EOLI
Efficient Operation of Urban Wastewater Treatment Plants

Work Package 1
Third year Mid Term Report

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WP1 - UNAM - Third year mid-term report

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Introduction

This report presents the activities of the partner 6 (UNAM) concerning the WP1 of the EOLI project, realized during the period November 2004- April 2005. The experiments conducted could be divided in two sets.

- The exploratory experiments in order to investigate the behavior of the optimal strategy with the degradation of a mixture of municipal wastewater and phenol. These exploratory experiments will be used to anticipate the behavior of the industrial pilot plant of $1m^3$, which will be started up the next month.
- The experiments for the evaluation of the long-term stability of the system by the application of the ED-TOC strategy will be started.

Experiments conducted

Experiment 1. Exploratory experiments. Degradation of a mixture of municipal wastewater and phenol by ED-TOC strategy

- 1.1. Experiments with FTC strategy
  - 1.1.1. Standard condition degradation by FTC strategy
- 1.2. Experiments with ED-TOC strategy
  - 1.2.1. Degradation of Standard condition by ED-TOC strategy (385 mg/L)
  - 1.2.2. Degradation of 723 mg/L in the mixture by ED-TOC strategy
  - 1.2.3. Degradation of 1596 mg/L in the mixture by ED-TOC strategy
  - 1.2.4. Degradation of 3058 mg/L in the mixture by ED-TOC strategy
  - 1.2.5. Degradation of 7000 mg/L in the mixture by ED-TOC strategy

Experiment 2. Experiments for the evaluation of the long-term stability of the system by the application of the ED-TOC strategy

- 2.1. Acclimation
  - 2.1.1 Structure community analysis by DGGE
- 2.2. EDTOC strategy operation under standard conditions

Methodology

Experiment 1. Exploratory experiments

The reactor was operated with a synthetic toxic wastewater prepared with municipal wastewater and phenol (TWW). For the standard condition, 350 mg/L of phenol were added to the wastewater. For all the cases, wastewater was colleted from a wastewater treatment plant of the University campus. The reactor was operated under the FTC strategy. The performance of the ED-TOC strategy was studied applying different phenol concentrations to the municipal wastewater (385, 723, 1596, 3058 and 7000 mg/L of phenol). Kinetics of the TWW degradation was followed. Total phenols, COD, VSS, TSS, SVI, DO, pH and T were monitored.

Activated sludge from the aeration tank of the UNAM WWTP was used as inoculum for the acclimation. For all the cases, VSS was measured and the necessary quantity was retired in order to obtain 250 mgVSS/L. This quantity was maintained in order to avoid aeration problems due to the high oxygen uptake as discussed in the previous EOLI report (WP1 UNAM, 2004).

Experiment 2. Long-term stability experiments
2.1. Acclimation

The reactor was inoculated with activated sludge from a municipal wastewater treatment plant containing 2000 mgVSS/L. The biomass was acclimated using a variable cycle strategy, i.e., the reaction phase duration was variable and stopped when the removal of 4CP was equal or greater than 95%. Initial concentration was maintained at 350 mg4CP/L in the influent during all the acclimation time. Due to the dilution (exchange volume of 4L from a total volume of 7L, i.e. 4/7, the resulting initial concentration in the tank was 200 mg/L.

2.1.1 **Changes in the community by the acclimation**

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, in order to determine the diversity and dynamics of the microbial community on the activated sludge. PCR, DGGE, cloning and sequencing techniques were used. Total DNA was extracted from 2 mL samples of activated sludge using an Ultra clean^{TM} 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 is being processed at this moment. A search of GenBank database will be conducted using the BLAST software (National Center for Biotechnology Information).

Experimental results

Experiment 1. Exploratory experiments. Degradation of the toxic wastewater (TWW) with the ED-TOC strategy

Exploratory experiments were carried out in order to investigate the degradation of the TWW using the ED-TOC strategy. These exploratory experiments will be used to anticipate the behavior of the industrial pilot plant of 1 m^3.

1.1 Experiments with FTC strategy

1.1.1 Degradation of the toxic wastewater (TWW) with FTC strategy Standard condition

Once the biomass was acclimated to the TWW, the degradation time took around 2.5 h, for a total cycle time of 4 h. Figure 1 presents the behavior of the TWW degradation during a standard cycle using the FTC strategy. Substrate concentration, measured as total phenol concentration, COD and DO were followed.

