10th International IFAC Symposium on
Computer Applications in Biotechnology
Preprints Vol.2, June 4-6, 2007, Cancún, Mexico

# ACCLIMATION MODEL OF AN AEROBIC BIOREACTOR FOR THE TREATMENT OF TOXIC WASTEWATER

**Fajith Martínez[1], Manuel J. Betancur[1*], Jaime A. Moreno[2], Germán Buitrón[3] and Iván Moreno-Andrade[3]**

*[1]A+D, Universidad Pontificia Bolivariana, Cq. 1#70-01, Of. 11-259 Medellín, Colombia, [*](+57)44159020 x 9584, manuel.betancur@upb.edu.co Coordinaciones de [2]Automatización y [3]Bioprocesos Ambientales, Instituto de Ingeniería, Universidad Nacional Autónoma de México, Cd. Universitaria, 04510 Coyoacán, México DF, Mexico.*

Abstract: The optimal automation of the acclimation process of a biorreactor, for treating toxicant wastewaters, may be possible if a sound mathematical model is developed in order to cope with the instrumentation limitations that exist nowadays. This work presents a first step in creating such a model. Experimental data was used to identify the changing kinetic parameters of the model as acclimation progresses. It was found that only one key parameter changed during the acclimation process. Then an acclimation model, i.e. a model of such a parameter progression in time, as a function of the total substrate treated, is proposed. 

Keywords: mathematical modeling, acclimation, bioreactor, wastewater treatment

## 1. INTRODUCTION

Many industrial processes in the chemical, pharmaceutical, plastic, and petrochemical industries generate wastewater (WW) containing organic toxic compounds. Due to its toxicity, biological treatment of WW containing a toxic substrate is difficult, since the microorganisms are initially not able to treat that substance. So to setup a WW treatment plant (WWTP), for biodegrading toxic substances, it is necessary to acclimate its biomass. In a favorable environment, when microorganisms are put in contact with toxic compounds, acclimation to these compounds may occur (Aelion et al., 1989). Different mechanisms have been described to explain such a process. Wiggings et al. (1987) suggested that there is a selection and a multiplication of specialized microorganisms during acclimation and that physiological transformations occur in the metabolic system of the microorganisms, i.e. alterations at the enzymatic level, regulation and production, mutations, etc. In aerobic microbial communities, the acclimation periods range from several hours to several days (Wiggings et al., 1987).

In an aerobic sequencing batch reactor (SBR), once acclimated, an appropriate control strategy (Moreno-Andrade et al., 2006; Betancur et al., 2006) can avoid the negative impact of the inhibition caused by the excess of the toxic substrate concentration *(S)*, maintain a high sludge activity, and avoid the negative effects of deacclimation. However, the initial acclimation, necessary at the set up of the SBR, or after a failure of the system, still has to be manually controlled. The automation of such an acclimation process, using a controller similar to that suitable for normal operation, would make it more reliable and would allow the full optimization of WWTPs. Hence a model of the acclimation process is necessary. Buitrón and Moreno (2004) proposed such a model for a SBR treating 4-chlorophenol (4CP). They assumed that the acclimation effect is

reflected in the change of the values of the kinetic parameters of the Haldane law that describes the specific biomass growth rate *(μ)*, and their model identification relied on *S* kinetics measurements. In the present work, under similar conditions, the approach to identify the model is modified by using, on top of the *S* kinetics, the dissolved oxygen concentration (DO) measurements for the determination of the kinetic parameters of $\mu$ during the acclimation. Since such parameters are badly identifiable with batch experiments (Holmberg, 1982; Buitrón and Moreno, 2004) from the *S* measurements alone, the introduction of DO measurements increases identificability. This is explained because more and qualitatively better samples of DO are available than samples of *S*, since DO is measured online, whereas *S* is determined offline, making it more prone to experimental errors.

For the final objective of automating the acclimation phase in a WWTP, it is required for the controller to be able to determine the acclimation state of the biomass using only online measurements, and thus the DO is one suitable candidate because of its availability. The present work, by including the DO in the model identification process, is a first step forward towards that final objective.

