (two hours after the aeration has been stopped) is shown in Figure 2.2.5. It is noticed that during the period where the TSS concentration increased, the sludge level increased also. Then, at the 60th day, the sludge level stopped to increase and both the settling capacity and the sludge concentration began to increase.

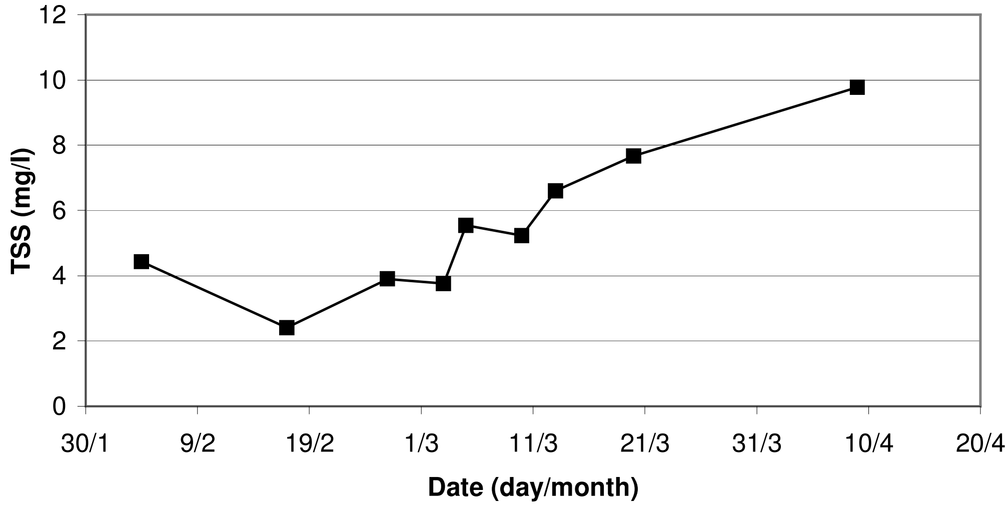


Figure 2.2.4*: Suspended solid in the SBR reactor*

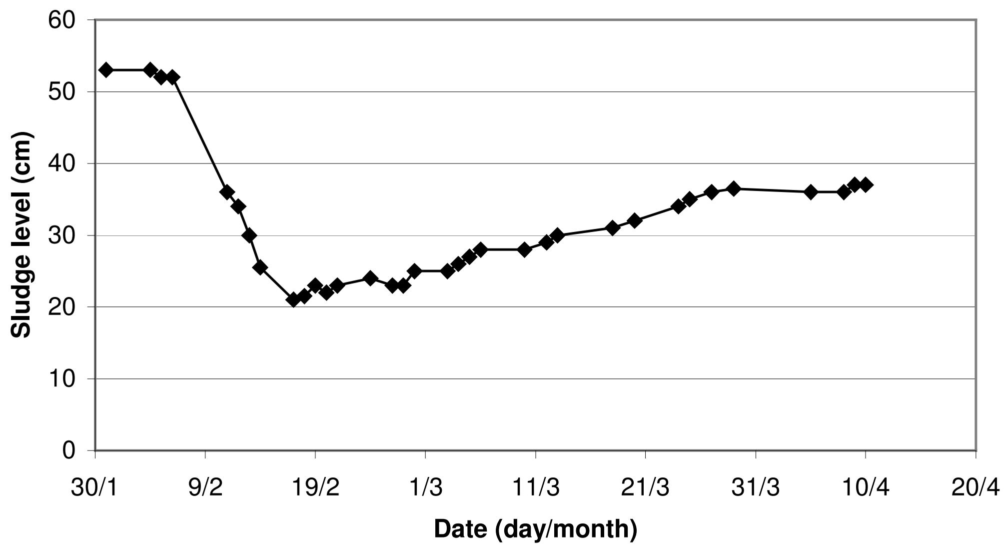


Figure 2.2.5: *Sludge level evolution after 2h of decant*

Figure 2.2.6 shows the sludge index evolution. This parameter present the volume occupied by 1 g of sludge after tow settling hours. It allows quantifying the settliability: as this index is low as settliability is better. This signifies that we can have a high VSS concentration witch occupies a low volume after settlement.

