EOLI
Efficient Operation of Urban Wastewater Treatment Plants

Deliverable D1.2
Report on evaluation of the process improvement with the application of the optimal control law: Shock loads and the long-term stability of system

WP1. Process experiments
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I. INTRODUCTION

This deliverable provides the evaluation of the process improvement with the application of the optimal control law. Two cases were considered: case 1 toxic compounds and case 2 nutrient removal.

In the case of toxic removal the Event Driven Time Optimal Control developed in the UNAM was tested for the biodegradation 4-cholophenol and phenol. The ED-TOC strategy was evaluated against the introduction of shock loads. The long-term stability of system under the standard conditions was also evaluated for the degradation of 4CP.

For the case 2, the deliverable presents the evaluation of the process improvement with the application of the control strategy by the comparison with a non optimal (conventional) strategy and the evaluation of the influence of shock loads on the reactor stability for both optimal and non optimal strategies. The optimal control law (CL) described in Deliverable 5.2 was implemented for a laboratory scale reactor. At UU, Reactor M fed with synthetic dairy effluent was employed to test the CL. Standard reactor operation was characterized by an F/M ratio of 0.5 kgDQO/kgSSV/d. Shock load was performed by feeding the Reactor with more concentrated influent resulting in a F/M ratio of 0.75 kgDQO/kgSSV/d.

CASE I. TOXIC WASTEWATER

II. METHODOLOGY

II. 1. Description of the pilot reactor

A pilot reactor of 10L (7L of working volume) 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 recycle water pump was used to maintain a constant temperature of 20°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, in addition there are a Temperature and Dissolved Oxygen Sensor (Endress + Hauser, COS4). The aeration flow was 1.5 L/min.

II. 2. Details of the Experimental Protocol

The synthetic wastewater contained 4-chlorophenol (4CP) as carbon source and complemented with nutrients as is decribed by AFNOR (1985). The results for each one of the experiments report the data on line (Temperature, DO, and the Influent_FR) and the data off line (Conc of 4CP, CODs, DOCs, MET, DO, VOLreact, InfluentFR, TSS, VSS, SVI, SV, 4CP_in, CODt_inf, CODs_inf, pH_inf, pH (in the reactor at the end of the reaction phase), AirFR_in, CODT_ef, CODS_ef, TSSef, Kla, SOUR (End,To 4CP, To acetate), rpm (mixer), Temp (in the reactor), InfluentFR_in, the phases duration (AWOF, FWA, Aeration, Settling, Draw and the Total time). Two different control strategies were used: Fixed Time Control (FTC) and Event Driven Time Optimal Control (ED-TOC) as is described in the D1.1.

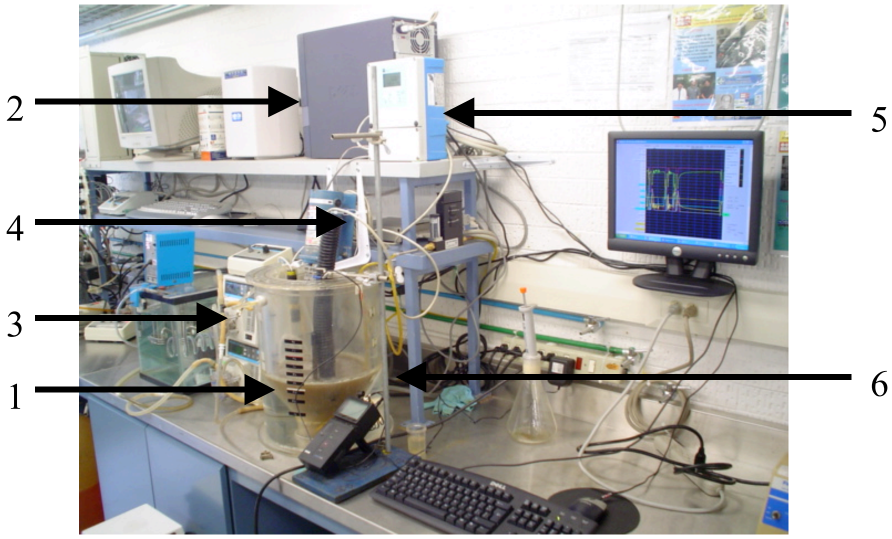


Figure 1. Pilot reactor utilized for the toxic compounds degradation. 1) Acrylic reactor with double wall and water recycling to maintain constant temperature at 20°C, 2) PC, 3) Peristaltic pumps (Master flex), 4) Temperature and Dissolved Oxygen Sensor (Endress + Hauser, COS4), 5) Temperature and Dissolved Oxygen Transmisor (Endress + Hauser, COM 223), 6) Switch Level (Flow line LV10 series)

II. 3. Event Driven Time Optimal Control (ED-TOC)

Moreno and Buitrón (1998) and Moreno (1999) proposed a control strategy to maintain the substrate removal rate at an optimum. The strategy called Time Optimal Control Observed Based (OB-TOC) tries to maintain the substrate concentration at S*, by feeding the exact amount of dissolved substrate with the input water flow, for as long as the physical volume constraints of the reactor allow. After completely filling the reactor, one must only wait enough time as for the biomass to complete the degradation of the remaining substrate until its concentration is below a certain minimum. In synthesis, this control strategy determines the fill phase by controlling the input flow to the reactor and ends the reaction phase when almost all substrate has been degraded. Note that the optimal operation is fed-batch and not batch. This control law has a main problem: one must continually measure the biomass and substrate concentrations in order to implement it. This is either impossible or too expensive; therefore, other alternatives have been sought. One solution is to estimate asymptotically the unmeasured variables based on the mathematical model of the system and the measurement of other variables involved in the process; this constitutes a state observer. The drawback is that it needs to know exactly the inflow substrate concentration (S_{in}) and other critical SBR parameters as S*. A slight mistake in estimating such parameters renders the observer useless. This makes the OB-TOC very difficult and unreliable to apply.

The Event Driven Time Optimal Control, ED-TOC (Betancur *et al.,* 2004) (Figure 2) solves the problems by not looking at S, but instead finding a variable named □, which is linearly related to μ, such that the latter is maximized too, i.e. equation 1.

$$\square = k_1 \square + k_2 \qquad (1)$$

The parameters k_1 and k_2 are unknown, but their values are not necessary for control purposes. Betancur *et al.,* 2004 show in particular that:

$$\square = \frac{XV}{Y_{X/O}}\mu + bXV = K_l a\left(O_s - O\right)V - OQ_{in} + V\frac{dO}{dt} \quad (2)$$

This can be derived from the mathematical description of the fill and reaction phases (consider equations 3 and 4 and assume that the term *XV* is almost constant).

$$\frac{dX}{dt} = \mu X - K_d X - X\frac{Q_{in}}{V} \quad (3)$$

$$\frac{dS}{dt} = -\frac{1}{Y_{X/S}}\mu X + \left(S_{in} - S\right)\frac{Q_{in}}{V} \quad (4)$$

$$\frac{dO}{dt} = -\frac{1}{Y_{X/O}}\mu X - bX + K_l a\left(O_s - O\right) + \left(O_{in} - O\right)\frac{Q_{in}}{V} \quad (5)$$

$$\frac{dV}{dt} = Q_{in} \quad (6)$$

Where,

X: Biomass concentration inside the reactor; S: Substrate concentration inside the reactor; V: Water volume level in the reactor; O: Dissolved oxygen concentration, YX/S: Biomass/substrate yield coefficient; $Y_{X/O}$: Biomass/oxygen yield coefficient; Kla: Oxygen mass transfer coefficient; b: Specific endogenous respiration rate; Kd: Decay biomass rate; μ: Specific growth rate, S_{in}: Influent substrate concentration; O_{in}: Influent dissolved oxygen concentration; Q_{in}: Influent flow rate; Os: Dissolved oxygen concentration at saturation.

Such _ can be estimated in real time by using O and V measures, that is why it is feasible to control Qin so that the reaction's speed stays inside the fast zone in Figure 2. Such zone is above a P% of the maximum speed. Associated with that there is a shaded rectangle to explain how controlling the speed indirectly keeps the unknown S oscillating in a gap between some Spl (lower than S*) and Sph (higher) all the way trough the SBR filling. Every time S tries to leave this gap, an event is issued and the ED-TOC then switches Qin on/off, to prevent it. This is why the volume-substrate plane trajectory zigzags inside the shaded envelope. As P→ 100% the zigzags will be finer and then the ED behavior will approach the theoretical TOC. This will work regardless of the Sin value and despite changes in most of the SBR parameters. Only Kla and Os are needed to estimate _ and they do not even need to be known precisely. This makes the ED-TOC robust. In control terms, this means that increasing the error in obtaining those parameters will only degrade the ED-TOC performance gradually. Even when those parameters are badly given, the ED-TOC will behave acceptably. This solution is therefore a good candidate for uses in real industrial environments. Table 1 show the standard conditions used in the experiments with the FTC and ED-TOC strategies.

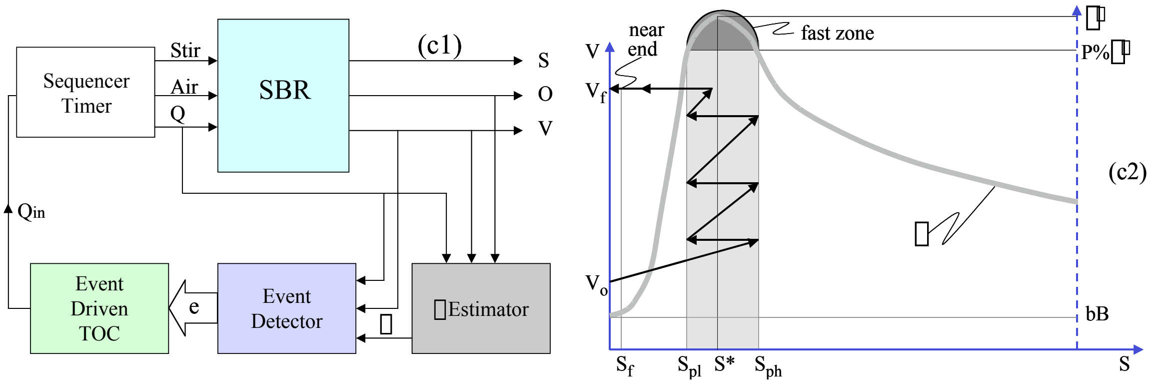


Figure 2. ED-TOC; 1) Control Layout, 2) V-S plane trajectory (VSpt); Secondary dashed line for μ or _ , respectively. A dark area marks the speed related zone in which the process spends most of its time. Initial S=0 and V=Vo conditions are assumed.

II. 4. Control System

The system was developed to introduce the selected type of operation strategy for each of the realizing experiments. The process register window (figure 3) shows the dissolved oxygen concentration, the reactor volume, the phase and the time elapsed of the phase, the file name, pH, temperature, estimated substrate, movements in the control register and another parameters that are used in the control strategies.