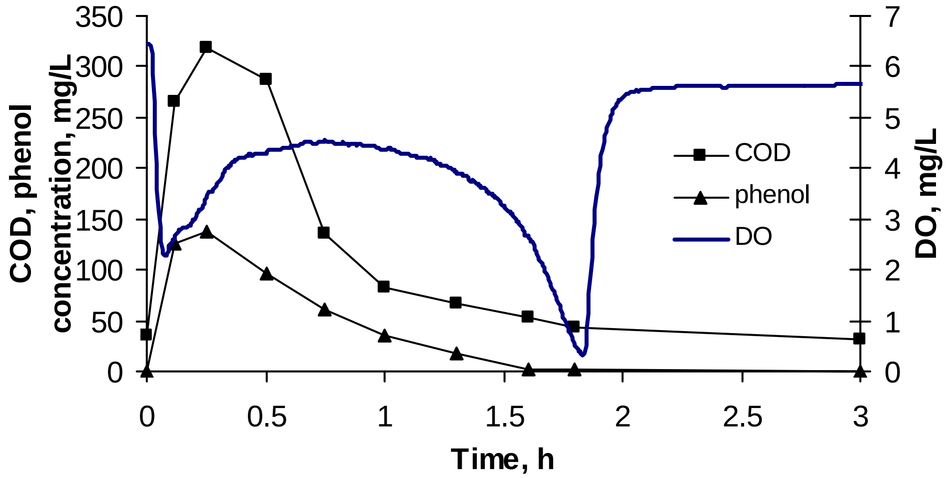

Figure 2. *Degradation kinetics of the TWW under the standard conditions with the FTC strategy.*

1.2 **Experiments with ED-TOC strategy**

The application of the ED-TOC strategy showed that it is possible to degrade the TWW containing a mixture of municipal wastewater and phenol. The TWW presented a total COD of 1227. 2087, 4350, 8017 and 14470 for the five initial phenol concentrations. Note that the increase in COD is due to the increase of phenol. The municipal wastewater presented 310 ± 36 mgCOD/L . The reaction time took around 2 h when the phenol concentration was 350 mg/L in the influent. It was found that it was possible the degradation of shock loads of TWW as high as 7000 mg/L of phenol without any inhibition problems. The removal efficiencies were higher than 98% as COD and 100% as phenol. This contrast with the results obtained by Yoong *et al.,* (2000) who reported that the degradation of phenol concentrations as high as 1300 mg/L, using SBR, caused problems of inhibition and a total loss of the activity of the microbial consortia.

Figures 2 and 3 show the operation data for the period of use of the ED-TOC strategy in the degradation of the TWW. It is possible to note that the reactor operates in a stable form during all the operated under ED-TOC strategy period. In the figure 2 are presented the volatile suspended solids (VSS) in the reactor, the VSS in the wastewater (SSV WW), the Total suspended solids (TSS) in the effluent, a sludge volumetric index (SVI). Figure 3 presents the phenol concentration in the influent (phenol-in), the COD in the influent in the TWW (COD-in TWW), COD in the municipal wastewater (COD-in WW) and phenol concentration in the efluent (phenol-ef).

