OPTIMAL DEGRADATION OF INHIBITORY WASTEWATERS IN A FED-BATCH BIOREACTOR

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Short title: **OPTIMAL DEGRADATION OF INHIBITORY WASTEWATERS**

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Abstract

The application of a new control strategy to optimize the degradation rate of organic toxic compounds is presented. An optimal control strategy called Event Driven Time Optimal Control was applied to biodegrade, in a discontinuous reactor, wastewater containing 4-chlorophenol as inhibitory compound model. The strategy estimates the oxygen mass uptake rate, which is linearly related to the substrate uptake rate, by measuring only the dissolved oxygen concentration and the volume of the reactor. Using this estimation, the optimal strategy sets the influent flow rate such that the substrate degradation rate is maintained around its maximal value as long as possible and thus minimizing the reaction time. The strategy was tested for the degradation of different initial concentrations of 4-chlorophenol between 175 and 613 mg L^{-1}. Also, the robustness of the strategy under different airflow rates was studied. It was concluded that the practical implementation of the optimal strategy was possible and very successful.

Key words

Automation, biodegradation, chlorophenol, fed-batch, respirometry, wastewater

Nomenclature

b: Decay biomass rate, h^{-1}

f_D: Fraction of biomass contributing to biomass debris, dimensionless

K_i: Inhibition coefficient, mg L^{-1}

k_La: Oxygen mass transfer coefficient, h^{-1}

K_s: Half-saturation coefficient for substrate, mg L^{-1}

O: Dissolved oxygen concentration, mg L^{-1}

O_{in}: Influent dissolved oxygen concentration, mg L^{-1}

O_s: Dissolved oxygen concentration at saturation, mg L^{-1}

Q_{in}: Influent flow rate, L h^{-1}

S: Substrate concentration inside the reactor, mg L^{-1}

S_{in}: Influent substrate concentration, mg L^{-1}

S*: Substrate concentration where μ reaches a maximal value, mg L^{-1}

V: Water volume level in the reactor, L

X: Biomass concentration inside the reactor, mg L^{-1}

$Y_{X/O}$: Biomass/oxygen yield coefficient [$Y_{X/O} = Y_{X/S}/(1-Y_{X/S})$], dimensionless

$Y_{X/S}$: Biomass/substrate yield coefficient, dimensionless

Greek symbols

Gamma γ: Oxygen mass uptake rate, mg h^{-1}

mu μ: Specific growth rate, h^{-1}

μ*: Maximum specific growth rate in the Haldane law, h^{-1}

μ_{max}: Maximum specific growth rate, h^{-1}

Introduction

A general problem found in industrial wastewater treatment plants is the variation in the content of toxic compounds generated by several chemical and petrochemical facilities. In the case of wastewater with the presence of toxic compounds, biological processes are applied because total mineralization of the organic compounds is indeed possible. The activated sludge process has been traditionally applied to treat several industrial wastewaters, but the oscillatory nature of such discharges often causes operational problems, which are present even when an equalization tank exists. In the chemical and petrochemical wastewaters, the toxic compounds concentration

could be very high and variable. For example, typical phenols concentrations varied from 35 to 400 mg L^{-1} for oil-refining, pharmacy, electroplating, paper making, cooking and iron smelting industries.[1] But, in some petrochemical wastewaters, like the spent caustic liquors, the total phenols concentration may reach values up to 30000 - 50000 mg L^{-1}.[2] It has been reported, for the case of a Mexican refinery, producing 14 m^{-3} d^{-1} of spent caustic wastewater, that the phenol concentration was as high as 30000 mg L^{-1}.[3]

The toxic compounds can cause inhibition of the microorganisms, which is reflected in a decrease of the bacterial activity or even death.[4] To avoid this problem several strategies, as the discontinuous processes (controlled unsteady state processes), have been explored in order to increase the biotreatment efficiencies of industrial wastewaters. The term Sequencing Batch Reactor (SBR) is used as a synonym for the wastewater treatment technology where the volume in the reactor tank is variable in time. In these systems there is a cyclic feed and a cyclic discharge.[5] There are wide variations in the variable-volume activated sludge technologies and distinct process types just as is the case for the continuous flow activated sludge systems. Usually a SBR-type bioreactor operates under five well defined phases: fill, react, settle, draw, and idle. Differences between the various versions of the SBR include continuous versus periodic influent and distinct phases for react, settle and idle. Details about SBR system variations can be found in Wilderer *et al.*[6]