Section 2 introduces the mathematical model for $\mu$. Section 3 describes the methodology. Section 4 describes the experiments performed. Section 5 describes the identification of the parameters of $\mu$ for any given single batch. Section 6 proposes an acclimation model using the group of such individual results. Finally some conclusions are presented.

## 2. BIOREACTION MATHEMATICAL MODEL

A description of the reaction model used can be found in Betancur et al. (2006). A key element in that reaction model is $\mu$, which greatly influences both *S* and DO kinetics. All other model parameters, with the exception of $\mu$, are assumed to be invariant during the acclimation process. Hence understanding how $\mu$ evolves as a function of the history of exposure of the biomass to the toxic substance seems to be the key to modeling the acclimation process.

The typical function used for $\mu$ is the Haldane model. It may be used to express the dependence of the biomass growth on *S* when the oxygen is not a limiting reactant, i.e. when there is plenty of DO present, which is a normal operation condition.

$$\mu(S) = \frac{\mu_0 S}{K_S + S + S^2 / K_I} \qquad (1)$$

where $K_I$ is the inhibition constant (mg 4CP/l), $K_S$ is the Michaelis–Menten saturation constant (mg 4CP/l), and $\mu_0$ is the apparent maximum "non-inhibited" specific biomass growth rate ($h^{-1}$).

An alternate parameterization of the Haldane model was used. Such proposed parameters do have a clear physical meaning (see figure 1) and are easily calculated as follows:

$$S^* = \sqrt{K_I K_S} \qquad (2)$$

is the Substrate concentration (mg4CP/l) at which the biomass growth rate ($h^{-1}$) reaches its maximum value

$$\mu^* = \frac{\mu_0}{1 + 2K_S / S^*} \qquad (3),$$

and the Substrate concentration for Half Inhibition is

$$S_m : \frac{K_I + 4S^* + \sqrt{K_I^2 + 8K_I S^* + 12S^{*2}}}{2} \qquad (4).$$

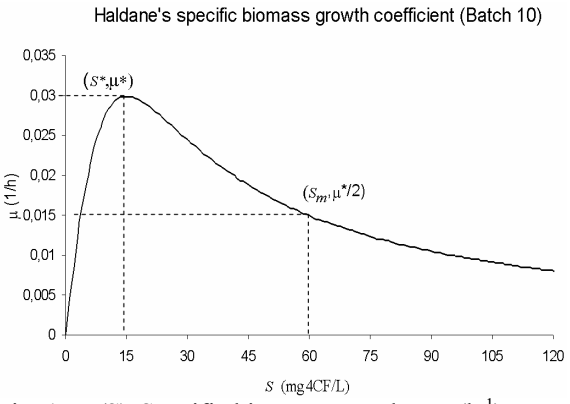


Fig. 1. *μ(S)*: Specific biomass growth rate ($h^{-1}$)

Assuming that the biomass decay is negligible, and that neither biomass nor oxygen are fed via the influent, leads to the mass balance based model for the filling and reaction phases of the SBR:

$$\dot{X} = \mu(S)X - XQ/V \qquad (5.1)$$

$$\dot{S} = -k_1 \mu(S)X + (S_i - S)Q/V \qquad (5.2)$$

$$\dot{O} = -(k_2 \mu(S) + b)X + k_L a(O_{sat} - O) - OQ/V \qquad (5.3)$$

$$\dot{V} = Q \qquad (5.4)$$

where

$0 < Q < Q_{max}$ : Inflow volumetric flow (l/h)
$V_0 \le V \le Vf$ : Volume level of the liquid in the tank (l)
$S$ : Toxic concentration in the tank (mg 4CP/l)
$S_i$ : Toxic concentration in the influent (mg 4CP/l)
$O$: Dissolved oxygen concentration (mg $O_2$/l)
$O_{sat}$ : Saturation value of the dissolved oxygen concentration in the liquid medium (mg $O_2$/l)
$k_L$a : Oxygen mass transfer coefficient ($h^{-1}$)
$b > 0$ : Endogenous respiration coefficient ($h^{-1}$)

## 3. METHODOLOGY

An aerobic automated SBR system with a capacity of 7 L and an exchange volume of 57% (4L) was used.