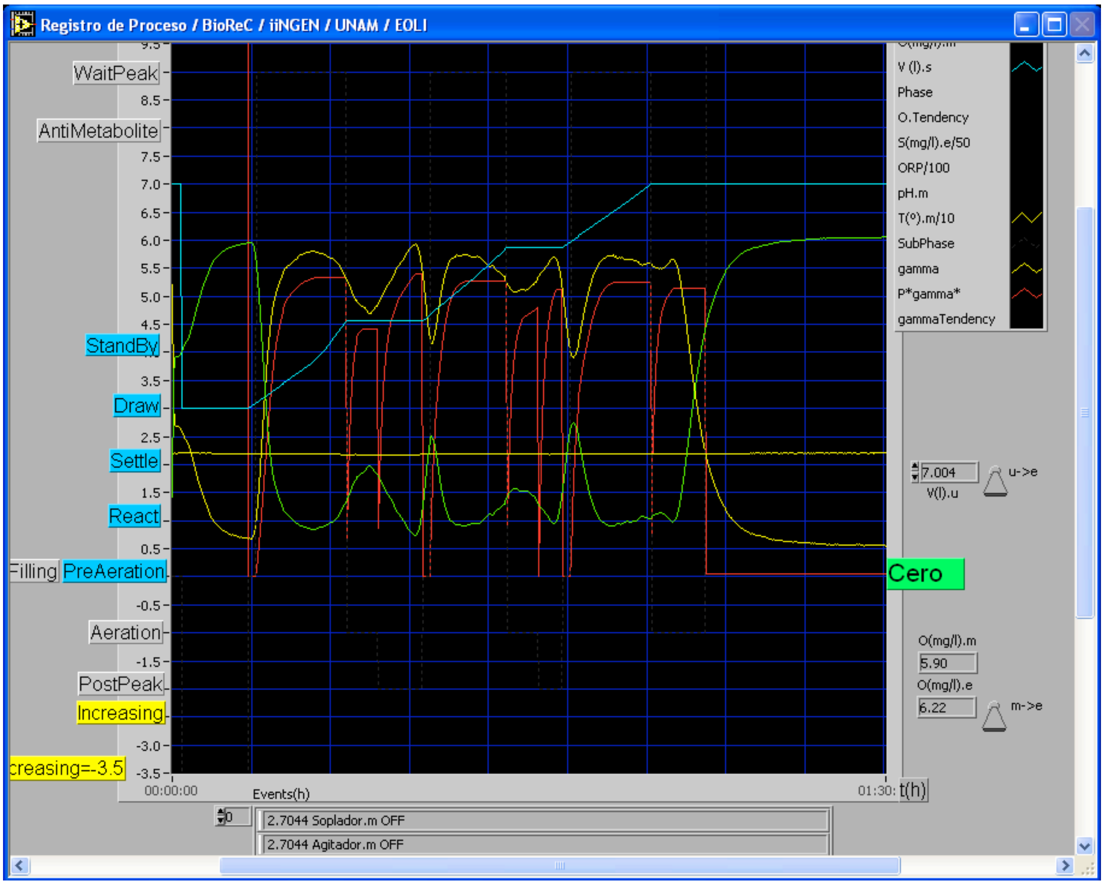


Figure 3. Process graphic register window

Table 1. Standard conditions used for the strategies employed

Conditions	ED-TOC
Filling/Reaction Ratio, %	Aprox. 100
Cin [mg COD/L] C(t=0)	375 (350)* 214 (200)*
Type of compounds	Synthetic wastewater. 4-chlorophenol
X(t=0) [mg/L]	2,000
Volume loading rate, gCOD/L-d	2.25
Mass loading rate, gCOD/gVSS-d	1.12
O2 [L/min.]	1.5
pH	7
T [°C]	20
V(t=0) [L]	3
V(t=tfin) [L]	7
Total cycle time [h]:	4
SRT [days]	20 measured
Operational times	
Fill	variable
Aerobic	variable
Settling	30 min
Draw	6 min
Idle	15 min
Total time	variable

* as 4CP

II. 5. On Line Measurements

The on line measurements were obtained with the next devices:

Dissolved Oxygen and Temperature sensor, Hendress+Hauser (Liquisys-M)

Influent 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).

II. 6. Off Line Measurements

Total phenols (4CP or phenol concentration), Chemical Oxygen Demand (COD), Dissolved Organic Carbon (DOC), Metabolite (5-chloro-2hydroxy-muconic acid semialdehyde), Volatile Solid Suspended (VSS) and Total Solid Suspended (TSS), Activity of microorganism, sludge volumetric index (SVI), settling velocity (SV).

II. 7. Analytical Methods

II. 7. 1.Total phenols (4CP or phenol concentration)

Total phenols were determined using the 4-aminoantipyrine method (colorimetric method). The phenolic compounds have a reaction with the 4-aminoantipyrine at a pH of 7.9 +/- 0.1 in presence of alkaline medium for form a aminoantipyrine colored complex with color. This is read at 500 nm.

II. 7. 2. Chemical Oxygen Demand (COD)

The method used is according to HACH (method 420 COD Low Range). The sample was filtered using Durapore Membrane filters of 0.45 µm (Millipore).

II. 7. 3. Dissolved Organic Carbon (DOC)

The DOC is used in order to detect the degradation of 4CP (organic compound) during the degradation kinetics. The sample was filtered using Durapore Membrane filters of 0.45 μm (Millipore), the analysis was carried out with a Carbon Organic Total Analyzer (Shimatzu 5050)

II. 7. 4 Metabolite

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

II. 8. 5 Volatile Solid Suspended (VSS) and Total Solid Suspended (TSS)

The VSS and TSS were done according to the Standard Methods (APHA, 1992).

II. 8. ED-TOC experiments (evaluation of the process improvement with the application of the optimal control law)

In order to evaluate the process improvement with the application of the optimal control law two sets of experiments were tested: The evaluation of the optimal control strategy in the shock loads and in a long-term stability of system

II. 8. 1. Shock loads

Many industrial processes generating wastewater containing toxic compounds are characterized by their variability. In chemical, pharmaceutical, plastics, and petrochemical industries, many production processes are in batch. Because of the high variations in flow and concentration of contaminants in industrial wastewater, usual treatment processes do not obtain satisfactory removal efficiencies. Besides, due to its toxicity, the biological treatment of industrial wastes containing a high concentration of toxic compounds like phenols is inefficient because of the inhibition of the microorganisms (Buitrón *et al.*, 2003).

The SBR operated under the FTC presents several constraints when it is applied to toxic wastewater degradation: inhibition of the microorganisms, problems with shock loads of toxic compounds, deacclimation and problems of starvation of the microorganisms, and low toxic removal efficiencies (Buitrón and Moreno 2002; Buitrón et al, 2003).

The ED-TOC strategy was tested in order to overcome the problems discussed above. In this case two set of experiments were tested in order to evaluate the process improvement with the application of the optimal control law in the response of the ED-TOC strategy to shock loads.

The first one evaluates the degradation of 3 shock loads concentration of 4CP:
Experiment 1. Degradation of a shock load concentration of 3000 mg4CP/L
Experiment 2. Degradation of a shock load concentration of 5000 mg4CP/L
Experiment 3. Degradation of a shock load concentration of 7000 mg4CP/L

The second set of experiments evaluates the degradation of a mixture of municipal wastewater and shock loads concentration of phenol:
Experiment 4. Degradation of a shock load concentration of 3000 mg/L of phenol
Experiment 5. Degradation of a shock load concentration of 5000 mg/L of phenol
Experiment 6. Degradation of a shock load concentration of 7000 mg/L of phenol

II. 8. 2. Long-term stability of the system

In order to test the long-term stability of SBR system operated by the ED-TOC strategy, the bioreactor was operated during more than 400 cycles (100 days) by the ED-TOC strategy. The reactor was operated under the standard conditions as is described in the methodology, i.e., the wastewater degraded in this periods were synthetic wastewater containing 4CP (350 mg/L) and nutrients as a sole carbon source.

II. 8. 2. 1. Changes in the community by the acclimation and the long-term stability of the system using the ED-TOC strategy using molecular biology techniques

The acclimation of the biomass was carried out according with the Deliverable D1.1 of this project. The evolution of the microbial community was followed using PCR (Polymerase Chain Reaction), DGGE (Denaturing Gradient Gel Electrophoresis), cloning and sequencing techniques. Total DNA was extracted from 2 mL samples of activated sludge using an Ultra clean™ Soil DNA isolation kit (MoBio). PCR amplifications were conformed with a BioRad temperature cycle, with the following program: initial activation 95°C (15 min), 35 cycles of: denaturation 94°C (1 min), annealing 53°C (1 min) and elongation 72°C (1 min) and a final extension of 72°C (1 min). The universal primers GC-338F and 518R were used for amplification (Muyzer *et al.,* 1995). The PCR products were separated by DGGE using a D-code universal mutation detection system (BioRad, Hercules, CA) as described by Muyzer *et al.* (1995). The central portions of selected bands were excised and extracted amplicons were used as template for the re-amplification. These PCR products were cloned into pCR2.1-TOPO plasmid (Invitrogen, USA). The positive clones were sequenced with the primer M13 reverse (5′-CAGGAAACAGCTATGAC) in an ABI Prism 3100 DNA sequencer. The identification of the clones was done using the GenBank database by the BLAST software (National Center for Biotechnology Information).

Samples of the reactor, for the acclimation period, were taken each 24 h during 5 days, i.e., samples were collected at 24, 48, 72, 96 and 120 h. For the case of the long term stability the samples were collected each week of operation. These analyses were performed in order to determine the diversity and dynamics of the microbial community on the activated sludge.

II. 8. 2. 2. Elimination of the toxicity of wastewater using Microtox® techniques

During the operation of the reactor with the ED-TOC strategy, and during the stable phase, the toxicity of different influent and effluent samples was testing with the Microtox ® Model 500 Analyzer. This method is a useful tool to estimate the toxicity of different chemicals and effluents by measuring the reduction of light production due to interactions between the luminescent bacteria *Vibrio fischeri* and toxic compounds. Total toxicity of the samples was measured with the Microtox ®Acute Toxicity Test and the Basic Method. EC50 values were determined using contact time of 5 min and the reference chemical was phenol. These analyses were carried out in order to verify the elimination of the toxicity during the process.

III. RESULTS AND DISCUSSION

III. 1. Degradation of Shock loads of 4CP by the ED-TOC strategy

The degradation of 350 mg4CP/L (standard condition) with the ED-TOC strategy was obtained in 2h. The total cycle time was 3.5h, 25% less than the case for the FTC strategy. Figure 4 presents the behavior of the substrate concentration, measured as 4CP, COD, and DOC during a cycle. It is possible to distinguish how the control operates if the substrate evolution and the DO curve are followed. When the substrate is filled the DO decreases until the substrate concentration is higher than S* and inhibition starts; then, the DO increases, indicating that the maximal degradation rate in the Haldane curve has been passed. At this point the fill is stopped and the degradation rate of the 4CP is near to its maximum. Once the substrate is degraded below S*, the DO starts to increase again and the filling pump is turned on. This cyclic behavior is maintained until the total volume of the reactor is reached. The operation mode can be explained as if in the reactor several mini-batches took place during a cycle. At the end of the reaction the DO increases to the saturation value. For the example showed the inhibition never occurred due to the fact that 4CP concentration in the reactor was around S* and never was higher than 50 mg/L during all the reaction, in this case the S* value was around 40 mg/L.