In this type of reactor, as a consequence of the substrate concentration variations in each cycle (decreasing in time), the growth rate of the microorganisms changes from high to low. There is a selection of a microbial community with a vast metabolic range in which the microbial species

can differ greatly in growth rate and yield. This is particularly important when toxic compounds are to be degraded, since the specialized microorganisms can be selected. The SBR processes can work with suspended or attached biomass, or the combination of both.[7] Also, their flexibility allows the application of control strategies and full automation.[8,9]

The operation mode in the SBR refers to the way the duration of these phases is set and has a fundamental impact in the SBR characteristics. In the standard operation mode, the duration of each of these phases is typically determined by an expert, based on his experience and exhaustive testing in the laboratory with a pilot plant. In particular, the reaction phase is set sufficiently long to allow the toxic substances to be degraded. The settle and draw phases are fixed in duration by the characteristics and constraints of the activated sludge and the reactor itself. This operational strategy, typically applied in a batch process, can be named Fixed Time Control strategy (FTC). Despite the inherent advantages of the discontinuous processes in relation to the biodegradation of toxic substances,[10] the SBR operated under the FTC strategy presents several difficulties when it is applied to toxic wastewaters: inhibition of the microorganisms, problems with shock loads of toxic compounds, deacclimation, and problems of starvation of the microorganisms. These phenomena cause low efficiencies regarding the removal of toxic compounds and a lack of robust performance of the plant under realistic operating conditions.[11, 12]

To overcome these problems several alternative operation modes have been proposed, using either the dissolved oxygen (DO) concentration or the carbon dioxide evolution rate.[13-15] Moreno and Buitrón[16] presented a methodology and mathematical simulations for the time optimal control of a discontinuous reactor for toxic wastewater degradation. The time optimal control

law, described by Moreno [17], controls the input flow rate in such a manner that the total reaction time is as short as possible. This requires the substrate concentration inside the SBR to be maintained at the value where the microorganisms achieve maximal degradation velocity during the whole filling phase. A feedback implementation of the theoretical optimal control law requires measuring the substrate concentration on-line, and the knowledge of several plant parameters, but this is difficult in practice. Therefore this variable may be estimated from the dissolved oxygen concentration using an observer. Vargas *et al.*[18] propose to estimate the substrate concentration from the dissolved oxygen concentration using an observer (an Extended Kalman Filter). They presented the calibration of the observer and the experimental validation of the respective control strategy, named Observer-Based Time Optimal Control (OB-TOC). The drawback of this strategy is the need to know exactly the inflow substrate concentration and the value of the kinetic constants of the Haldane model, as well as other parameters from the mathematical model.[19] This could make the OB-TOC difficult to apply in practice. To overcome the OB-TOC limitations, Betancur *et al*.[20] described a new strategy called Event-Driven Time Optimal Control (ED-TOC). The ED-TOC strategy uses solely the measurements of the dissolved oxygen concentration and the volume to control the influent flow rate so that the substrate degradation rate oscillates around its maximal value during the whole filling phase. The ED-TOC requires neither an observer, nor a complete mathematical model of the reactor, nor the knowledge of all its parameters to be implemented. Moreover, the ED-TOC is stable and robust against influent concentration perturbations and parameter and model uncertainties. These properties have been mathematically proved by Betancur *et al.*[21,22]

The objective of this work was to study the practical implementation of the optimal strategy (ED-TOC) to biodegrade inhibitory wastewater containing 4-chlorophenol as a toxic model compound in a fed-batch reactor.

Materials and methods

Event-Driven Time Optimal Control Strategy

The ED-TOC strategy is a practical implementation of the Optimal Time Control.[17] To describe it, consider the following mathematical description of the fill and reaction phases[23]

$$\frac{dX}{dt} = \mu X - bX - X\frac{Q_{in}}{V} \quad (1)$$

$$\frac{dS}{dt} = -\frac{1}{Y_{X/S}}\mu X + (S_{in} - S)\frac{Q_{in}}{V} \quad (2)$$

$$\frac{dO}{dt} = -\frac{1}{Y_{X/O}}\mu X - (1 - f_D)bX + k_L a(O_s - O) + (O_{in} - O)\frac{Q_{in}}{V} \quad (3)$$

$$\frac{dV}{dt} = Q_{in} \quad (4)$$

where all concentrations are in COD units, except oxygen concentrations given as O_2. It is known that the behavior of the biomass growth rate (μ), as a function of the toxic substrate concentration (S), can be described by the Haldane law (equation 5)

$$\mu = \mu_{max}\frac{S}{S + K_s + S^2/K_i} \quad (5)$$

In this model μ reaches a maximal value, μ^*, when the substrate concentration value is S*, that are given by the expressions 6 and 7.