The airflow rate was 1.5 L/min and the temperature was maintained at 20°C inside the reactor. The reactor was inoculated with microorganisms coming from a municipal activated sludge WWTP. The biomass concentration was set to 2,000 mgVSS/L. A synthetic WW containing 4CP, as a toxic model compound, was used as the sole source of carbon and energy. Nutrients such as nitrogen, phosphorus, and oligoelements were added following the techniques recommended by AFNOR (1985). The SBR was operated under the following strategy: preaeration time (15 min), filling time (5 min), reaction time (variable depending on the necessary time to reach 99% of removal efficiency of 4CP), settling time (12–30 min) and draw time (1 min). Degradation time was determined using the dissolved oxygen (DO) concentration present in the reactor (Buitrón et al., 2003).

The substrate concentration in the tank, *S,* was measured manually taking samples and processing them offline using the colorimetric technique of 4-aminoantipyrine (Standard Methods, 1992). Total and volatile suspended solids analyses were determined according to the Standard Methods (1992). Dissolved organic carbon was determined with a Shimadzu TOC-5050 and chemical oxygen demand according to Standard Methods (1992). These analyses were performed to evaluate 4CP mineralization.

## 4. ACCLIMATION EXPERIMENTS

Two sets of acclimation experiments were made, using two different initial concentrations of 4CP ($S_0$=50 and 100 mg4CP/l, were $S_0$ is the value of $S$ just after filling the tank). Each set was initiated with fresh non-acclimated sludge inoculum. In both cases enough successive reaction batches were performed until the reaction-time stabilized, thus indicating that the biomass was acclimated to the respective affluent toxic concentration. Such an acclimated state was reached after 10 batches in both of the cases. For some of the batches $S$ kinetics were monitored, offline, and the DO *(O)* kinetics were registered, online. The other batches in between were performed but not monitored.

Figure 2 shows the evolution of DO kinetics as the biomass gets acclimated to $S_0$=100 mg4CP/l. The final of each reaction coincides with the time of the raising of the DO curve after its last valley. Note that the final DO value is close to saturation. It depends on temperature, atmospheric pressure and endogenous respiration. However, the final DO value itself is not relevant, but the fact that it reaches a plateau after a rather abrupt change is. Such a final increase of the DO is related to the diminution in oxygen consumption, by the biomass, as $S$ approaches zero i.e. once the toxic removal is almost completed. Note such a reaction-time decreases from

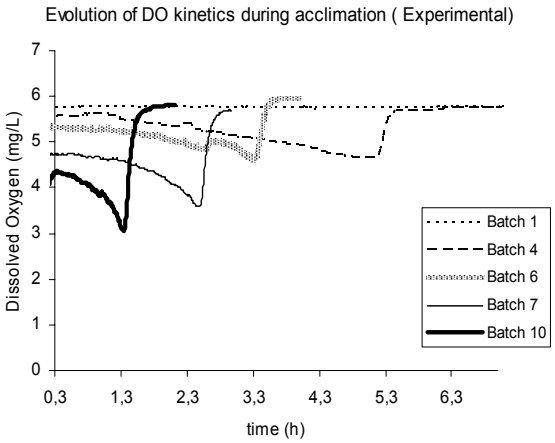


Fig. 2. Dissolved Oxygen experimental kinetics evolution, for the selected monitored batches, during acclimation to $S_0$=100 mg4CP/L.

one batch to the next, indicating that the biomass acclimation progresses. This behavior leads to the motivation for modeling, trough the evolution of the parameters of $\mu$, the acclimation state as a function of the DO kinetics.

## 5. KINETIC PARAMETERS IDENTIFICATION

The kinetic parameters were identified for those batches in which the kinetics of $S$ was monitored. The working hypothesis is that $\mu$ changes during the successive batch reactions needed to complete the acclimation process but, for the duration of each single reaction, it is assumed to be constant.