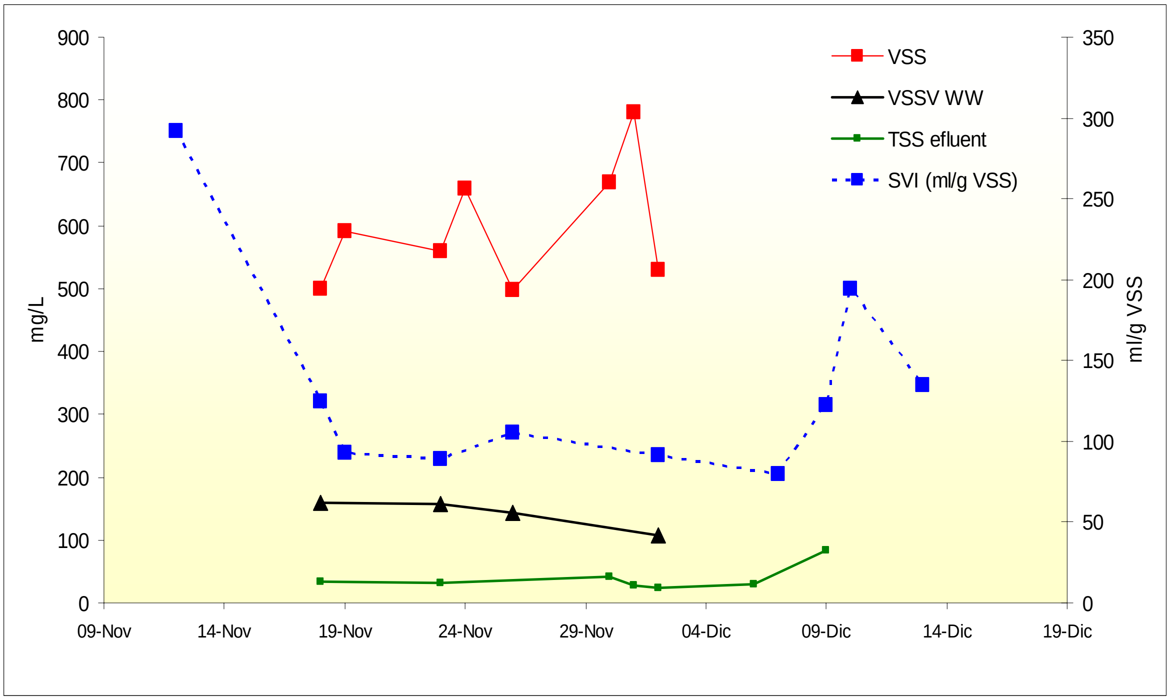

Figure 3. *Operational data for the period of use of the ED-TOC strategy in the degradation of the TWW*

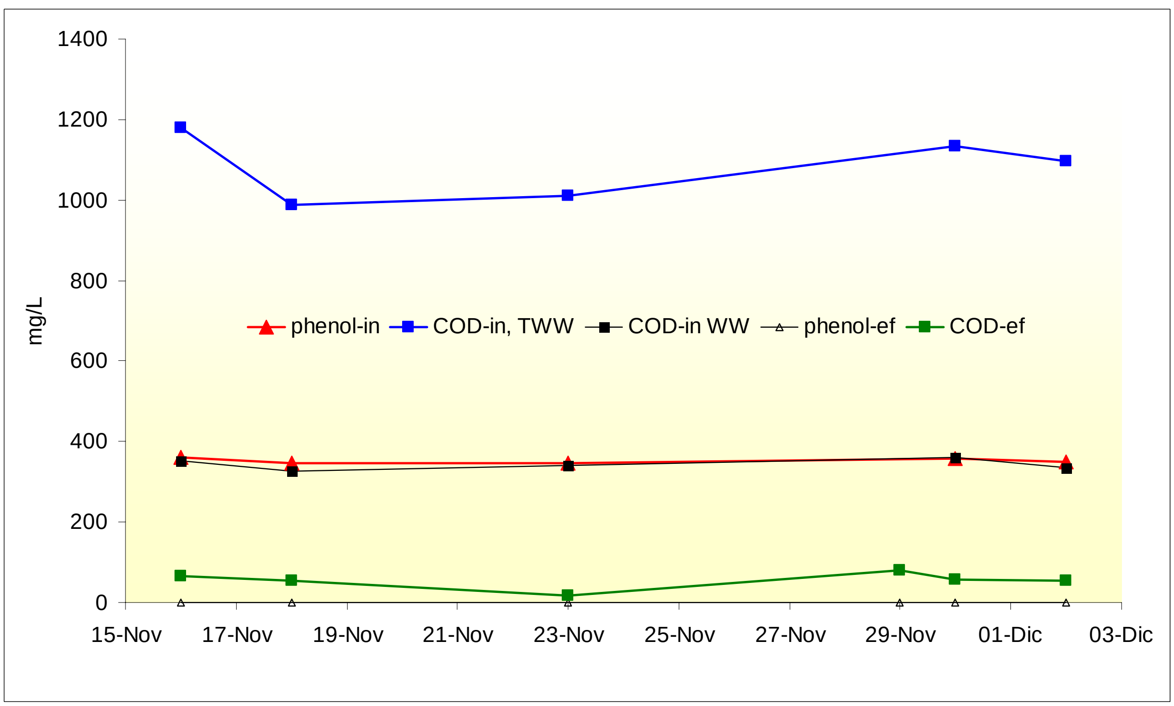

Figure 4. *Operational data for the period of use of the ED-TOC strategy in the degradation of the TWW*

Figure 4 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). When the substrate is filled the DO decreases (Figure 4, point 1) until the substrate concentration is higher than S* and an inhibition starts; then, the DO increases (point 2), 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 phenol is near its maximum. Once the substrate is degraded, the DO starts to increase again (point 3) and the filling pump is turned on. This 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 phenol concentration in the bulk of the liquid in the reactor was around S* and was never higher than 120 mg/L during the whole reaction. 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.