$$\mu^* = \frac{\mu_{max}}{1 + 2\sqrt{\frac{K_s}{K_i}}} \quad (6)$$

$$S^* = \sqrt{K_i K_s} \quad (7)$$

Toxic concentrations above or below μ^* will reduce the growth rate and, consequently, the reaction rate. The time optimal control law [17] reduces to a minimum the reaction phase, and consists in acting on the influent flow rate (Q_{in}) to maintain S at the value S* (or equivalently to maintain μ at the value μ^*) during the whole filling phase. It is however impractical, since it requires the measurement of all state variables of the model and the knowledge of the parameters.

To overcome these difficulties Betancur *et al.*[20] introduced the oxygen mass uptake rate (γ), defined by

$$\gamma = \left(\frac{1}{Y_{x/o}} \mu + (1 - f_D) b \right) XV \quad (8)$$

and that corresponds to the oxygen uptake rate multiplied by the volume (V). Note that in equation (8) XV corresponds to the total biomass, which in a reaction cycle changes very slowly compared to the changes of S and O. For the present description it will be considered as a constant, although the results are valid if XV does not change very fast.[21, 22] Figure 1 presents the behavior of γ as a function of the substrate concentration. It is clear from equation 8, that γ is a linear function of μ, so that both variables μ and γ reach their maxima μ^* and γ^*, respectively, at the same value S*. Note that maintaining γ at its maximum value γ^* during the filling phase corresponds to the time optimal control strategy.

Rearranging equation (3) it is easy to see that the oxygen mass uptake rate can be expressed as

$$\gamma = k_L a(O_s - O)V + (O_{in} - O)Q_{in} - V\frac{dO}{dt} \qquad (9)$$

This shows that the oxygen mass uptake rate can be estimated in real time by measuring on-line the dissolved oxygen concentration in the reaction tank, since all the variables in the right hand side of equation (9) are measurable or easily computable. Only the mass transfer coefficient, k_La, the dissolved oxygen saturation concentration, O_s, and the influent dissolved oxygen concentration, O_{in}, are needed as parameters to estimate γ. Note that, due to the presence of bacteria in raw wastewaters, the O_{in} value is almost to zero and can be neglected.

The ED-TOC strategy approximates the time optimal control strategy by switching on/off the influent flow rate in such a way that the oxygen mass uptake rate, and therefore the reaction rate too, remains close to the maximal value (gray zone in Figure 1). This is done in the following form: suppose that S is below S* and so γ* is below γ. The influent pump is switched on and therefore γ and S increase. When S reaches and exceeds the value S*, γ will attain its maximum γ* and will begin to decrease. Once γ arrives at a fraction P% of the maximum γ* the pump will be turned off, and S will begin to decrease. γ will increase again, reach its maximum γ* and, when it arrives at P% of the maximum γ*, the pump will be turned on. As a consequence of this control, the substrate concentration will oscillate within a range, determined by the value of P%, during the SBR filling.

Since only a correct estimation of γ is required for this strategy to work properly, it will succeed regardless of the knowledge or variation of all variables and parameters not included in equation (9). For that reason the ED-TOC is robust with respect to variations or uncertainties in almost all parameters, and also against influent perturbations or shocks. However, it offers all the advantages of the Time Optimal Control, i.e. it minimizes the reaction time and avoids inhibition. This solution is therefore a good candidate for use in an industrial environment.