Figure 3 shows, for one single given batch, the typical fitting difference of two identification procedures, for model in (5): one that identifies the parameters using both $S$ and DO experimental data, and the other identified using only $S$ data. Note that, although a better fit of $S$ dynamics was possible when letting out the DO data then, as expected, the DO kinetics adjust is poorer. As the final objective of the model is to allow for the design of an automatic acclimation controller, based on DO online measurements, including the DO data for identification makes sense.

Figure 4 depicts the identification results, for the 3 parameters of $\mu$, for the two acclimation runs. Batch number 10, for both cases, was considered as the first batch in which the acclimation process was practically completed. Figure 4a) shows $\mu^*$ evolution as acclimation progresses. Note that its final value is different for both runs. Instead, the values of $S^*$ and $S_m$, in Figures 4b) and 4c) respectively, are almost the same, and their variations are comparatively small. This result is very interesting as it suggest that the shape of the model of $\mu(S)$ may remain unchanged during the acclimation process, and that the only thing that evolves is its scale, i.e. its amplitude changes proportionally to changes in $\mu^*$.

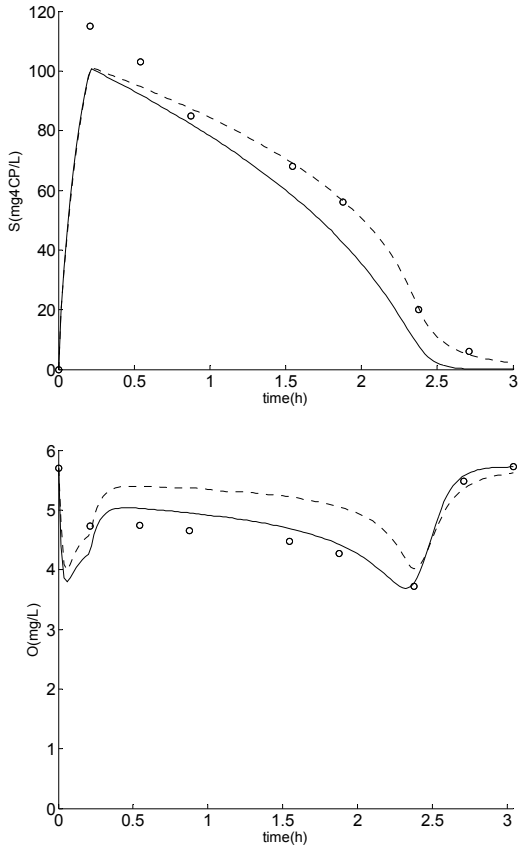


Fig. 3. Model simulation of filling and reaction, for batch 7 ($S_0$=100 mg4CP/L)
a) Dotted line: Model identified using *S* only $S^*$=23.9867,$S_m$=48.1241, $\mu^*$=0.0172
b) Continuous line: Model using both *S* and DO $S^*$=15.0660,$S_m$=60.2778, $\mu^*$=0.0124
c) Circles: Experimental data

## 6. ACCLIMATION MODEL

A sigmoid type model is proposed for the evolution of $\mu^*$ during acclimation (fig. 5), assuming that all other parameters in the bioreactor model itself are invariant in time:

$$\mu^* = \left(\mu^*_{\max} - \mu^*_{\min}\right)\frac{\left(\frac{W}{K_w S_i}\right)^n}{1+\left(\frac{W}{K_w S_i}\right)^n} + \mu^*_{\min} \qquad (6)$$

where

| | |
|---|---|
| $\mu^*$ | Maximum Value of $\mu$ *(S)* during a single batch |
| $\mu^*_{\min}$ | Minimum Value of $\mu^*$ i.e. before acclimating |
| $\mu^*_{\max}$ | Maximum Value of $\mu^*$ i.e. once acclimated |
| $S_i$ | Toxic substrate concentration in the affluent |
| $W$ | Total mass of processed substrate (mg4CP) i.e. since the inoculation of the biomass to the bioreactor |
| $K_w$ | Half acclimation constant |
| $n$ | Exponent constant |

The total affluent volume fed to the reactor in each batch is the exchange volume *($V_e = V_f - V_0 = 4$L)* i.e. the difference between the final and initial volume. Then the total treated affluent mass, *W,* is easily computed by multiplying the number of batches performed times the input (affluent) substrate concentration *($S_i$)* and the exchange volume *($V_e$)*.