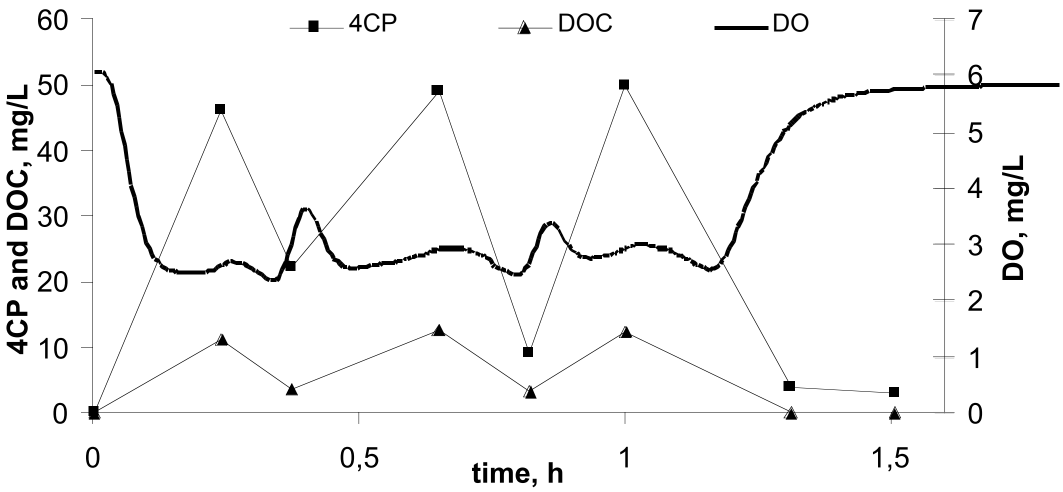


Figure 4. Behavior of the substrate concentration measured as 4CP, COD and DOC during the degradation of 350mg4CP/L using the ED-TOC strategy

The degradation of a shock load of 3000 mg4CP/L (Figure 5) was reached in 22 h (the total cycle duration was 23 h). It is possible to distinguish how the control operates if the substrate evolution and the DO curve are followed as in the Figure 4. The behavior of the control and chemical parameters (DO, COD, DOC, 4CP) was similar as the degradation of 350mg4CP/L. In the reactor the concentration of 4CP was never superior to 50 mg/L, except in the first filling period (the first mini-batch) 70 mg/L where the ED-TOC strategy first tried to find the conditions indicating S is near and above S* (around 40 mg/L).

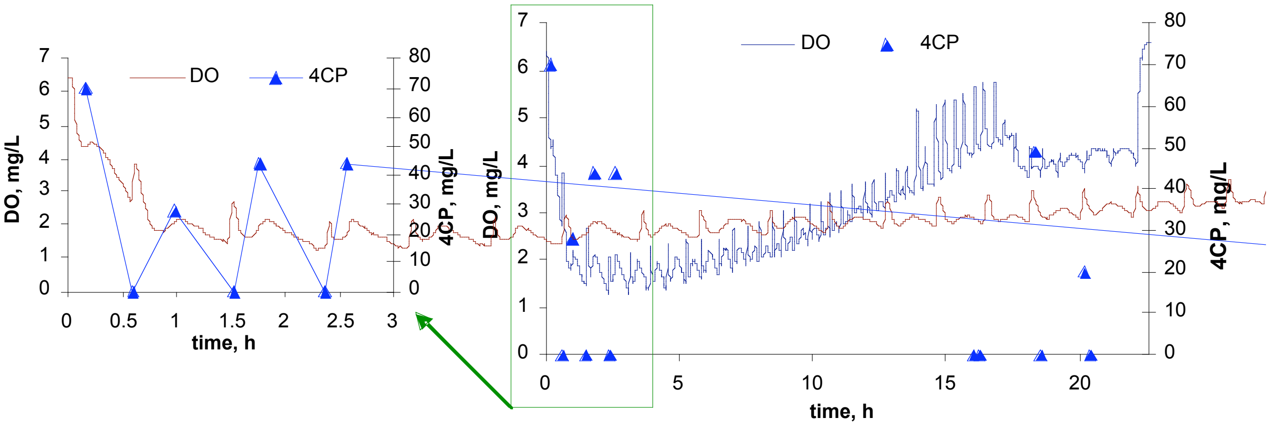


Figure 5. Behavior of the substrate concentration measured as 4CP and DO during the degradation of 3000 mg4CP/L using the ED-TOC strategy

The degradation of 5000 mg 4CP/L (Figure 6) was achieved in 30h. The quantity of 4CP in the reactor was never superior to 80 mg/L, except in the first period of fill (110mg/L), this concentration of 4CP did not caused biomass inhibition problems. Similar results were observed for the case of 7000 mg 4CP/L (Figure 7) the reaction time was extended to 42h and the total cycle duration was 43h. It is interesting to note that independently of the influent concentration, no inhibition problems of the biomass were observed for any case.

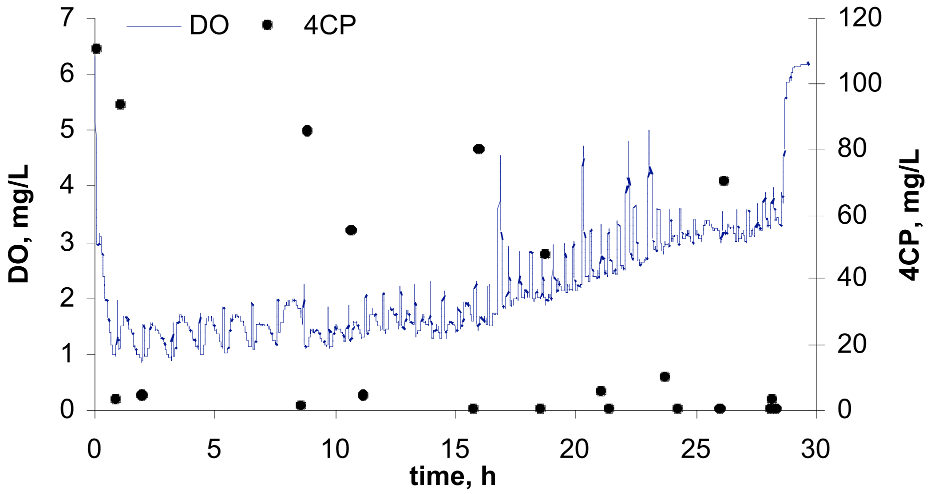


Figure 6. Behavior of the substrate concentration measured as 4CP and DO during the degradation of 5000 mg4CP/L using the ED-TOC strategy

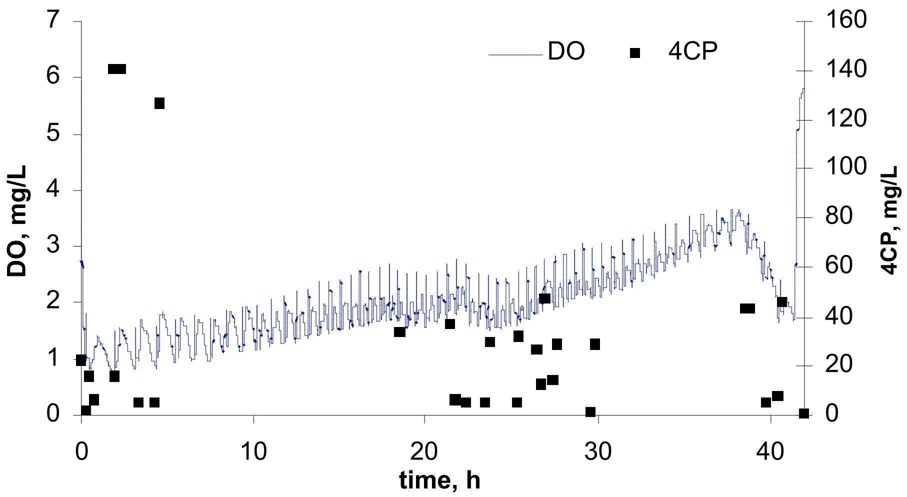


Figure 7. Behavior of the substrate concentration, measured as 4CP and DO during the degradation of 7000 mg4CP/L using the ED-TOC strategy

III. 1. 1. Comparison of FTC and ED-TOC strategies

With the results obtained it was possible to compare the performances for both strategies and, also, it was verified the capacity of the ED-TOC strategy to degrade high concentrations of toxic compounds. In order to degrade the shock loads of 4CP by the FTC strategy it was necessary to dilute the initial wastewater to 350 mg/L. Buitrón *et al.* (2003) found that concentrations higher than 350 mg/L produced inhibition problems. For example, for the degradation of a wastewater containing 7000 mg 4CP/L, it is necessary to dilute 20 times, thus 20 FTC cycles will be needed. On the contrary, using the ED-TOC strategy there is no need of dilution, since in the bulk of the reactor the controlled amount of wastewater is fed by the strategy. As the feeding is divided in several mini-batches there is no chance of inhibition. Theoretically any initial concentration can be fed to the reactor since only the adequate concentration will be introduced to maintain the degradation velocity as close as possible to the maximal degradation rate.

Another advantage of the ED-TOC strategy is that it is not necessary to know the initial toxic wastewater concentration because the control of the ED-TOC is based on the oxygen consumption of the microorganisms and thus, directly related to the microbial activity. This is significant because in the FTC strategy it would be necessary to know the initial concentration when a shock load is arriving in order to make the necessary dilution to avoid inhibition and to proceed with the satisfactory degradation of the wastewater.

The comparison of the strategies showed that the necessary time to degrade the same concentration of 4CP is lower with the use of the ED-TOC strategy (figure 8). The time required by the ED-TOC is lower from 25% to 52% for the 350 and 7000 mg4CP/L, respectively that the FTC strategy. The higher the concentration of 4CP the lower the equivalent degradation time with the ED-TOC strategy was observed. The maximal specific degradation rate, q_{max}, did not show significant differences when comparing both strategies. For FTC strategy the q_{max} was 50 mg4CP/gVSS/h, and for ED-TOC it varied between 46 and 50 mg4CP/gVSS/h, for the four concentrations studied. This can be explained because although there is a great difference in the concentration of the wastewater (from 350 to 7000 mg/L), the concentration of the toxic in the bulk of the reactor is not inhibitory (between 50 and 140 mg/L) for either case.