Pilot reactor

An aerobic automated Sequencing Batch Reactor (SBR) system with a capacity of 7L and an exchange volume of 4L (57%) was used (Figure 2). The airflow rate was 1.5 L min^{-1} and the temperature was maintained at 20 ºC. The reactor was inoculated with microorganisms from a municipal activated sludge treatment plant (2000 mgVSS L^{-1}). A synthetic wastewater containing 4-chlorophenol, 4CP, (Aldrich, purity ≥ 99.0%) was used as a sole source of carbon and energy. Nutrients (nitrogen and phosphorous) and oligo-elements were added as recommended by AFNOR.[24]

As standard condition an influent concentration (S_{in}) of 350 mg 4CP L^{-1} was selected, because it was observed that acclimated biomass could biodegrade, in a batch reactor, wastewater containing 350 mg 4CP L^{-1} without inhibition problems.[25] The strategy was tested using different influent concentrations of 4CP between 175 and 613 mg L^{-1} (which represents variations of -50, +25, +50 and +75% with respect to the standard condition of 350 mg L^{-1}). To study the robustness of the strategy under different airflow rates, two conditions were examined (± 50% with respect to the standard condition of 1.5 L min^{-1}, i.e. 0.75 and 2.25 L min^{-1}). The SBR was operated under the following strategy (except for the acclimation period): pre-aeration time (15

min), filling and reaction time (variable depending on the influent concentration and set by ED-TOC), settling time (30 min) and draw time (6 min). 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%. Acclimation was done using an initial 4CP concentration of 175 mg L^{-1} in the influent. When degradation time remained constant, initial concentration was doubled to 350 mg L^{-1}. In batch reactors, there exists a volumetric exchange ratio, i.e. a fraction of the reactor volume which is removed during draw and replaced during fill. In the case studied, the volumetric exchange ratio was 4/7, i.e. in each cycle 4 L were drawn and filled in of a total volume of 7 L. Thus, due to the volumetric exchange ratio selected, during the acclimation the influent concentration gave an initial concentration in the reactor (S) of 100 (175 x 4/7) and 200 (350 x 4/7) mg 4CP L^{-1}, respectively. After acclimation, the experiments with the ED-TOC strategy started.

Analytical methods

The substrate concentration was measured via samples processed off-line by using a modified colorimetric technique of the 4-aminoantipyrine method.[25] Total and volatile suspended solids (TSS and VSS) analyses were done according to the Standard Methods.[26] Dissolved organic carbon (DOC) was determined with a Shimadzu TOC-5050 and Chemical Oxygen Demand (COD) according to Standard Methods.[26] These analyses were performed to evaluate the 4CP degradation. The metabolite (5-chloro-2hydroxy-muconic acid semialdehyde), formed by an alternate degradation route of 4CP by the microorganisms, was also determined at 380 nm using a HACH spectrophotometer. [27]

Results and discussion

Biomass acclimation to standard conditions

Acclimation to 175 mg 4CP L^{-1} was obtained after 10 degradation cycles (125 h). During the acclimation, 4CP was degraded with efficiencies higher than 99% as 4CP and 95% as DOC and COD. The relationship between residual 4CP concentration and incubation time gradually changed, and then stabilized. For 175 mg 4CP L^{-1}, degradation times were reduced from 52 h to 1.16 h, after 125 h (cycle 1 to 10). When the initial concentration increased 100% (350 mg L^{-1}), degradation was only slightly affected. The first degradation cycle for this concentration took only 2.5 h to remove 100% of the initial 4CP. After 5 h, degradation times were reduced to 1.75 h (in 6 cycles). This result indicated that in the acclimation to the second concentration, the required microorganisms reproduced and they also developed the necessary enzymatic activity. Doubling the initial concentration only generates a proportional increase in the degradation time. After the acclimation period, the total suspended solids in the effluent were below 14 mg L^{-1}, the sludge volumetric index was around 38 mL g^{-1}, and the settling velocity of the sludge was 6 m h^{-1}.

ED-TOC strategy under standard conditions

Figure 3 presents the behavior of the substrate concentration, measured as 4CP and DOC during a cycle operated under the ED-TOC strategy with the standard condition (350 mg 4CP L^{-1}). It is possible to distinguish how the control operates following the substrate and the DO evolution curve. Once the influent begins to be fed to the reactor, the reaction starts. The DO decreases (first 0.2 h, figure 3) as the metabolic activity of the biomass increases to degrade the 4CP. Simultaneously, the estimator calculates γ and follows its value. When a maximal point of γ is

detected (before a minimum in the DO concentration), the feeding pump is turned off. The microorganisms degrade the substrate. At this point, γ decreases because the concentration of substrate is lower than S* (it has passed from 48 to 22 mg 4CP L^{-1}, at 0.4 h in Figure 3). Thus, the feeding pump is switched on again, and a new charge of substrate is fed to the reactor. This procedure is repeated until the maximal volume has been reached and then, the degradation proceeds in a batch manner, ending the reaction when the DO concentration increases to a constant value. It is possible to observe in Figure 3 how the internal concentration of 4CP in the reactor is maintained around 30 mg L^{-1}, which corresponds to a concentration close to S* and thus, around γ*. The metabolite production increases as the reaction takes place, but after the cycle has finished, the metabolite production decreases, indicating a good operation of the system. It has been observed that the metabolite (5-chloro-2hydroxy-muconic acid semialdehyde) is formed by an alternate degradation route of 4CP by the microorganisms, and can be inhibitory for the microorganisms if it accumulates.[27]