The identified parameters for the $S_0$=100mg4CP/L case are $n$=6.56 and $K_W$=27.63. The model, and its generating data, is shown in figure 5a). The validation of such a model was made using the results of acclimation to $S_0$=50mg4CP/l (figure 5b).

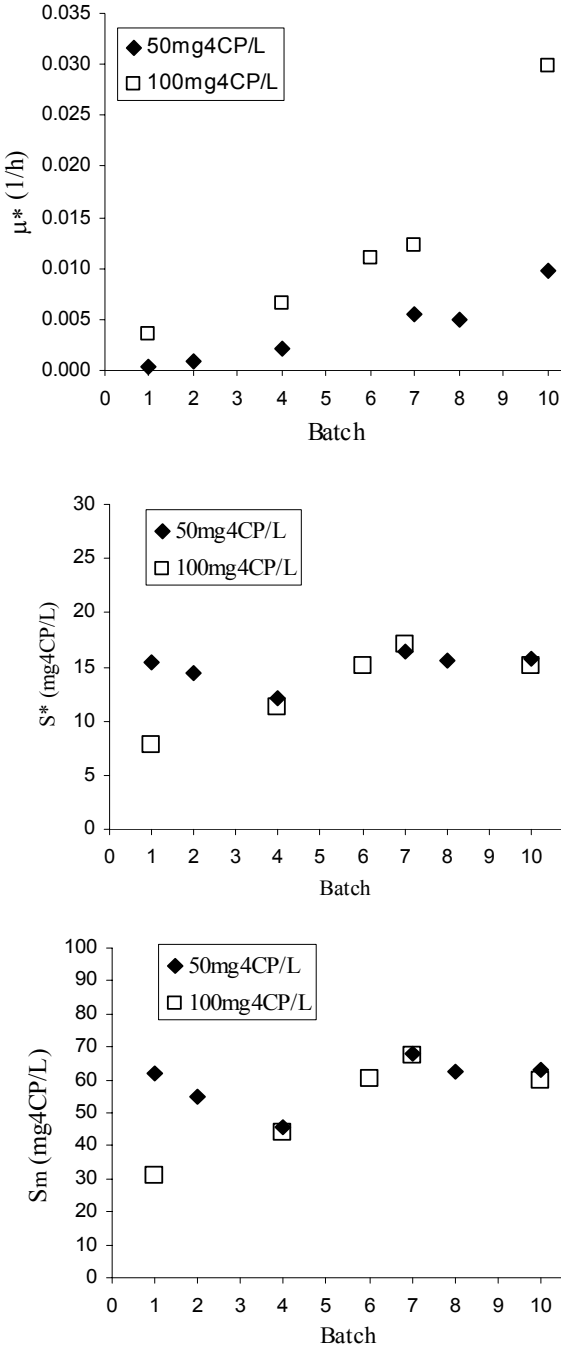


Fig. 4. Identification for acclimation to $S_0$=50 and 100mg4CP/L of a) $\mu^*$, b) *S**, and c) *Sm.*