At the beginning the sludge index was 120 ml/g and it start decreasing. At the date: 10/4, the sludge index was at 38 ml/g. It represents a good settliability performance.

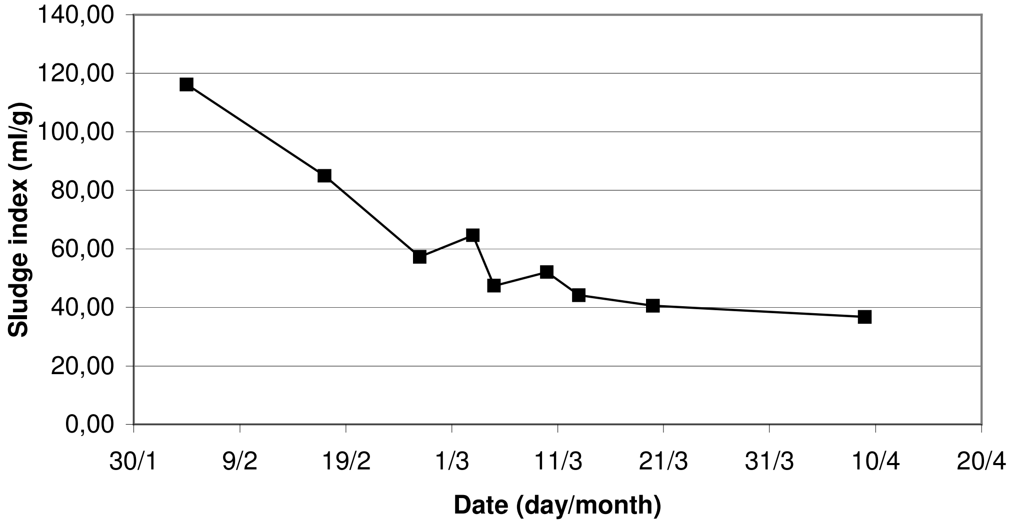


Figure 2.2.6 sludge index evolution

2.3 Toxic Compounds. Universidad Nacional Autónoma de México (partner 6)

2.3.1 Operation Data

Up to now, more than 82 cycles have been conducted. The results presented here correspond to the study of acclimatization/deacclimatization (cycles 1 to 10) to 50 mg 4-CP/L. The reactor was operated to maintain the biomass during the holydays period (Cycles 11-58).

2.3.1.1 Cycles time

The cycles' duration was determined depending on the necessary time to achieve a complete degradation of 4-CP (>99 of 4-CP removal). Figure 2.3.1 presents the evolution of the acclimatization time. From cycles 1 to 4 the degradation time decreased 40 to 4 hours. After this point the reactor was conducted with cycles of 4 h and 50 mg 4-CP/L:

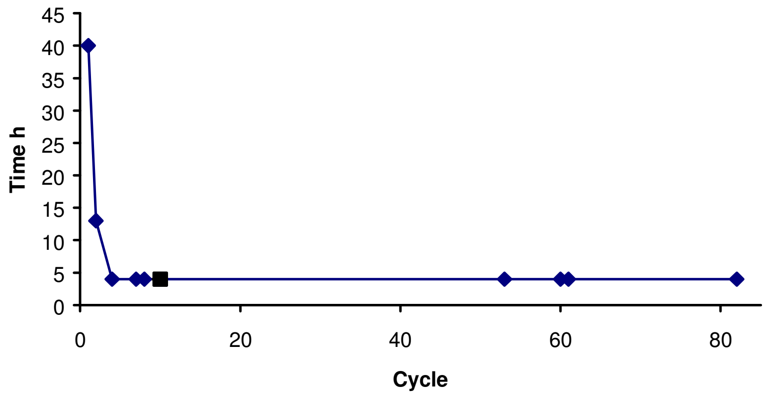


Figure 2.3.1 Evolution of the cycle time

2.3.1.2 SSV, SST and INT evolution

The SST showed a diminution from 2560 to 2330 mgVSS/L, furthermore the SSV diminished from 2000 to 1810 mgVSS/L in the acclimatization phase, nevertheless when the microorganisms acclimatized to the degradation of 4-CP, the SSV increased to1900 mgVSS/L (fig 2.3.2).