Performance of the ED-TOC strategy under different influent concentrations

To study the robustness of the control strategy against the variation of the influent substrate concentration, four conditions were tested, i.e., 175, 438, 525 and 613 mg 4CP L^{-1} in the influent (Figure 4). An increase of the 4CP concentration in the influent generates a proportional increase of the degradation time. For all cases the removal efficiency of the toxic was superior to 95% as COD, and 99% as 4CP. Results indicate that there was no influence on the performance of the reactor when an initial concentration as high as 613 mg 4CP L^{-1} was applied. Note that the maximal concentration in the reactor was around 50 mg 4CP L^{-1}, irrespective of the concentration present in the feed. The reactor really never was exposed to a shock load, since S

inside the reactor oscillates around the value S*. It has been reported that 4CP could be degraded and mineralized by aerobic bacteria[28] within a wide range of concentrations, ranging from 10 mg L^{-1} in a continuous activated sludge reactor[29] to 350 mg L^{-1} using a pure culture of *Arthrobacter chlorophenolicus* A6.[30] With the ED-TOC strategy it was possible to degrade without problems almost twice the maximal concentration reported for a pure culture.

Dissolved Oxygen Variation

As the ED-TOC strategy depends on the DO concentration to control the influent flow rate, it is important to study how much the variation parameters related to the control could affect the performance of the reactor. Two experiments were conducted varying the oxygen flow rate with respect to the standard value of 1.5 L min^{-1}. In all the cases the k_La was determined for the different value of the oxygen flow rate tested (11.5, 13.0 and 18.5 h^{-1} for the flow rates of 0.75, 1.5 and 2.25 L min^{-1}, respectively). Figure 5 presents the evolution of the dissolved oxygen during the variation of ±50% of the airflow with respect to standard conditions. For all the cases, the initial concentration of 4CP was 350 mg L^{-1}. For the case of the air flow rate of 0.75 L min^{-1}, the DO in the tank was always superior to 2 mg L^{-1}. Thus, it can be considered that microorganisms had no oxygen limitations.

Figure 6 shows the influence of the air flow variation on the substrate degradation (6A) and on the metabolite production (6B). In general, there was not a significant influence on the strategy operation when the air inflow rate was varied ±50%. However, it is very interesting to note that in the case where the airflow was reduced by 50% there was an increase in the quantity of metabolite produced (Figure 6B). As a consequence, the degradation time for this same

condition has a small increase (30 min) to achieve the 4CP degradation (Figure 3A). It was observed that this toxic by-product was not removed after the degradation cycle, possibly causing problems due to its accumulation in the next cycles.

Repeatability and stability of the ED-TOC

In addition to the different influent concentration experiments, the SBR has been operated for more than 160 cycles using the standard condition with the ED-TOC strategy in a stable, reproducible and robust manner. Removal efficiencies for 4CP during this period were always superior to 99% and COD varied between 96 and 98%. Sludge volumetric index was 80 ± 6.8 mL $gVSS^{-1}$ and suspended solids in the effluent were 17 ± 8 mg L^{-1}. This shows that the practical performance of the control strategy, based on the control of the filling and reaction phases, is feasible for the degradation of toxic wastewaters, and can be implemented in an industrial environment.

In the literature, other filling strategies to improve the operation of the batch reactors have been studied for the problem of nitrogen removal in wastewaters or for the maximization of the productivity in the field of biotechnology. Coelho *et al.*[31] studied the optimization productivity in a SBR biological nitrogen removal process. Their results showed that the use of a discrete fill strategy, consisting of symmetric pulses for wastewater reduced up to 36% the total batch time for a given nitrogen removal level. In the field of biotechnology, an optimization method for repeated fed-batch fermentation with the aim of improving the recombinant human serum albumin production in *Pichia pastoris* was presented by Ohya *et al.*[32] A simulation model for fed-batch fermentation was formulated and the optimal methanol-feeding policy calculated by

the method of dynamic programming using five different methanol feeding periods was. The production of recombinant human serum albumin in optimal fed-batch and the 4-times-repeated fed-batch fermentation was 28% and 47% higher, respectively, than in established fermentation method.