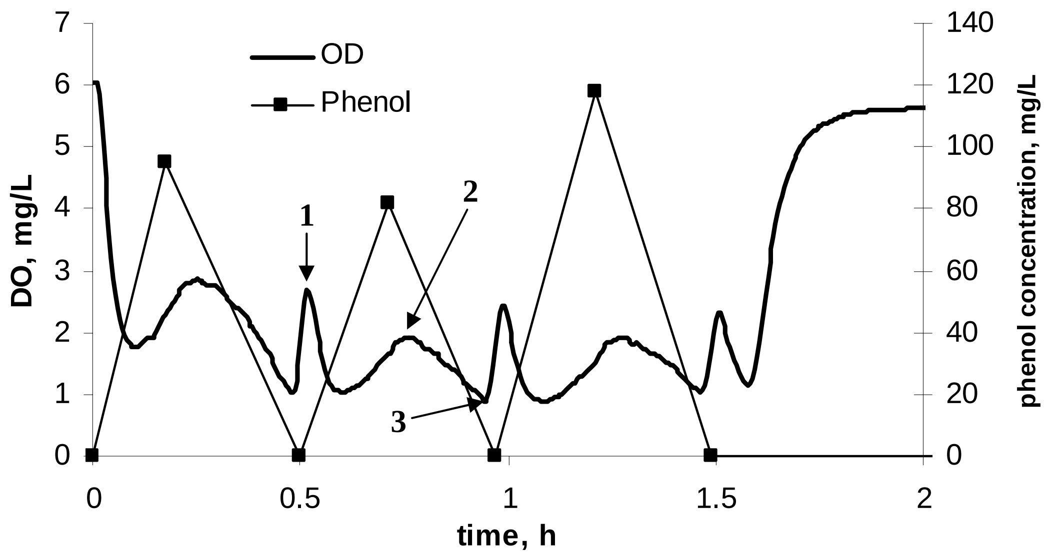

Figure 4. *Degradation kinetics of a mixture of municipal wastewater and 350 mg/L of phenol (standard condition) using the EDTOC strategy. Phenol concentration 350 mg/L; CODof the TWW: 1227 mg/L, COD for the municipal wastewater alone: 320 mg/L;*

Figure 5 shows the kinetic degradation of a shock load for degradation of 723, 1596, 3058 and 7000 mg of phenol in the TWW. It is possible to observe the same behavior in the control strategy as in the figure 4.