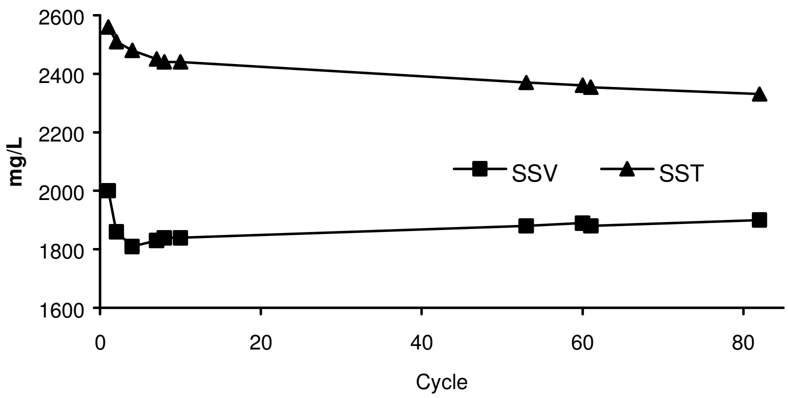


Figure 2.3.2. Evolution of the SSV and SST across the cycles

2.3.1.3 Sludge Volumetric Index (SVI)

The SVI was diminishing during the acclimatization (cycle 1 to 10) from 68 to 38 mL/g, indicating a beneficial effect by the SBR operation (figure 2.1.3).

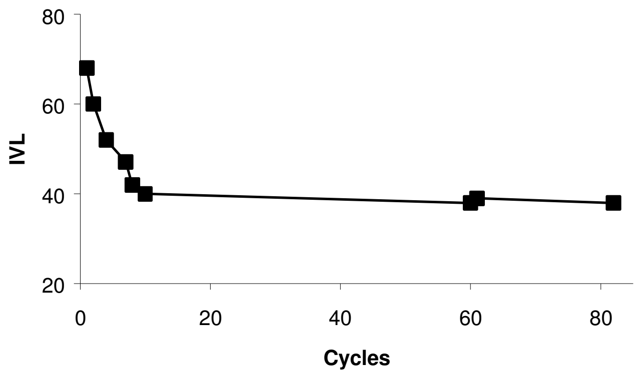


Figure 2.3.3. Evolution of the IVL across the cycles

2.3.1.4 Degradation efficiencies

The efficiencies of degradation of 4-CP were around 100 % (4-CP was below the detection limit of 0.1 mg/L). The efficiencies of COD removal were always superior to 95 % for the evaluated cycles (fig 7), and DOC removal efficiencies of 91 % in average.

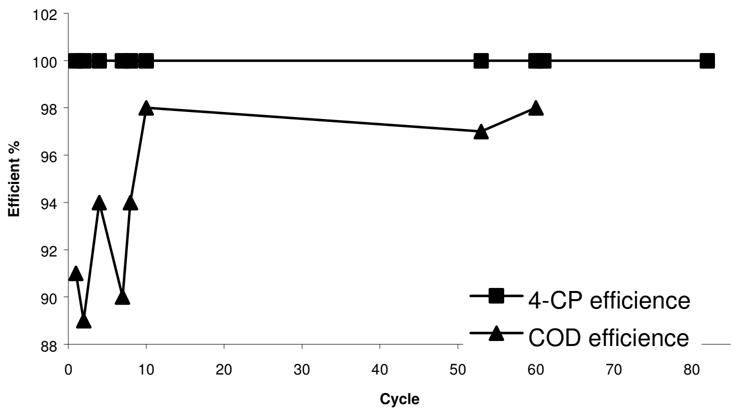


Figure 2.3.4. Evolution of the efficient degradation of 4-CP and COD

2.3.2 Acclimatization

The acclimatization was monitored following the kinetics of the first 10 cycles. The next parameters were followed of line: 4-CF, COD, DOC, activity, solids and production of metabolite (figures 2.3.5 to 2.3.9).

2.3.2.1 Total phenols (4-CP)

The degradation time of 4-CP decreased from of 40 hours in cycle 1 to 1 hour in the cycle 10, with efficiencies of 100 % (fig 2.3.5).