Conclusions

The application of a new control strategy that optimizes the degradation rate of toxic compounds was presented. The event-driven time optimal control strategy was applied to biodegrade in a fed-batch reactor a synthetic wastewater containing 4CP as model of an inhibitory compound. It was found that a practical implementation of the optimal strategy was possible with stable and reproducible results. A good performance of the reactor operated with the ED-TOC strategy was obtained since the degradation of the 4CP was efficiently completed.

During the operation of the reactor with the optimal strategy, average removal efficiencies were 97%, as chemical oxygen demand, and 99.9% as 4CP. The ED-TOC strategy was also able to manage increments of toxic concentrations in the influent up to 613 mg 4CP L^{-1}. Theoretically any influent concentration of toxic in the influent wastewater can be treated since in the ED-TOC strategy there is no need to know the influent substrate concentration and the input flow rate is controlled in such a manner to maintain the degradation rate close to its maximal value (no inhibition).

In general, there was not a significant influence on the performance when the air inflow rate was varied ± 50% with respect to the standard condition, but care must be taken when aeration is low, since harmful by-products may be formed.

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List of figures

Figure 1. Substrate-Volume trajectory for the ED-TOC strategy. V_o indicates the initial volume in the tank. The flow is fed to the reactor in such a manner to keep the reaction rate around the optimal value (gray zone). Once the final volume is reached, V_f, the reactor acts as a batch process and the reaction rate decreases

Figure 2. Pilot reactor utilized for the toxic compounds degradation

Figure 3. Evolution of the substrate concentration, measured as 4CP and DOC during a cycle operated under the ED-TOC strategy. Note that the changes in the γ value agree with the changes in the DO concentration, since the DO is used to estimate γ.

Figure 4. Degradation kinetics for different 4CP initial concentrations (175, 438, 525 and 613 mg 4CP L^{-1}) and dissolved oxygen evolution, using the ED-TOC strategy

Figure 5. Evolution of the dissolved oxygen concentration as a function of time using different oxygen flow rates (0.75, 1.5 and 2.25 L min^{-1}.) with the ED-TOC strategy

Figure 6. Evolution during the degradation cycle for (A) the 4CP concentration and (B) the metabolite production for different airflow rates

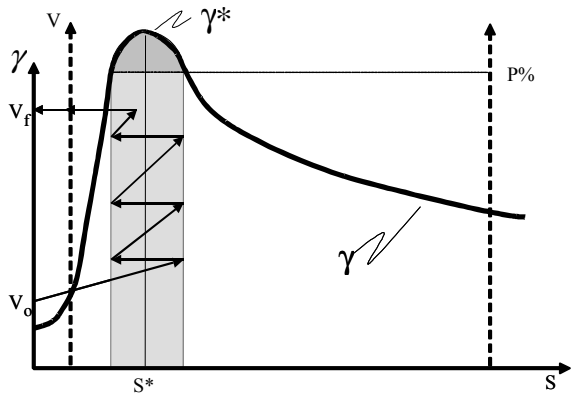


Figure 1. Substrate-Volume trajectory for the ED-TOC strategy. V_o indicates the initial volume in the tank. The flow is fed to the reactor in such a manner to keep the reaction rate around the optimal value (gray zone). Once the final volume is reached, V_f, the reactor acts as a batch process and the reaction rate decreases