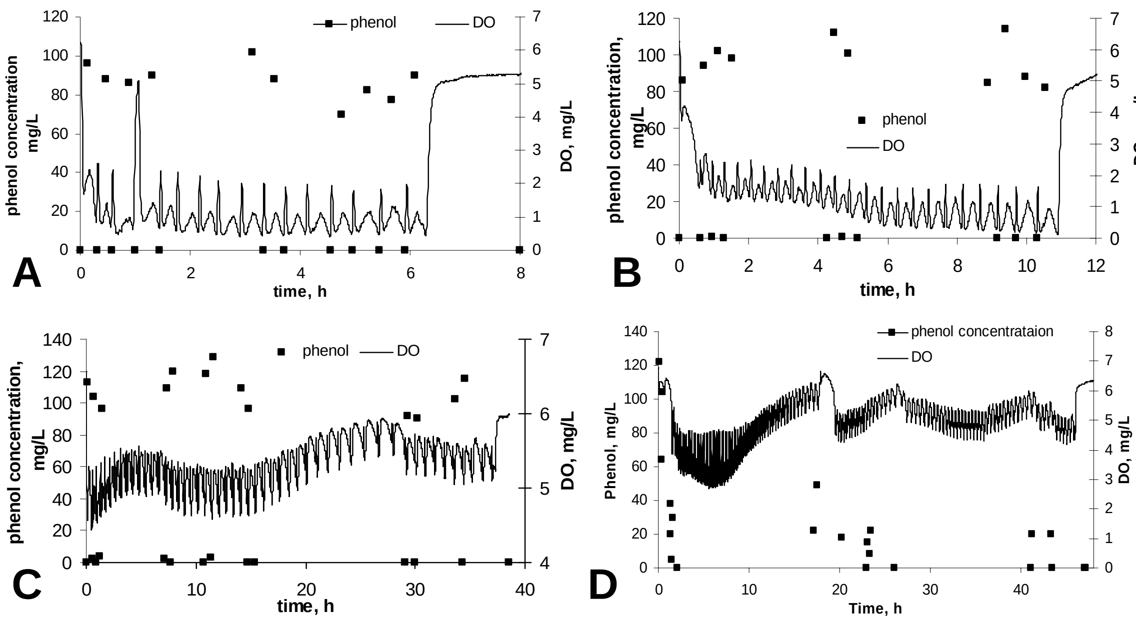

Figure 3. *Degradation kinetic of a mixture of municipal wastewater and A)700, B) 1500, C)3000 and D)7000 mg/L of phenol mixed in a municipal wastewater using the EDTOC strategy.*

Figure 6 shows a detail of the degradation kinetics of a shock load of 7000 mg of phenol in the mixture. It can be observed that phenol concentration never was higher than 120 mg/L, as in the case of 350 mg/L. These results confirm that higher phenol concentrations that otherwise in a usual batch operation would be inhibitory can certainly be treated with the proposed strategy. In theory any phenol concentration could be degraded by this control strategy.

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

Results were very promising for the operation of the field-scale pilot plant. With the laboratory experiments it was possible to evaluate the conversion yield (Y_{TWW}) for the biomass. The overall value of Y_{TWW} varied from 0.08 to 0.19 mgVSS/mgCOD, depending on the composition of the TWW, i.e., the different phenol concentration present in the municipal wastewater. Computing the individual contribution for the phenol (Y_{ph}) and the municipal wastewater (Y_{ww}) gave as a result of 0.15 and 0.43 mgVSS/mgCOD, respectively.

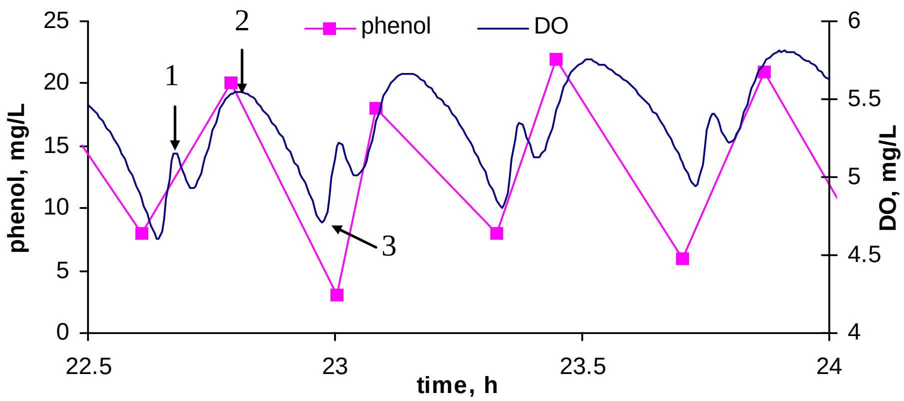

Figure 6. *Detail of degradation kinetic of a mixture of municipal wastewater and 7000 mg/L of phenol using the EDTOC strategy. The points correspond to the explanation of the points in figure 2*

Experiment 2. Long-term stability experiments

2.1. Acclimation

The acclimation was carried out in one week and the behavior corresponding to the explanation of the WP1 first and second year reports. Once the acclimation was obtained the ED-TOC experiments were started.

2.1.1 DGGE analysis of the bacterial community in the consortium and bacterial 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. Figure 5 shows the DGGE community fingerprint of the consortium. 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 (The results are processing at this moment).