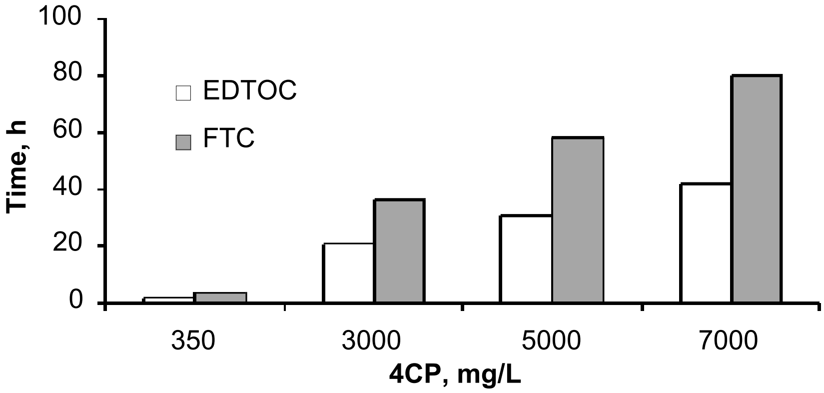


Figure 8. Comparison of the time necessary for degrade the same quantity of 4CP by the FTC and ED-TOC strategies

It can be observed that for ED-TOC strategy the applied load is of almost the double (table 2) than for the FTC strategy. For the degradation of phenols Herzbrun *et al.*, (1985) reported a value of 0.26 kgCOD/m^3/d for an SBR pilot scale reactor. Also, Buitrón *et al.*, (1994) reported a value of 1.2 kgCOD/m^3/d for the degradation of 4CP using a strategy similar to the FTC in a discontinuous reactor.

Table 2. Applied load used with the different strategies

4CP, mg/L	Applied load (kgCOD/m^3/d)	
	FTC (for each cycle)	ED-TOC
3000	2.52	4.11
5000		4.80
7000		4.80

An important point of discussion is that with the FTC strategy it is not possible to degrade influents with concentrations superiors to 500 mg/L. In addition, it has been observed that inhibition and a total loss of the activity of the microbial consortia was found, when a concentration of 1050 mg/L was applied to the reactor (Buitrón *et al.*, 2003). However, ED-TOC strategy could theoretically degrade any amount of toxic in the influent, without even measuring it. It was showed that it is possible to degrade a concentration of 7000 mg4CP /L. This concentration is 10 times higher to the value that would cause an inhibition using the FTC strategy in the sequential discontinuous reactors.

The energy consumption by the aeration pump by the two strategies was compared. As the ED-TOC strategy reduces the total cycle time, and in particular de degradation time, there is also a reduction in the quantity of air supplied to the system. The volume of air supplied was computed considering the degradation time for each shock load applied when using the FTC and the ED-TOC strategies were used (Table 3) and taking into account that the air flow rate was 1.5 standard L/min. In general there is a reduction for the supplied air from 24 to 37 %.

Table 3. Comparison of the supplied air volume during the degradation of different concentrations of 4CP using the FTC and ED-TOC strategies

4CP, mg/L	Volume of air, m^3		Percentage of save by the ED-TOC strategy (%)
	FTC	ED-TOC	
350	0.29	0.18	37.9
3000	2.63	1.98	24.8
5000	4.39	2.70	38.4
7000	5.85	3.87	33.8

III. 2. Degradation of a mixture of municipal wastewater and shock loads of phenol by the ED-TOC strategy

The application of the ED-TOC strategy showed that is possible to degrade a mixture of municipal wastewater and phenol. The mixture had presented a total COD of 1227. 2087, 4350, 6380 and 14470 for the five initial phenol concentrations (350, 700, 1500, 3000 and 7000 mg/L. Note that the increase in COD is due to the increase of phenol. The municipal wastewater alone has 270 mgCOD/L in average. When the phenol concentration was 350 mg/L in the influent, reaction rate took around 2 h. It was possible to biodegrade shock loads of 700, 1500, 3000 and 7000mg/L of phenol contained in the municipal wastewater without any problem of inhibition. The removal efficiencies were up to 98% as chemical oxygen demand and 100% as phenol. In other studies using SBR it has been reported that the degradation of phenol concentrations as high as 1300 mg/L cause problems of inhibition and a total loss of the activity of the microbial consortia is presented (Yoong *et al.,* 2000).

Figure 9 shows the degradation of the mixture of toxic wastewater for the case of 350 mg/L of phenol (1227 mgCOD/L in the mixture with wastewater). For the example showed the inhibition never occurred due to the fact that phenol concentration in the bulk of the liquid in the reactor was around S* and never was higher than 120 mg/L during all the reaction.

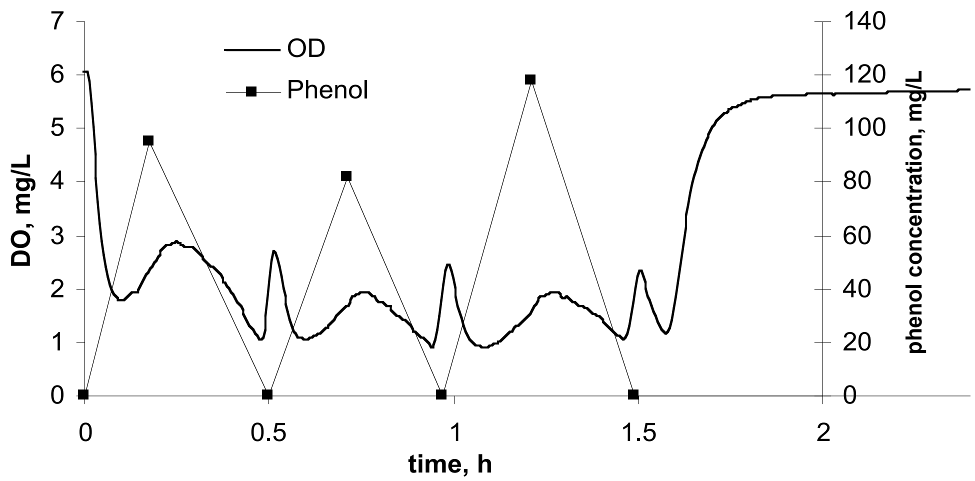


Figure 9. Degradation kinetic of a mixture of municipal wastewater and 350 mg/L of phenol using the EDTOC strategy. See the explanation in the text.

Figure 10 shows the kinetic degradation of a shock load for degradation of the of 700, 1500, 3000 and 7000 mg of phenol in the mixture. It is possible to observe the same behavior in the control strategy as in the figure 9.

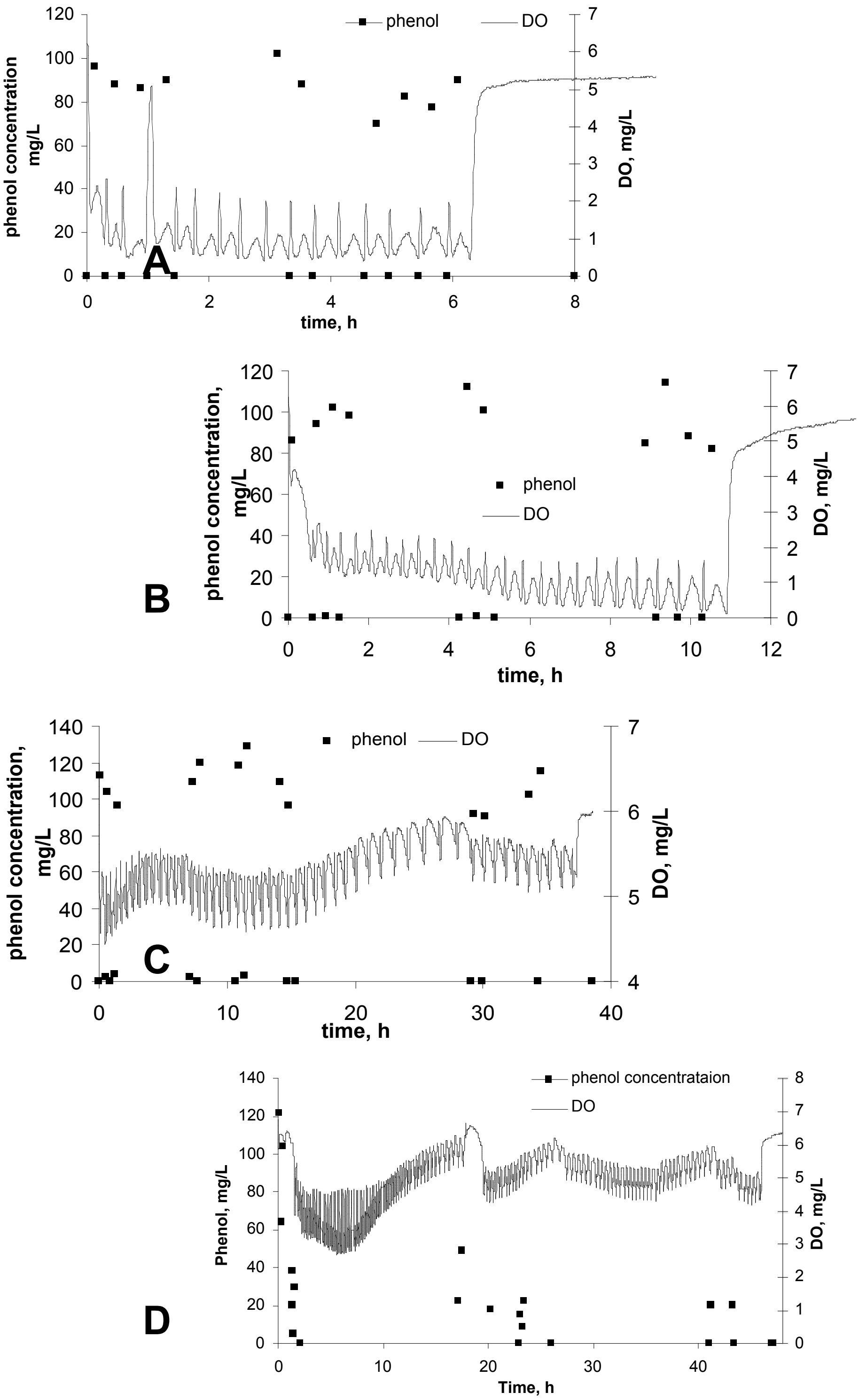


Figure 10. Degradation kinetic of a mixture of municipal wastewater and A)700, B) 1500, C)3000 and D)7000 mg/L of phenol mixed in a municipal wastewater using the EDTOC strategy.

Figure 11 show a detail of the kinetic degradation of a shock load of 7000 mg of phenol in the mixture. It was observed that the phenol concentration never was higher than 120 mg/L as in the case of 350 mg/L. These results confirm that higher phenol concentrations could be inhibitory. In theory any phenol concentration could be degraded by this control strategy.

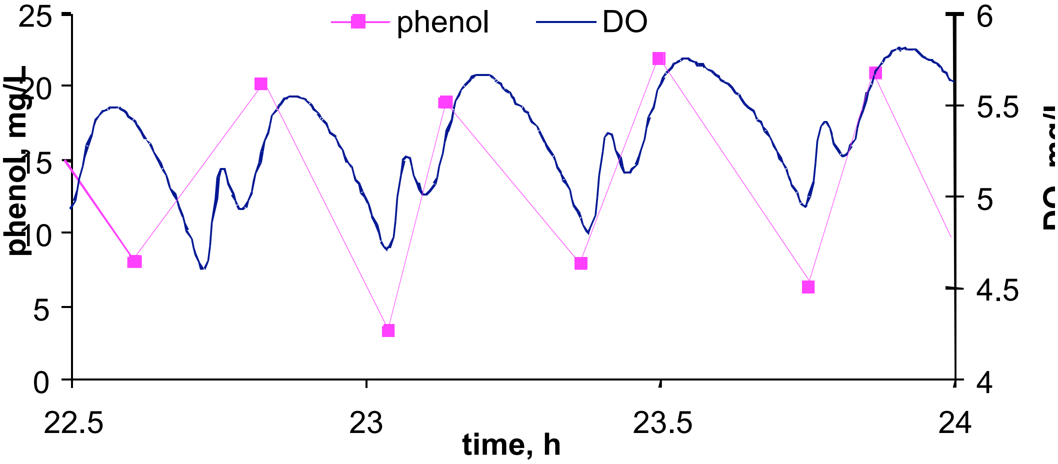


Figure 11. Detail of degradation kinetic of a mixture of municipal wastewater and 7000 mg/L of phenol using the EDTOC strategy.