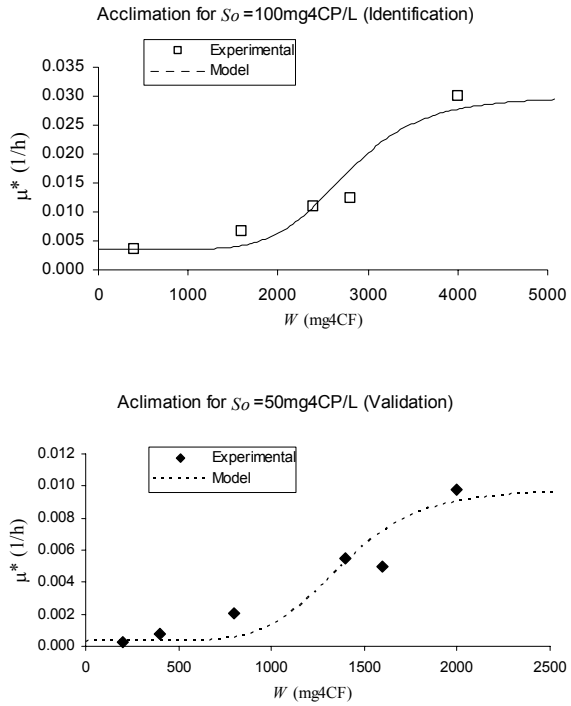


Fig. 5. $\mu^*$ acclimation model, as a function of processed substrate *(W)* for
a) Identification, using $S_0$=100mg4CP/l
b) Validation, using $S_0$=50mg4CP/l

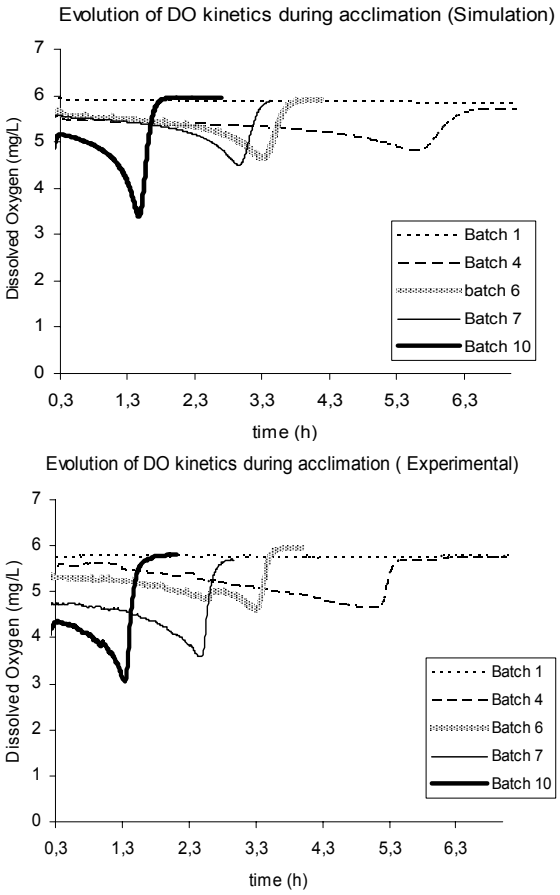


Fig. 6. Dissolved Oxygen kinetics evolution, for the batches in acclimation to $S_0$=100 mg4CP/L.
a) Simulated reaction phase results, not including the filling phase, using the acclimation model for $\mu^*$ (see figure 5).
b) Copy of figure 2 (for easy comparison)

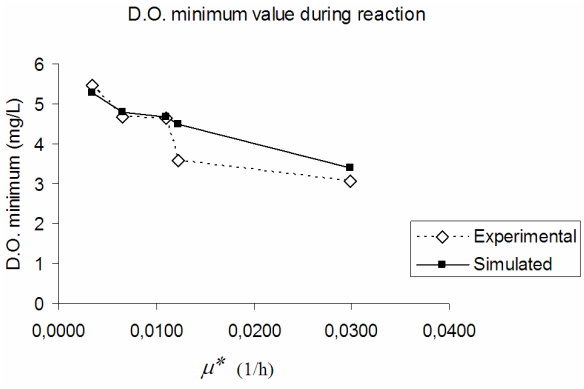


Fig. 7. Minimum value of D.O. of each selected batch reaction as a function of $\mu^*$, i.e. the acclimation state (comparison of experimental vs. simulated results during acclimation to $S_0$=100 mg4CP/L).

Using the model identified for the acclimation of $\mu*$ (figure 5), all experiments in figure 2 were simulated. Results are shown in figure 6a. Figure 2 is repeated as figure 6b to allow for easy comparison of simulation versus experimental results. It can be seen that the simulation reflects accurately the shape, the timing, and, particularly (see figure 7), the minimum values of DO during the experimental results.