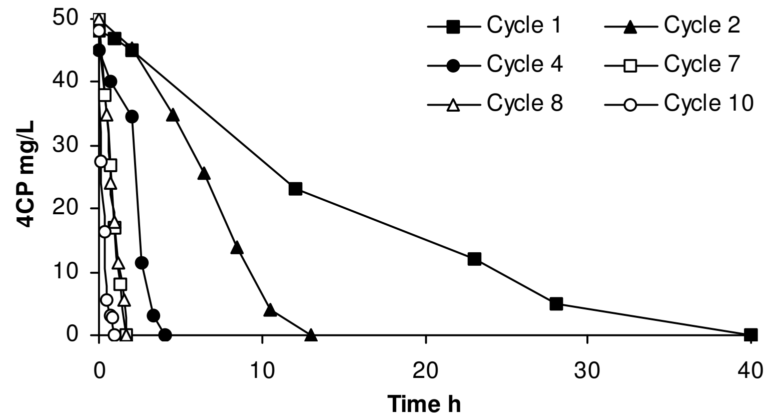


Figure2.3.5. Degradation of 4-CP in the acclimation process

2.3.2.2 Chemical Oxygen Demand

The initial COD was between 105 and 115 mgCODs/L, with removal efficiencies major of 95 % (fig 2.3.6).

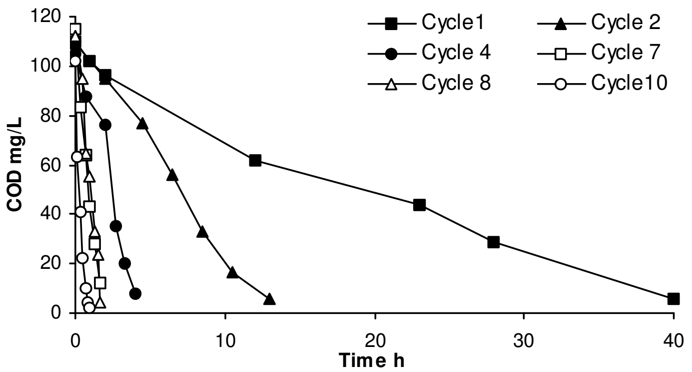


Figure 2.3.6. Degradation of COD in the acclimatization process

2.3.2.3 Dissolved Organic Carbon

Figure 2.3.7 presents the evolution of the kinetics of acclimatization for DOC removal.

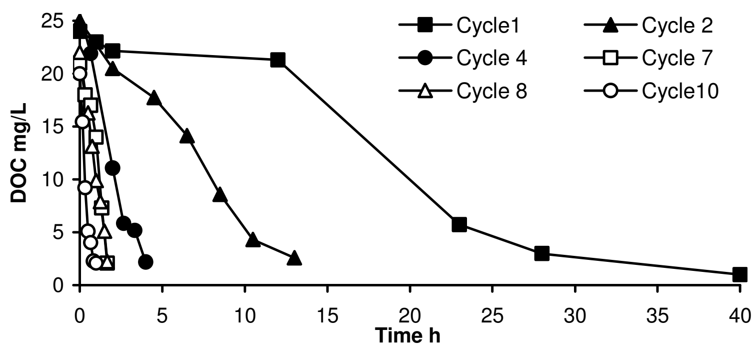


Figure 2.3.7. Degradation of DOC in the acclimatization process

2.3.2.4 Activity of microorganisms

The respirometric activity variation is showed in figure 11. It is observed an increase in the activity to 4CP from 6.5 to 28 $mgO_2/gVSS$ due to the microorganisms acclimatization in the degradation of this substrate. Activity to acetate decreases as the acclimation took place.