An important point of discussion is that with the FTC strategy it is not possible to degrade influents with concentrations superiors to 1300 mg/L (Yoong *et al.,* 2000). However, EDTOC strategy could theoretically degrade any amount of toxic in the influent, without even measuring it. It was showed that it is possible to degrade a concentration of 7000 mg of phenol/L in the mix. This concentration is more than 5 times higher to the value that would cause an inhibition using the FTC strategy in the sequential discontinuous reactors.

III. 2. Long term stability

The reactor was operated during 450 cycles (100 days). The figures 12 to 15 show the operation data, corresponding to the physicochemical parameters evaluated. It is possible observed that the operation of the reactor was stable, with efficiencies higher than 99% for the 4CP degradation, and 97% as a COD degradation. The specific degradation rate was 95.3±8.2 mgCOD/mgVSS/h for the COD and 50.8±4.7 mg4CP/mgVSS/h for the 4CP during the operation with the ED-TOC strategy.

The reactor operates in two sets of experiments:

Set 1:

- **Acclimation**: cycle 1 to 40
  During this period the reactor was acclimated to the degradation of 4CP as is described in the D.1.1
- **Operation with the ED-TOC strategy**: cycle 41 to 188
  The reactor operated during all the period with efficiencies higher than 99% for the 4CP degradation, and 97% as a COD.
- **Experiments with variation of pH.** From the cycle 101 to the cycle 110 the pH of the reactor was adjusted to 3.5 (±0.5). In the cycle 111 the pH was neutralized to 7.0 and the reactor continued operating under the standard conditions. With this experiment the robustness of the reactor to variations on the pH values was tested. In the figures 15 it is possible to observe that this stress periods not affect the efficiency of the reactor (degradation of the 4CP and the COD), but the specific degradation rate

(q) decreases in 35% mgCOD/mgVSS/h and 40% mg4CP/mgVSS/h for the qCOD and q4CP respectively. When the reactor operated again at pH of 7, the biomass was recovered in 6 cycles (from cycle 111 to 117) After this period the specific degradation rate was recovered to 50.8 mg4CP/mgVSS/h.

At the end of the cycle 194, the biomass was discharged and a second set of experiments was started.

Set 2:

- **Acclimation:** cycle 195 to 218
- **Operation with the ED-TOC strategy:** cycle 223 to 450

The reactor operated of the reactor during all the period with efficiencies higher than 99% for the 4CP degradation, and 97% as a COD.

- **Experiments with high biomass concentration.**

From the cycle 356 to the cycle 404 the biomass was not purged, with this, the VSS increases 135% (from 2000 to 4700 mg/L). At this point the ED-TOC strategy was robust and the degradation was effectively achieved. The degradation efficiencies were conserved (higher than 99% for the 4CP degradation and 97% as a COD). However the ED-TOC strategy took more time than the usual degradation time (75% more), and the specific degradation rate decreased 80%, due to the microorganisms stress of deficiency of oxygen, thus the strategy was not optimal. The ED-TOC strategy maintained an efficient degradation but if the limitation of oxygen continues, the ED-TOC strategy will present problems related to the control strategy since it is based in the measurement of the dissolved oxygen in the reactor.

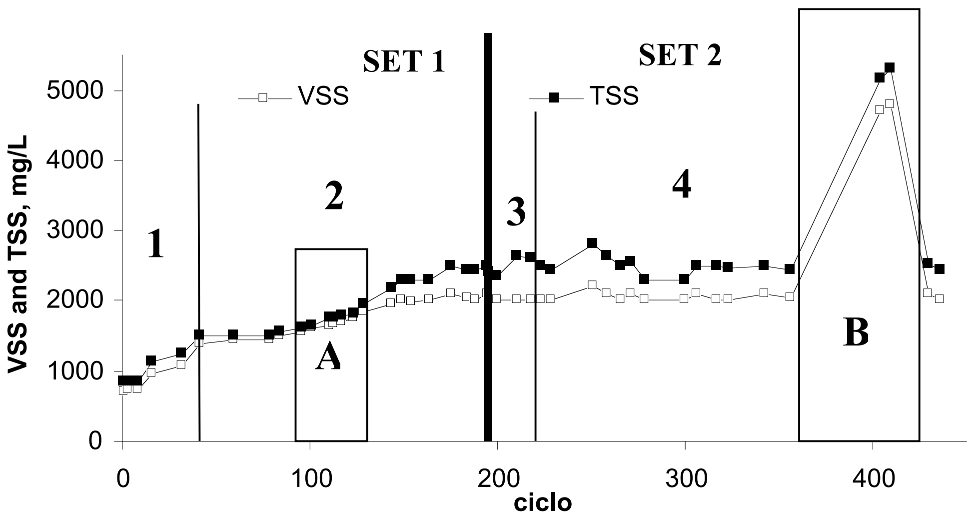


Figure 11. Experimental data of the VSS and TSS during the long term degradation by the ED-TOC strategy. In the figure is showed the sets of experiments: **1)** Acclimation, set1, **2)** ED-TOC, set 1, **3)** Acclimation, set2, **4)** ED-TOC, set 2. **A)** Low pH, **B)** Increase of biomass.

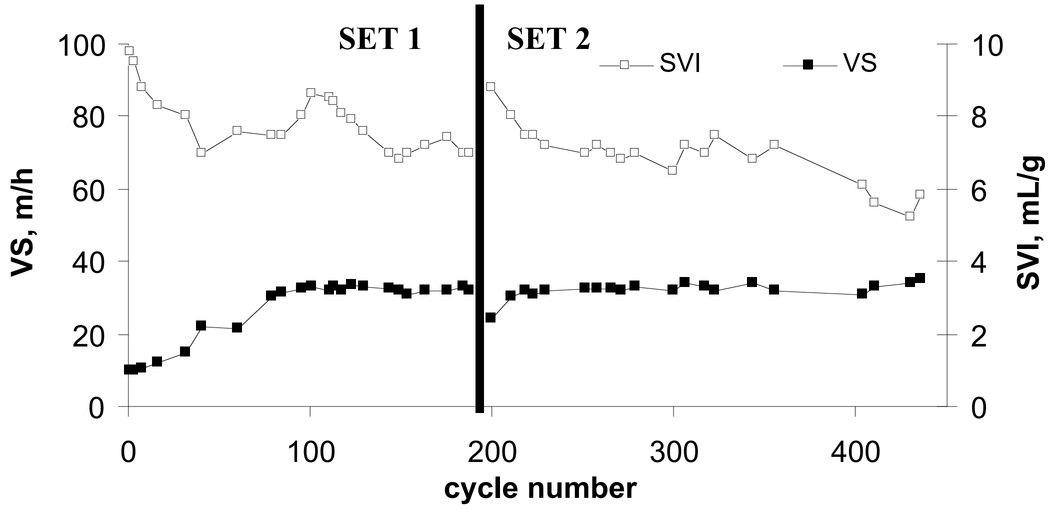


Figure 12. Experimental data of the VS and SVI during the long term degradation by the ED-TOC strategy

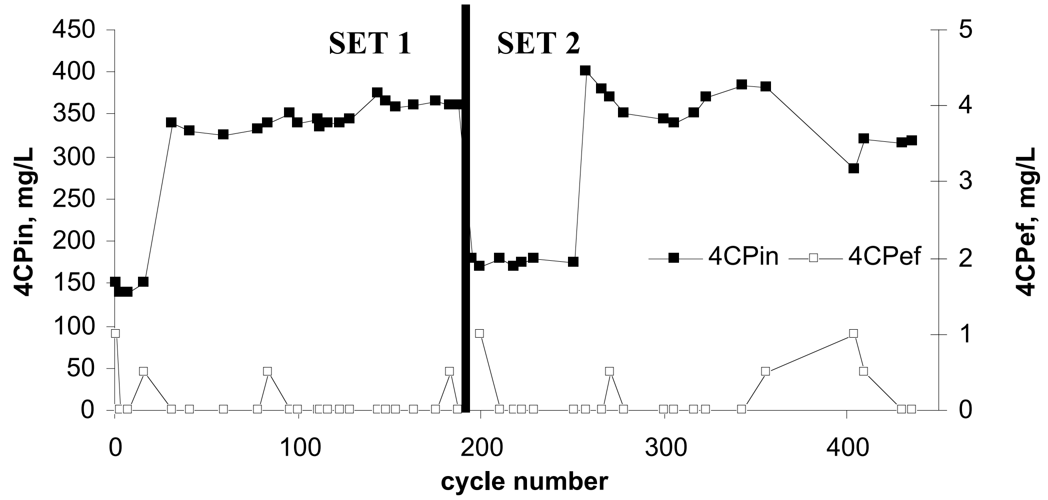


Figure 13. Experimental data of the 4CP in the influent (4CPin) and in the effluent (4CPef) during the long term degradation by the ED-TOC strategy

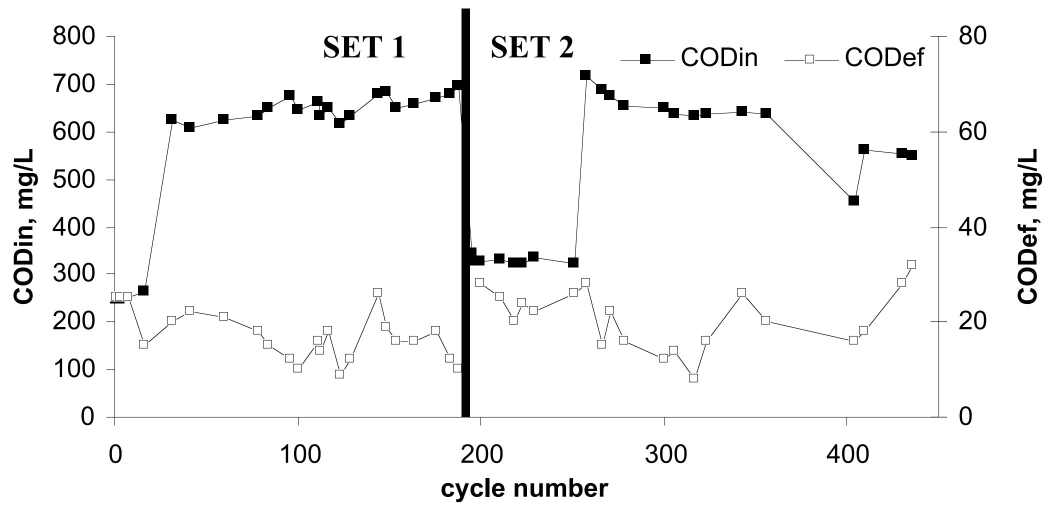


Figure 14. Experimental data of the COD in the influent (CODin) and in the effluent (CODef)during the long term degradation by the ED-TOC strategy