## 7. CONCLUSIONS

A model to explain the evolution of the specific biomass growth rate *(μ),* during acclimation of an aerobic biomass to an inhibitory toxic substrate, was presented. The method used for identification includes the use of the dissolved oxygen concentration (DO) experimental data. Such a method yields a fit less optimum for the substrate concentration data, but allows a better fit for the DO kinetics. This model is a first step, setting the basis for a future work, towards the final objective of linking the DO online measurements to the acclimation status of the bioreactor, in order to develop a control law able to automate the acclimation process.

A Haldane type model for $\mu(S)$ was estimated using 3 alternate parameters with clear physical meaning: $\mu$ maximum value $(\mu^*)$, the substrate concentration at which such a maximum occurs $(S^*)$, and the substrate concentration $(S_m)$ at which inhibition reduces $\mu$ value to half its maximum.

Identification results show that, for the objectives at hand, $S^*$ and $S_m$ exhibit little sensitivity to the acclimation process. On the other hand, $\mu^*$ evolves, from an initial minimum to an acclimated state maximum, in a way that may be approximated by a sigmoid function of the total treated substrate mass.

The acclimation model was identified using one set of acclimation data, and validated with another acclimation run for a different toxic concentration. However, it is still necessary to generate, in a future work, sufficient acclimation experiments to fully validate the proposed model.

## ACKNOWLEDGMENT

The CIDI-UPB is gratefully acknowledged. This paper includes results of the EOLI project that is supported by the INCO program of the European Community, Contract number ICA4-CT-2002-10012

The scientific responsibility rests with the authors.

## REFERENCES

Aelion, C.M., Dobbins, D.C. and Pfaender, F.K. (1989). Adaptation of aquifer microbial communities to the biodegradation of xenobiotic compounds: influence substrate concentration and preexposure. Environ. *Toxicol. Chem.*, **8**, 75–86.

AFNOR (1985). Evaluation en mileu aqueux de la biodegradabilité aérobie "ultime" des produits organiques solubles, *Normalisation française,* NFT 90-312.

APHA (1992). American Public Health Association/ American Water Works Association/ Water Environment Federation, Washington DC, *Standard Methods for the Examination of Water and Wastewater* 18th ed., USA.

Betancur, M.J., Moreno, J.A., Moreno-Andrade, I. and Buitrón, G. (2006). Practical Optimal Control of Fed-Batch Bioreactors for the Waste Water Treatment. *International Journal of Robust and Nonlinear Control*, **16**, 173-190.

Buitrón G. and Moreno J. A. (2004). Modeling of the acclimation/deacclimation process of a mixed culture degrading 4-chlorophenol. *Water Science and Technology*, **49**(1), 79-86.

Buitrón, G., Schoeb, M.-E. and Moreno, J. (2003). Automated sequencing batch bioreactor under extreme peaks of 4-chlorophenol. *Water Sci. Technol.*, **47**(10), 175–181.

Holmberg, A. (1982). On the practical identifiability of microbial growth models incorporating Michaelis–Menten type nonlinearities. *Math. Biosci.*, **62**, 23–42.

Moreno-Andrade, I. and Buitrón, G. (2003). Influence of the initial substrate to microorganisms concentration ratio on the methanogenic inhibition test. *Water Sci. Technol.*, **48**(6), 17–22.

Moreno-Andrade I. and Buitrón G. (2004). Variation of the microbial activity during the acclimation phase of a SBR system degrading 4-chlorophenol. *Water Science and Technology*, **50**(10), 251-258

Moreno-Andrade, I., Buitrón, G., Betancur, M.J. and Moreno, J.A. (2006). Optimal degradation of inhibitory wastewaters in a fed-batch bioreactor. *Journal of Chemical Technology and Biotechnology*, **81**(4), 713-720.

Wiggings, B.A., Jones, S.H. and Alexander, M.A. (1987). Explanations for the acclimation period preceding the mineralization of organic chemicals in aquatic environments. *App. Environ. Microbiol.*, **53**, 791–796.