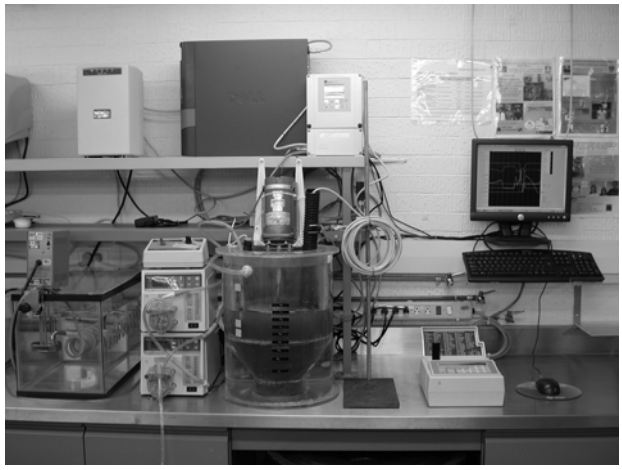


Figure 2. Lab scale reactor utilized for the toxic compounds degradation

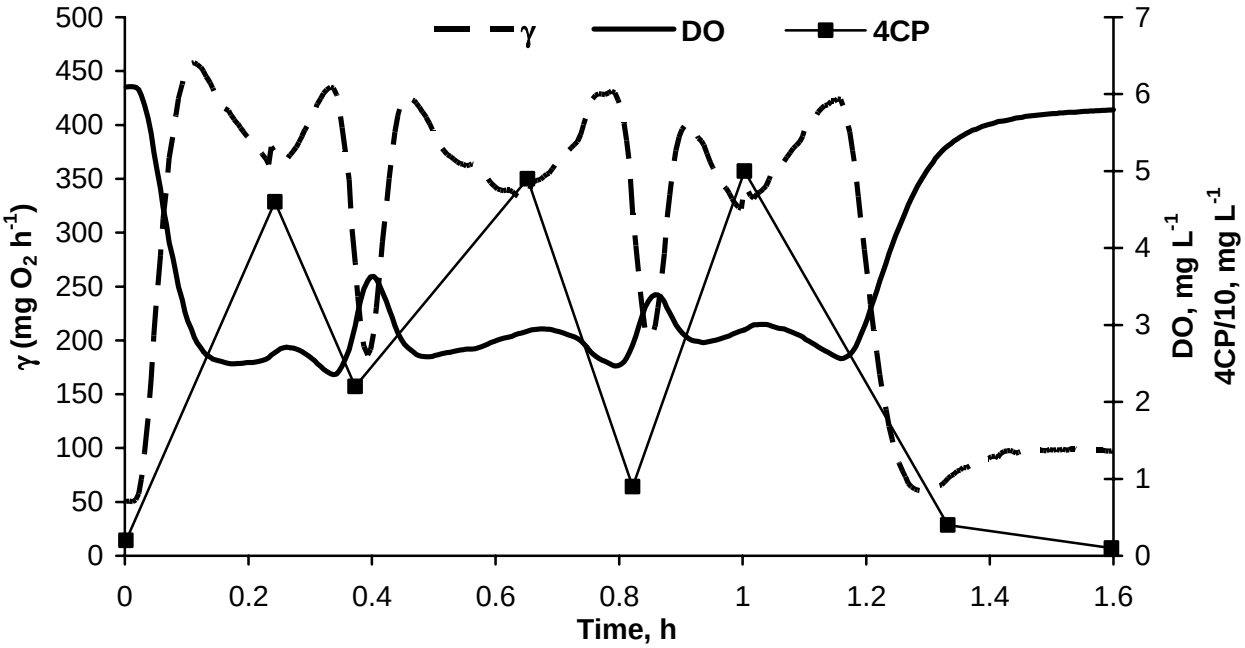
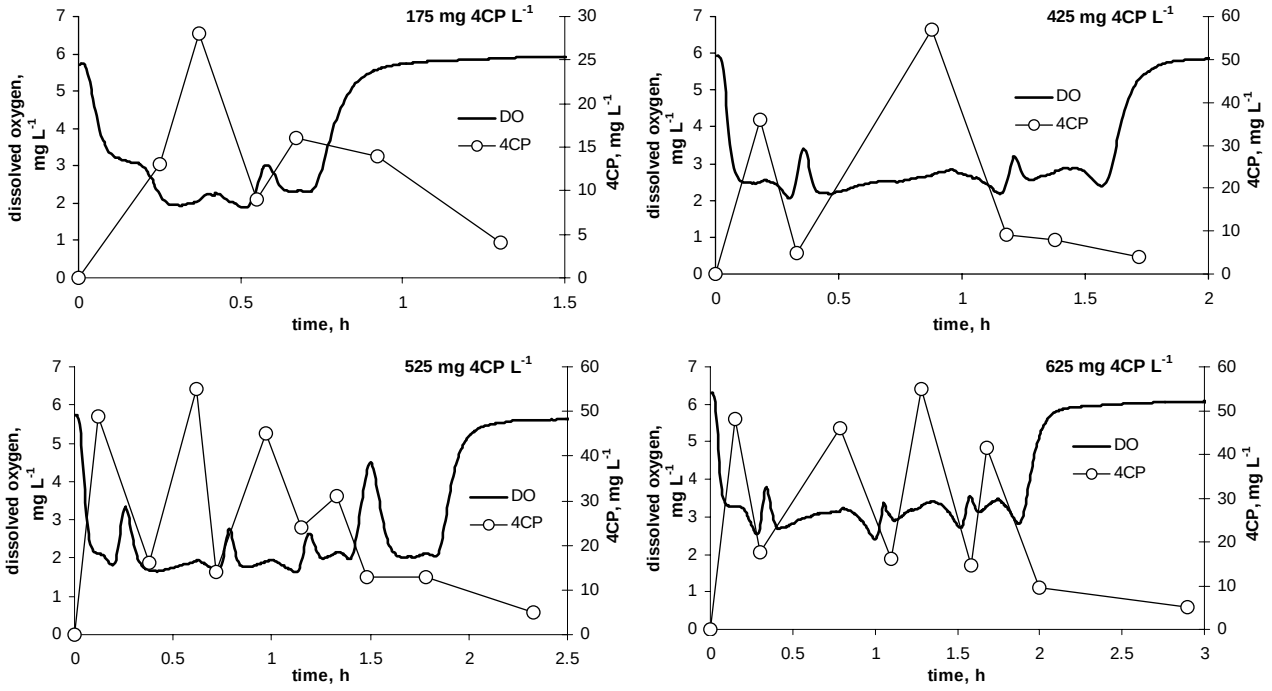