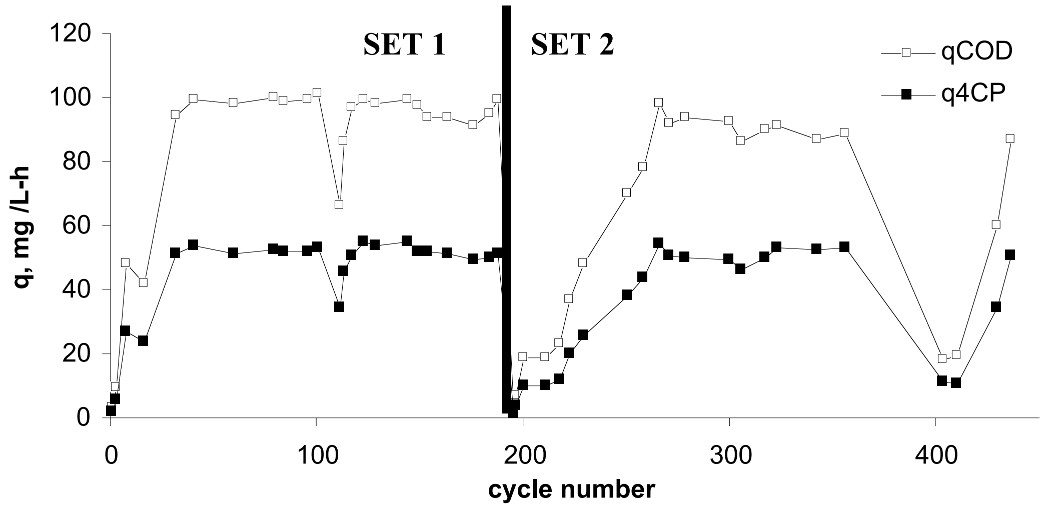


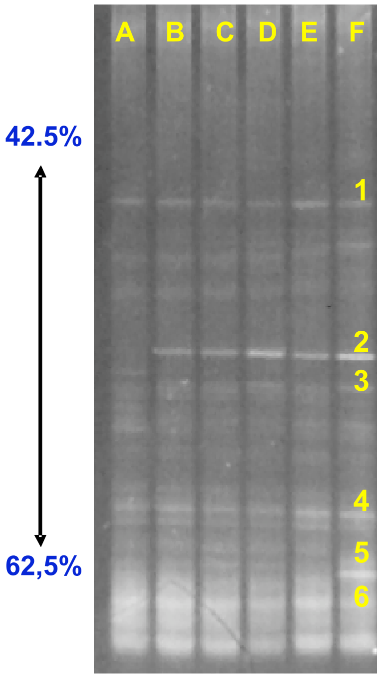
Figure 15. Experimental data of the specific degradation rate during the long term degradation by the ED-TOC strategy

III. 2. 1. Bacterial community analysis and identification

The V3 hypervariable region of the 16S rRNA gene was obtained by PCR amplification using the DNA obtained from the Easy-DNA kit method as template. The PCR amplification products of template DNA; 200-bp fragments were obtained as expected. The number of individual bands obtained in a DGGE analyses is related to the number of bacterial species in the tested sample.

III. 2. 1. 1. Changes in the bacterial community during the acclimation

Figure 16 shows the DGGE community fingerprint of the consortium during the acclimation process. This analysis revealed a moderately diverse bacterial community, eleven DGGE band were visible, these suggesting that minimum eleven different bacteria were present. Six of the detected bands were excised from the gel and sequenced. The most closely related sequences in the GenBank nucleotide database were determined. It is possible to observe in figure 3 that the species corresponding to the bands 1, 3, 4, 5 and 6 were maintained during all the acclimation period. Table 1 presents the microorganisms associated to each DGGE band. Band 2 (*Pseudomonas sp.*) was evident until the second day of acclimation, and it prevailed until the end of acclimation, indicating the reproduction of this specialized organism.



Band	Sequence Determined (bp)	Affiliation	Similarity (100%)	Accession NBCI GenBank
1	No determined	----	----	----
2	195	*Pseudomona s* sp.	100	AY269867.1
3	168	*Novosphingobium subarticum* strain	99	AY167828.1
4	195	Uncultured bacterium 1	100	UEU409001
5	170	Uncultured *Hyphomicrobiaceae*	100	AY710861.1
6	143	*Paracoccus* sp.	85	AY312056.1

Figure 16. DGGE community fingerprint of the consortium during the acclimation. The table shows the microorganisms determined by the DGGE analysis from the band showed in the figure. A) Initial condition (0h), B) 24h, C) 48h, D) 72h, E) 96h, F) 120h

III. 2. 1. 2. Evolution of the bacterial community during the long-term stability of the system using the ED-TOC strategy

The 16S rDNA fragments obtained after PCR amplification of total DNA extracted from the inoculum sludge and reactor at different operational periods (cycles 1, 41, 101, 144 and 188) of reactor were analyzed by DGGE. Figure 17 shows the DGGE community fingerprint of the consortium during the set 1 of the long term stability experiments. This analysis revealed a diverse bacterial community, more than fifteen DGGE band were visible, and thirteen of the detected bands were excised from the gel and sequenced.

The resulting patterns showed that the bands in the inoculum sludge (A) are different from those of all other samples, as is showed in the DGGE fingerprint of the consortium during the acclimation process (Fig.17). It is also evident that bands in the reactor at B and C are largely similar, but they are quite different from those of reactor at other days D and F. On the other hand, the band pattern at the beginning of the acclimation show that were present initially, disappeared on cycles 41 and 101 while others had become more intense (12 and 13) on cycle 188. Another interesting variation is present in the bands 6 and 9, which are present in a very low concentration in the beginning of the ED-TOC operation in cycle 41 (B, in figure 17), but the concentration of that increases after 60 operation cycles (C, D and D, figure 17).

Even though the community was not stable, the macroscopic parameters of the reactor operation were always constant. The species will be identified to determine their importance in the degradation of the toxic compound during the long-term stability of the system using the ED-TOC strategy.

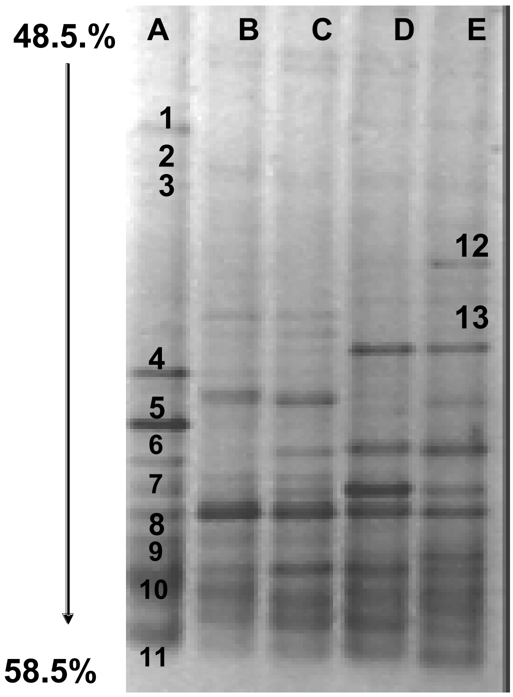


Figure 17. DGGE analysis of V3 fragments amplified by PCR of the set1 of experiments for the evaluation of the ED-TOC strategy operated in long period. A) Cycle 1, initial condition (inoculation), B) Cycle 41, 1 ED-TOC, at this point the biomass was acclimated to degradation of 4CP, C) Cycle 101 , D) Cycle 144, E) Cycle 188. Bands numbers 1-13 were excised and sequenced.

III. 2. 1. 3. Toxicity of the treated wastewater using the ED-TOC strategy

Toxicity of the punctual samples was measured with the Microtox ®Acute Toxicity Test and the Basic Method. First, the toxicity of the influent was evaluated. It was found that a feeding solution of 100 mg/L of 4CP and nutrients in the influent presented an $EC50_5$ = 0.8532 mg/L with a 95 % of confidence range. This result indicates that 4CP is a very toxic compound (Figure18) with 80 % inhibition.

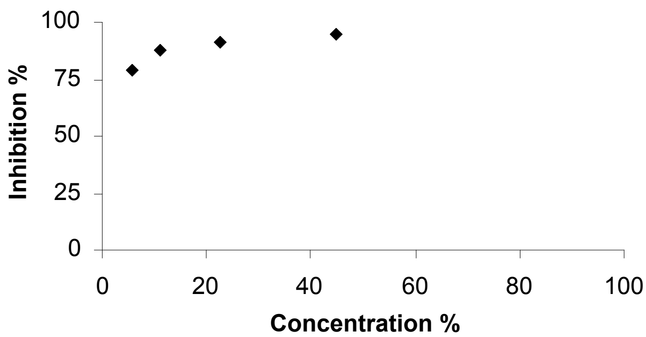


Figure 18. Toxicity measure of the influent sample. 100 mg 4CP/L

In contrast the toxicity of the effluent samples was no detected ($EC50_5$ = N.D). Figure 19 showed that an increase in the effluent concentration has no effect on the toxicity evaluation. The effluent sample caused some increase in luminescence after 5 min incubation time. According to Lappalainen *et al*., (2001) this is probably due to nutrients available for the bacteria in the effluent samples.

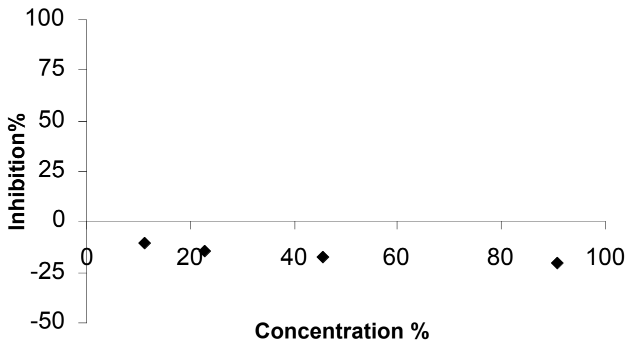


Figure 19. Toxicity measure of the effluent sample from the SBR reactor treating 200 mg 4-CP/L.

CASE II. NUTRIENTS REMOVAL

IV. METHODOLOGY

The conventional strategy under evaluation has been conducted before the implementation of CL, during two months, after a re-inoculation of Reactor M and allowing reaching its steady state which was practically completed in 3 weeks. Five standard conditions and three shock loads were evaluated by on line and off line measurements.

The CL was evaluated during three weeks by on line measurements. During the first week, the standard condition was evaluated by off line and on line measurements. During the second and third week, a shock load condition followed by a standard one was conducted (off line and on line measurements).