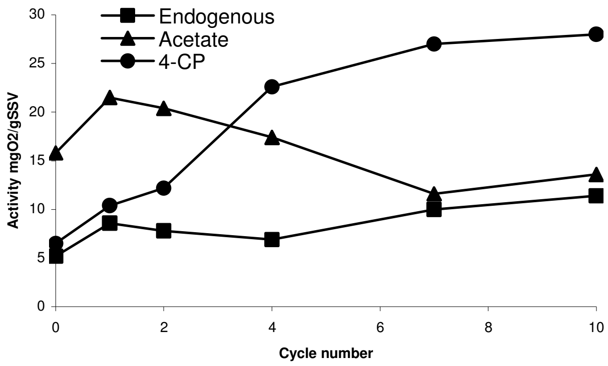


Figure 2.3.8 Activity of the microorganism in the acclimation process

2.3.2.5 Metabolite

The quantity of metabolite produces was superior during the first cycles. At the end of the acclimatization cycles the metabolite production was reduced (fig 2.3.9).

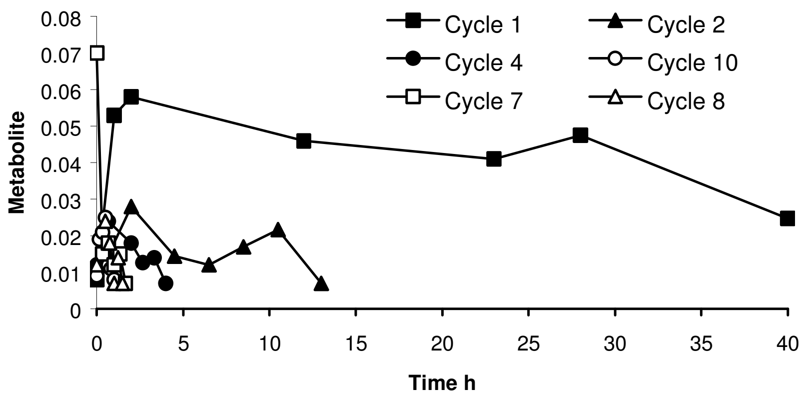


Figure 2.3.9. Metabolite production in the acclimatization process

3. CONCLUSIONS AND PERSPECTIVES

Pilot reactors and experimental work is being performed on time by partners 2, 4 and 6. Universidad de Uruguay does not have started with experimental so, they do not presented results either. It seems that next two months they are going to be on time. Next are presented conclusions, comments and perspectives formulated by each partner.

3.1 Carbon and Nutrients Removal. Politecnico di Milano (partner 2)

3.1.1 Planed activity for the Next six months

As agreed during the kick off meeting, the SBR operation is going to be mainly used to verify the feasibility of applying pH-stat and/or DO-stat techniques to monitor the SBR performance (WP3). For this purpose, during the following six months, the reactor performance will be monitored by titration experiments which will be compared with standard analytical determinations and on-line acquisition of pH, DO and ORP data. These last data will be made available for modelling, although with respect to the total set of data which are going to be collected by other units, only a subset will be considered (mainly ammonium, nitrite, COD and VSS data).

As soon as steady state conditions will be achieved, experiments will be performed to test the response of the system to: a) overload/underload conditions, b)presence of acute toxicants in the feed.

3.1.2 Comments on data format protocol

With respect to the protocol suggested, here are our comments:

- include TKN_inf (total Kjendal nitrogen in the influent) within the list of measured variables (Table 2)
- include ORP (redox potential in the reactor) within the list of measured variables (Table 2).

3.2 Sludge Level and purge automatization. LBE-INRA pilot (partner 4)

This first period was dedicated to the building, the instrumentation and to the acclimatation of the activated sludge to perform both carbon and nitrogen removal. To do so, a low laoding rate was applied. After a period of stabilization, the reactor performances have reached a steady state from an input-output point of view. At this low loading rate, both carbon and nitrogen removal as well as sludge settlement are correctly performed. The output concentrations is 35mg/l COD and 3 to 4 mg/l NTK. The output TSS is about 10 mg /l and we observe a good sludge settlement. The reactor is now ready to perform experiments necessary for model identification purposes.

2.3 Toxic Compounds. Universidad Nacional Autónoma de México (partner 6)

During this period the experiments were designed, analytical methods were implemented and the reactor was assembled. The first experiments of were conducted. According to the experimental plan results for the acclimatization/deacclimatization and general model will be finished on time. As we received the founding in February, at this moment we are in the equipment acquisition phase. To advance with the project equipment and supplies were borough from other projects conducted in the lab.

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