Figure 3. Evolution of the substrate concentration, measured as 4CP and DOC during a cycle operated under the ED-TOC strategy. Note that the changes in the γ value agree with the changes in the DO concentration, since the DO is used to estimate γ.



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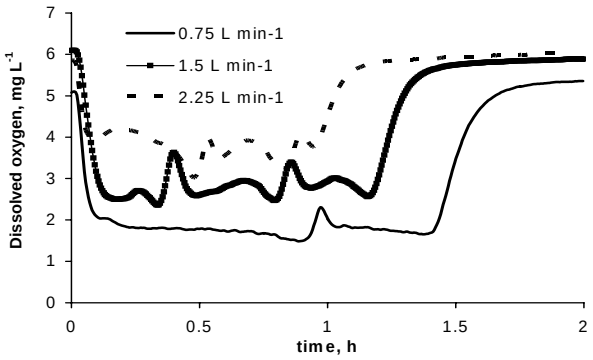

Figure 5. Evolution of the dissolved oxygen concentration as a function of time using different oxygen flow rates (0.75, 1.5 and 2.25 L min^{-1}.) with the ED-TOC strategy

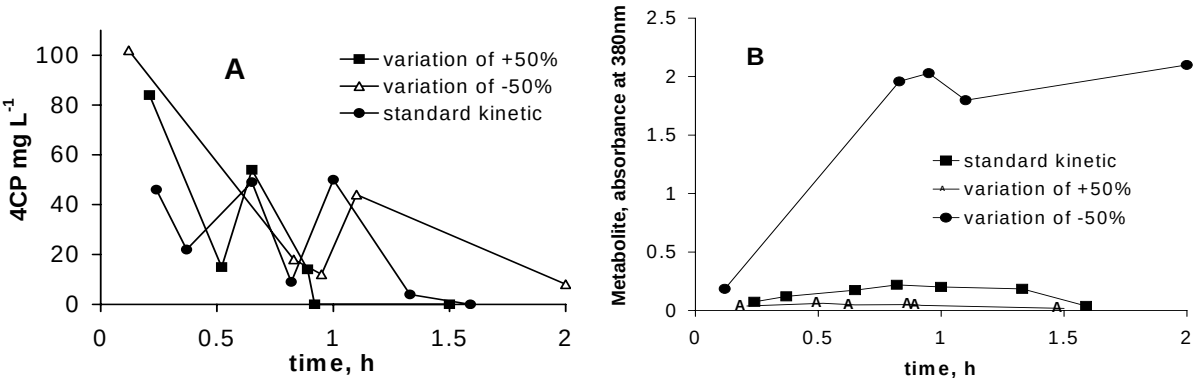

Figure 6. Evolution during the degradation cycle for (A) the 4CP concentration and (B) the metabolite production for different airflow rates