IV.1 Desirable effluent quality

The following table defines the desirable effluent quality:

Table 4. Set points for effluent quality

Parameter	Set point
COD (mg/l)	200
TKN (mg/l)	10
NOx (mg/l)	5

IV.2 CONVENTIONAL STRATEGY

Typical strategy employed for SBR operation during six months before control law implementation is described as following:

Table 5. Conventional strategy cycle phase duration

Stage	Duration
Anoxic Fill	~ 10 min.
Anoxic	~ 50 min.
Aerobic	6 hours
Settle	1 -2 hour
Draw	~ 10 min.
Idle	~ 2-3 hours 50 min.

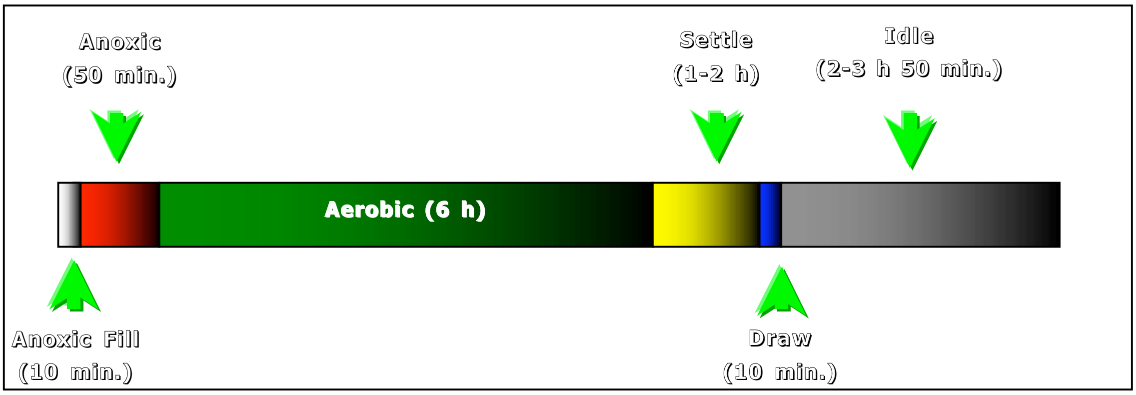


Figure 20. Conventional phase scheme

IV.3 OPTIMAL STRATEGY

The SBR operation under the CL, was characterized by the following work scheme (Table 6 and figures 2 and 3):

Table 6. CL cycle phase duration

Stage	Duration	
	Standard	**Shock Load**
Anoxic Fill	**~ 10 min.**	
1st Anoxic	**~ 20 min.**	**~ 5 min.**
1st Aerobic	**2 hours**	**2 hours 35 min.**
2nd Anoxic	**70 min.**	**2 hours 40 min.**
2nd Aerobic	**70 min.**	**1 hour 50 min.**
Settle	**2 hours**	
Draw	**~ 10 min.**	
Idle	**~ 5 hours**	**2.5 hours**

CL Standard operation:

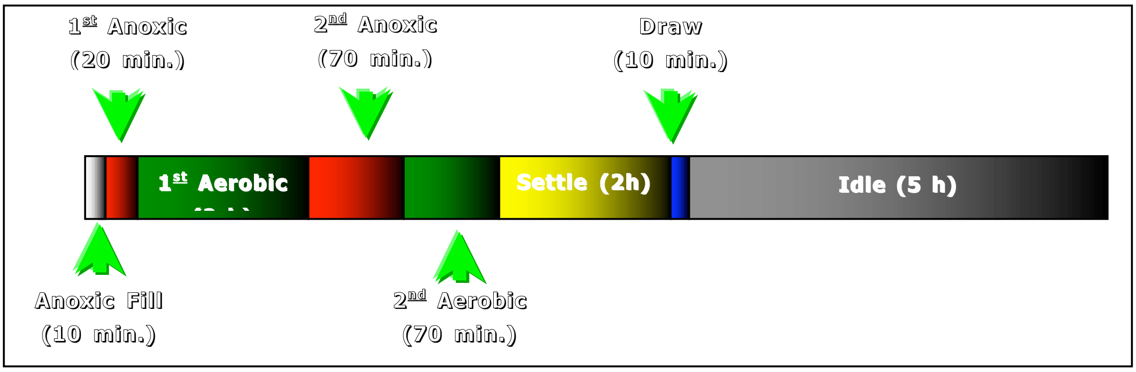


Figure 21. CL standard operation scheme

CL Shock Load operation:

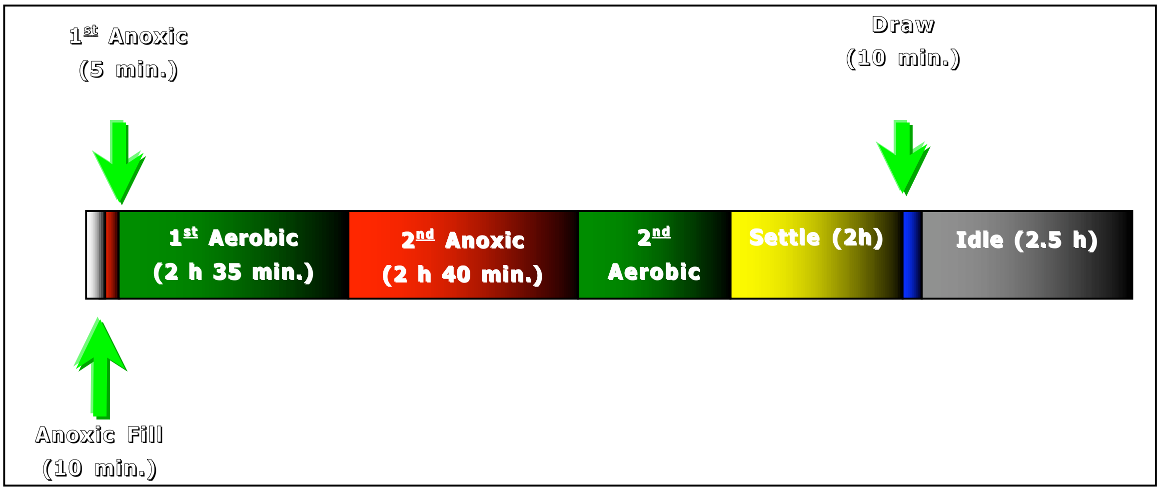


Figure 22. CL Shock load operation scheme

V. RESULTS

V.1 CONVENTIONAL STRATEGY

Reactor performance and effluent quality for standard conditions are resumed in the following tables (7 and 8):

Table 7. Conventional strategy standard condition performance

		Min.	Máx.	Average
Substrate Removal Efficiency (%)	COD	91.6	97.2	95.3
	TKN	86.8	92.6	89.1
	Total - N	92.9	96.8	94.9
Air Flow Rate (NLpm)		4.5		
Air consumption (NL)		1620		
Effluent Sludge Content (mgSSV/L)		[217 – 1136]		

Table 8. Average effluent quality for standard condition with conventional strategy

Parameter	Min.	Máx.	Average
COD (mg/l)	71	140	100
TKN (mgN/l)	4.7	6.6	6
Total – N (mgN/l)	4.7	13.2	7

As it is shown by the previous data, the SBR reactor presented good performance with this conventional strategy [average substrate removal efficiencies ≥ ~ 90% (COD, TKN and Total - N) and an acceptable effluent quality]. Effluent sludge content is high due to sludge bulking, frequently observed in Reactor M.

V.2 CONTROL LAW STRATEGY

The reactor performance and effluent quality are shown in tables 9 and 10.

Table 9. Global performance of the reactor under control law strategy.
Removal efficiency of 2 cycles is reported.

Substrate Removal Efficiency (%)	**COD**	**Standard Condition**	**91, 97**
		Shock Load Condition	**94 , 97**
	TKN	**Standard Condition**	**80, 95**
		Shock Load Condition	**71, 99**
	Total - N	**Standard Condition**	**91, 98**
		Shock Load Condition	**92, 99**
Air Flow Rate (NLpm)		**4.5 (idem for conventional strategy)**	
Air consumption (NL)		**Standard Condition**	**855**
		Shock Load Condition	**1193**
Effluent Sludge Content (mgSSV/L)		**~ 1000 (bulking phenomena)**	

Table 10. Average effluent quality with CL. Removal efficiency of 2 cycles is reported.

Parameter	**Standard**	**Shock load**
COD (mg/l)	**181, 71**	**207, 95**
TKN (mgN/l)	**12, 4**	**14, 2**
Total – N (mgN/l)	**12.3, 3.4**	**15.1, 2.4**

As it is shown by the previous data, the SBR reactor presented good performance under the CL strategy [average substrate removal efficiencies ≥ ~ 84% (COD, TKN and Total - N) and acceptable effluent quality], for both standard and shock load cases. Effluent quality under CL operation is comparable to the effluent quality under conventional operation.

As a result of control law implementation, cumulative air consumption, is appreciably reduced. Total cycle time duration for standard operation under CL (7 hours) is lower than conventional cycle (9 hours). Shock load cycle time under CL is similar to the conventional cycle duration

V.2.1 INDIVIDUAL CYCLE DATA ANALYSIS

Standard cycle

OD, COD, and NO_x (N-NO_2 + N-NO_3) concentration profiles of one standard cycle are shown in figures 23, 24 and 25 presented below :

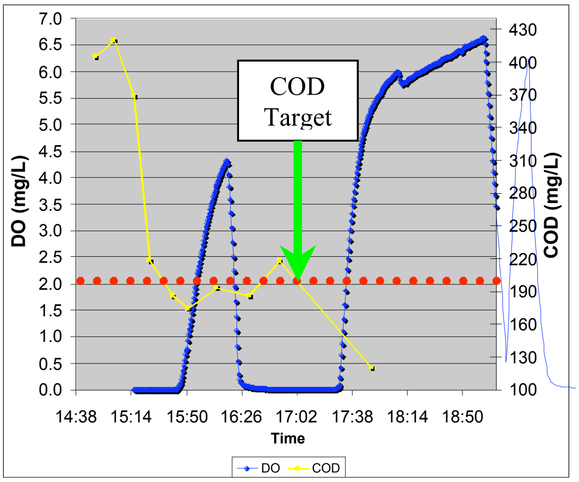


Figure 23. COD and DO profiles for standard CL cycle.