The most relevant results are show in figure 5. It is possible to observe that at the beginning of the acclimation the species corresponding to the bands 1, 3, 4, 5 and 6 prevailed during all the acclimation period. The band 2 at the beginning of acclimation was not present. Nevertheless, at second day this band appears and it was conserved until the end of acclimation, indicating the reproduction of this specialized organism. In the non acclimated activated sludge it is possible to observe a diffuse band (band **7**) which disappears once the acclimation occurs. It was not possible to cut this band it due to the diffuse presented.

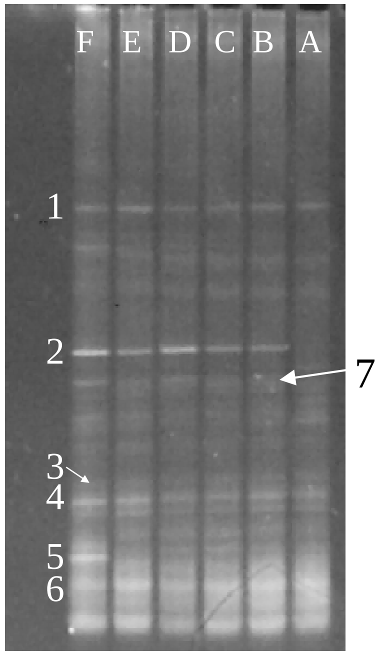

Figure 5 DGGE analysis of the bacterial community in the consortium during the acclimation. A) Initial condition, B) 24h, C) 48h, D) 72h, E) 96h, F) 120h

Conclusions

The results showed a reduction in the degradation time during the acclimation process. DGGE analysis show that the community varies before and after the acclimation process, mainly in for the bands 2 and 7. The specie of band 2 appeared during acclimation and the band 7 disappeared. The species will be identified to determine their importance in the degradation of the toxic compound. At this moment the identification of the bands is being conducted.

Future experiments

Last semester of third year (May 2005-October 2005)

Experiment 1.

1.1. Community evolution during the acclimation of the microorganisms to the 4CP by DGGE analysis.

1.2 Experiments for the evaluation of the long-term stability of the system by the application of the ED-TOC strategy

Experiment 2.

2.1. Start-up of the field scale reactor

Publication list

1. Vargas, A. and Buitrón, G. (2005) On-line concentration measurements in waste-water using nonlinear deconvolution and partial least squares of spectrophotometric data. 2nd IWA Conference on Instrumentation, Control and Automation, Busan, South Korea, May-June, 2005.
2. Moreno-Andrade I., Pérez J., Betancur M.J., Moreno J.A., Vargas A., Buitrón G. (2005). Degradation of a mixture of municipal wastewater and phenol in an aerobic optimally controlled SBR. 3rd IWA Leading-edge conference & exhibition on water and wastewater treatment technologies, 6-8/06/2005. Sapporo, Japón.
3. Moreno-Andrade I., Pérez J., Betancur M.J., Moreno J.A., Buitrón G. (2005). Biodegradation of high 4-chlorophenol concentrations in a discontinuous reactor fed with an optimally controlled influent flow rate. Sustainable Development of Chemical Industries, 14-16/07/2005. Tsukuba and Kashima, Japón.
4. Moreno-Andrade I., Buitrón G. (2005). The activity of the microorganisms acclimated to 4-chlorophenol vary when they are exposed to starvation. 4th IWA Activated Sludge Population Dynamics Specialist Conference. Microbial Population Dynamics in Biological Wastewater Treatment (ASPD4), 17-20/07/2005. Queensland, Australia.
5. Betancur M.J., Moreno-Andrade I., Moreno J.A., Dochain D., Buitrón G. (2005). Modeling for the optimal biodegradation of toxic wastewater in a discontinuous reactor. 4th IWA Activated Sludge Population Dynamics Specialist Conference. Microbial Population Dynamics in Biological Wastewater Treatment (ASPD4), 17-20/07/2005. Queensland, Australia.
6. Velázquez E., Moreno-Andrade I., Buitrón G. (2005). Community evolution during the acclimation process of activated sludge to p-chlorophenol. 4th IWA Activated Sludge Population Dynamics Specialist Conference. Microbial Population Dynamics in Biological Wastewater Treatment (ASPD4), 17-20/07/2005. Queensland, Australia.

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Yoong E.T., Lant P.A., Greenfield P.F. (2000). In situ respirometry in a SBR treating wastewater with high phenol concentrations. *Wat. Res.*, **34** (1), 239–245.