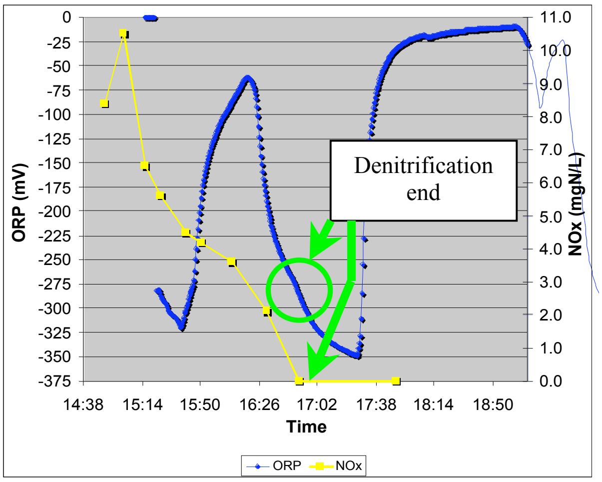


Figure 24.- ORP and NOx profiles for standard CL cycle

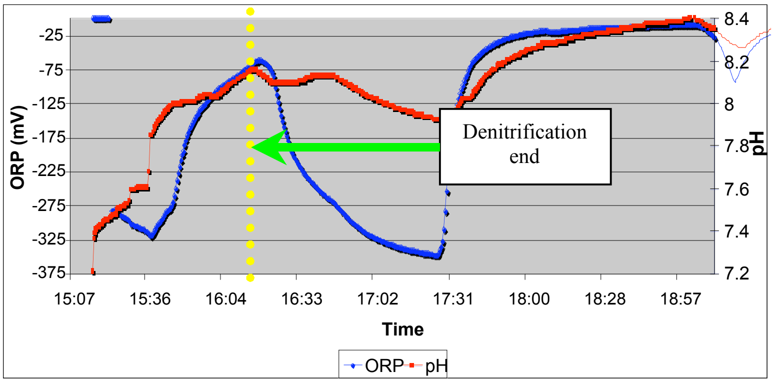


Figure 25.- ORP and pH profiles for standard CL cycle

Shock load cycle

OD, COD, and NO_x ($N\text{-}NO_2 + N\text{-}NO_3$) concentration profiles of one shock load cycle are shown in figures 26, 27 and 28 presented below:

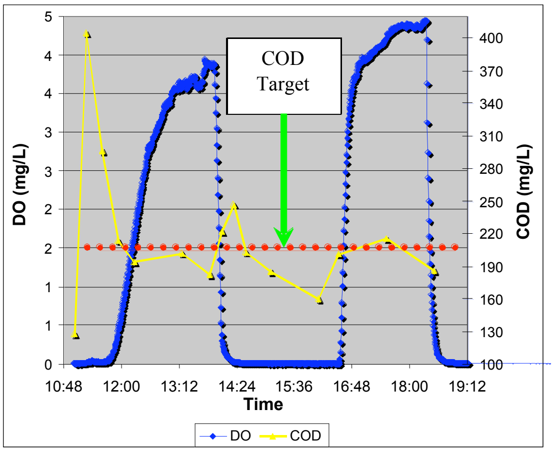


Figure 26.- COD and DO profiles for shock load CL cycle.

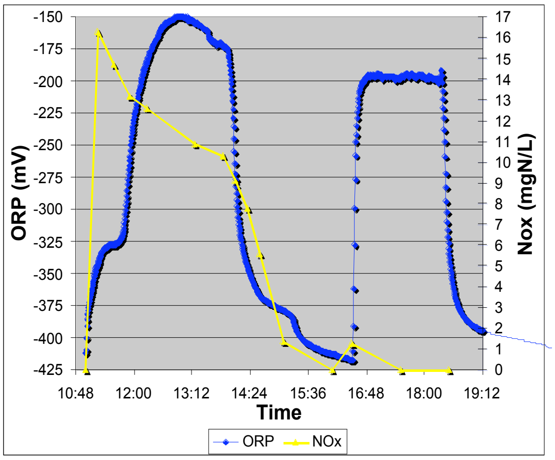


Figure 27.- ORP and NOx profiles for shock load CL cycle.

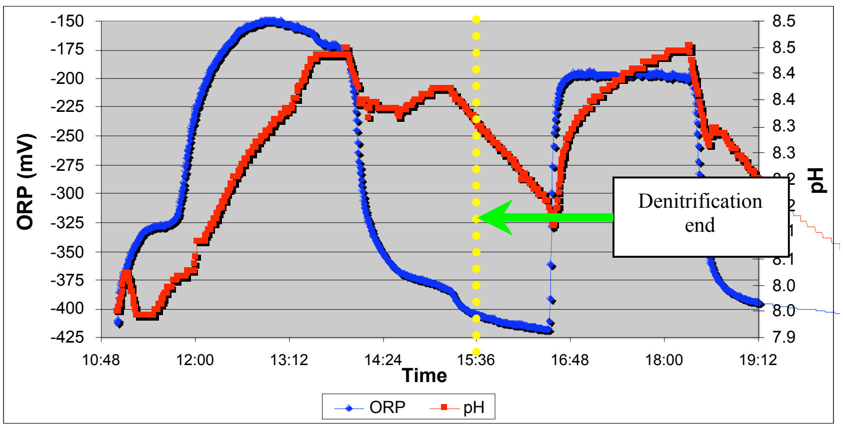


Figure 28.- ORP and pH for shock load CL cycle

VI. DISCUSSION

Final effluent quality is acceptable for both standard and shock load cycles under CL application. However, second anoxic phase duration in the reactor was excessive (around 30 minutes in standard cycle and 1 hours and 20 min extra time during shock load). This observation could be explained by some of the following effects:

- High $N\text{-}NH_4^+$ assimilation on biomass, and low nitrification extent in the reactor, neglected for the CL .
- Presence of $N\text{-}NO_x$ removal during aerobic stage (assimilative reduction, denitrification at not sufficiently inhibitory DO levels).

Regarding aerobic phase, parameter variation is detected within the first 60 minutes. Extra aeration time seems to be not necessary.

Influence of shock loads on the reactor stability

Conventional strategy No differences were appreciated in reactor operation at standard and shock loads conditions. Removal efficiencies (COD, TKN, etc.) and biomass activity remain stables at both reactor states.

Control strategy Standard cycle before and after 2 consecutive shock load cycles are compared. COD, total nitrogen and nitrite and nitrate evolution were compared and shown in the following figure 10. No signs of instability could be found: COD removal was even higher and nitrite consumption was faster after the shock load.

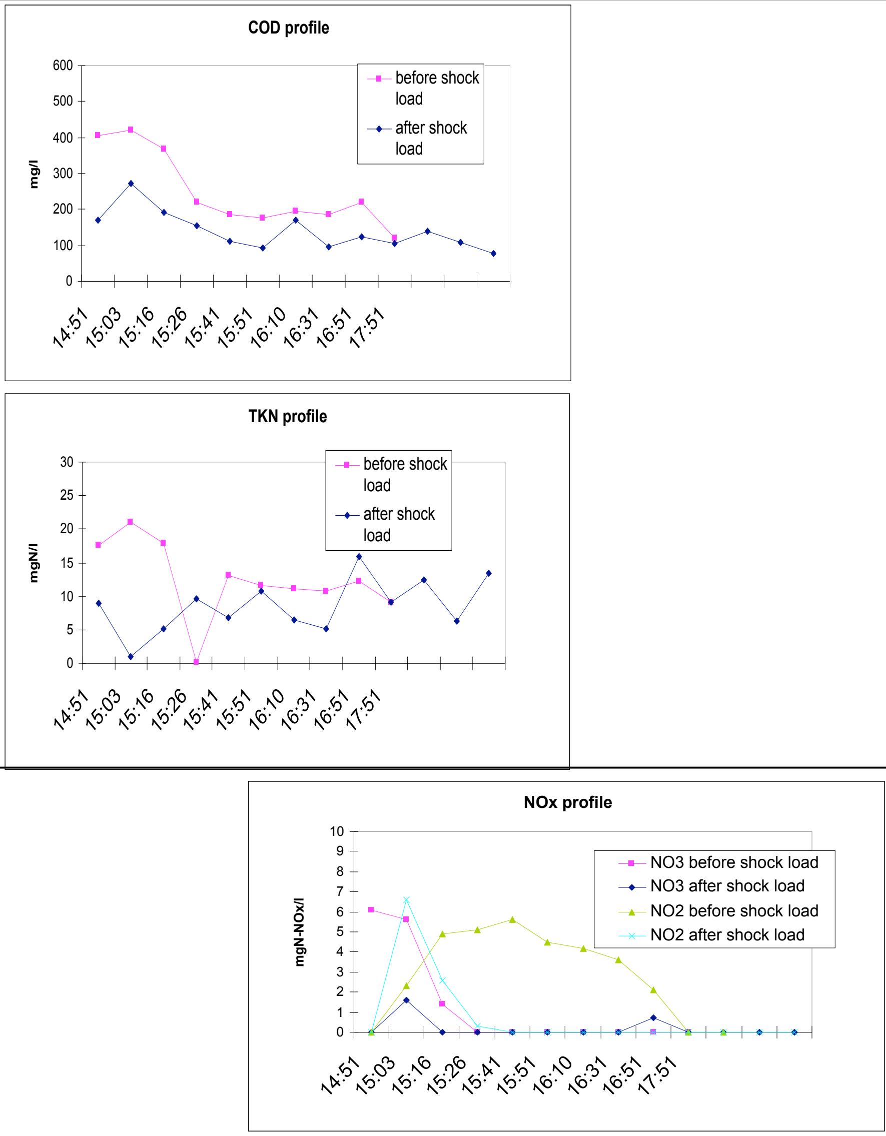


Figure 29.- COD, TKN and NOx profiles of standar cycles before and after a shock load.

After shock load condition, OD and ORP profiles a slow evolution could be detected, and profiles stabilized within 3 days.

VII. CONCLUSIONS

VII.1. CASE 1. Toxic wastewater

With the application of the ED-TOC strategy it was possible to degrade shock loads of 4CP concentrations and a mixture of municipal wastewater and phenol without any problem. A better performance of the reactor operated with the ED-TOC strategy was observed when it was compared to the response obtained with the reactor operated under the conventional strategy. It was shown that not only higher concentrations of toxic could be treated with the ED-TOC strategy, but also a reduction in degradation time (around 52%) and in the supplied air volume was obtained.

A good performance of the reactor operated with the ED-TOC strategy was obtained since the degradation and mineralization of the mixture with high load of the 4CP and phenol cases was efficiently completed. The ED-TOC strategy was able to manage increments of toxic concentrations in the influent up to 7000mg/L of phenolic compounds in the wastewater without any inhibition problem.

In theory the strategy could degrade any influent concentration of toxic wastewater without problems. The use of the ED-TOC strategy can minimize the volume of the reactors because it is not necessary to dilute high concentrated toxic wastewater as in the usual operation mode in order to avoid inhibition. The strategy can be applied for industrial wastewater containing high phenols concentrations or other inhibitory compound.

The reactor operated by ED-TOC strategy showed that is robust and stable in large periods of operation (100 days), with efficiencies of plus than 99% for the 4CP degradation, and 97% as COD degradation. The DGGE analysis showed that there are changes in the microbial population during the acclimation and during the stability in a long operation period by ED-TOC. The analyses of toxicity demonstrate that the bioreactor operated by ED-TOC eliminates the toxicity in the wastewater.

VII. 2.CASE 2. Nutrient removal

The application of the CL reduced the total cycle time from nine to seven hours for standard condition, and total air consumption is reduced in 50%, with comparable effluent quality. Shock load cycle duration is also shorted with respect to the conventional cycle, even when air consumption is similar. However, second anoxic and aerobic phase duration seems to be excessive. Nitrogen assimilation for biomass growth is the main mechanism of N reduction for this wastewater with a particularly high C/N ratio. A CL based in a model which specially consider this process could be necessary to optimize the cycle phase for this effluent.Reactor performance after a shock load didn’t show signs of instability as normal effluent quality and profiles were obtained.

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