

# BIODEGRADATION OF XENOBIOTIC COMPOUNDS AND THEIR INFLUENCE ON THE PERFORMANCE OF MUNICIPAL WASTEWATER TREATMENT PLANTS

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## ABSTRACT

A rising number of new synthetic chemicals (xenobiotics) has been produced over the last century by chemical and pharmaceutical factories. Most of them have the peculiarity to persist in the environment when discharged, because their low sensitivity to natural degradation phenomena as well as to conventional processes used in wastewater treatment plants. As consequence, their presence in the environment will increase if appropriate measures to limit their release will not be taken. To this aim is addressed the research described in this paper, where the biodegradation characteristics of some xenobiotic compounds have been evaluated, together with the performances of both a conventional activated sludge system and an innovative MBR to the removal of the para-nitrophenol from wastewater. A mathematical expression to predict the biodegradation rate of several organic compounds, using some characteristics of their molecular structure as descriptors, has been also implemented and calibrated.

## 1 INTRODUCTION

Xenobiotic compounds have had an impressively fast as well as large diffusion in the last century thanks to advances in science, technology and industry. Although they have positively contributed to improve, on the whole, the quality of life, their often uncontrolled use and discharge have been responsible, over the years, for a rising worsening of the environmental quality with consequences that, at the moment, is not possible to quantify yet with any certainty.

These compounds are widely produced by chemical and pharmaceutical factories for many purposes. For instance they are successfully used as catalysts in some manufacturing processes, constitute intermediate compounds of many production cycles, are used as pesticides in agriculture and as drugs to treat diseases that affect humans as well as livestock. Nevertheless, once they have performed their task, turn into a waste hard to be disposed of. Indeed xenobiotics containing sewage are often not suitable to be treated in conventional wastewater treatment plants because of the low

biodegradability that characterizes such compounds. For this reason and because of untreated discharges as well, more and more often and in higher concentration over the last years their presence has been detected in water bodies where (Aries *et al.*, 2001, Zuccato *et al.*, 2005) they can accumulate and persist for ages because of their low sensitivity to be degraded by microorganisms, causing serious damages to the aquatic ecosystem even in presence of low concentrations (Richardson and Bowron, 1985; Wollenberger *et al.*, 2000).

In the aim of avoiding the damages that these substances can cause, it is absolutely necessary to limit their release providing for the elimination of all untreated discharges that may contain xenobiotics, as well as for the identification of treatment systems and cycles suitable to remove these compounds from sewage (Joss *et al.*, 2005). For this reason, in recent years numerous studies have been carried out aimed at estimating the biodegradation kinetics of many xenobiotic compounds as well as at determining wastewater treatment systems capable to remove these compounds.

Focused on these issues, some research activities have been carried out at the University of Naples *Federico II*, whose main aspect, partially described in details in the following paragraphs, are summarized as follows:

- has been experimentally evaluated the biodegradability ( $K_{bio}$ ) of some xenobiotic compounds;

- has been determined, on a bench scale reactor, the effectiveness of a MBR (membrane bio-reactor) treatment system in removing from sewage 4-nitrophenol, a widely diffuse xenobiotic compound as it is an intermediate in the synthesis of paracetamol and also has been assessed, on another bench scale reactor, the effects due to the presence of the same compound on the biodegradation activity of microorganisms in an activated sludge process;

- has been developed a predictive model for the aerobic biodegradation of chemical substances by relating the rate of the consumption to the structure of the compounds.

## 2 BIODEGRADABILITY EVALUATION OF XENOBIOTIC COMPOUNDS

Twenty xenobiotic compounds were taken into account (Andreozzi *et al.*, 2006) and for each of them the kinetic constant of biodegradation ( $K_{bio}$ ) was experimentally evaluated. For this purpose experimental tests according to the methodology described in the following sub-paragraph were carried out.

### 2.1 Materials and methods

The methodology used to carry out the biodegradation tests is based on the Michaelis-Menten (1) and Monod (2) laws:

$$\frac{dS}{dt} = -\hat{v} \frac{S}{K_s + S} x \quad (1)$$

$$\frac{dx}{dt} = (\mu - K_d) x \quad (2)$$

where:

$S$  [ $\text{ML}^{-3}$ ] is the concentration of the substrate;

$t$  [T] is the time;

$\hat{v}$  [T<sup>-1</sup>] is the maximum consumption rate of the substrate;  
 $K_s$  [ML<sup>-3</sup>] is the semi-saturation constant, that is the concentration of substrate corresponding to a consumption rate equal to the half of the maximum;  
 $x$  [ML<sup>-3</sup>] is the concentration of microorganisms;  
 $\mu$  [T<sup>-1</sup>] is the rate of the microorganisms growth;  
 $K_d$  [T<sup>-1</sup>] is the rate of the microorganisms death.

Coupling equations (1) and (2) and assuming the rate of microorganism death negligible if compared to that one of their growth, the following equation (3) is obtained:

$$\frac{dS}{dt} = -\frac{1}{y} \cdot \frac{dx}{dt} = -\frac{1}{y} \cdot \mu x = -\frac{\hat{\mu}}{y} \frac{S}{K_s + S} x = vx \quad (3)$$

where:

$y$  [dimensionless] is the conversion coefficient of the substrate into microorganisms;  
 $\hat{\mu}$  [T<sup>-1</sup>] is the maximum rate of the microorganisms growth;  
 $v$  [T<sup>-1</sup>] is the current consumption rate of the substrate.

Furthermore, assuming that the substrate is in extremely low concentration (as very often occurs when the substrate is a xenobiotic compound, since they are usually present at trace level in sewage), the contribute of  $S$  to the sum at the denominator of (3) is negligible and therefore it is reasonable to consider a simplified form for the equation (3) represented by the equation (4):

$$\frac{dS}{dt} = -\frac{\hat{\mu}}{y} \frac{S}{K_s} x = \hat{v} \frac{S}{K_s} x \quad (4)$$

Comparing the (4) to the following expression modelling the consumption of substrate:

$$\frac{dS}{dt} = K_{bio} \cdot S \quad (5)$$

can be obtained the analytical expression of the pseudo first-order kinetic constant of biodegradation ( $K_{bio}$ ):

$$K_{bio} = \hat{v} \cdot \frac{x}{K_s} \quad (6)$$

Integrating the equation (5) according to the method of separation of variables, the (5) can be rewritten as follows:

$$\frac{dS}{S} = K_{bio} \cdot dt \quad (7)$$

and the integration process results in:

$$\ln \frac{S_0}{S} = K_{bio} \cdot t \quad (8)$$

The equation (8) is the analytical expression according to which has been evaluated, using the experimental data, i.e. the couples of points ( $t$ ,  $S_0/S$ ), the kinetic constant of biodegradation ( $K_{bio}$ ) for the 20 xenobiotic compounds considered.

The collection of data used to evaluate  $K_{bio}$  by the means of the expression (8) have been obtained carrying out several batch tests focused on the biological degradation of the 20 xenobiotic compounds considered. Each test was carried out in triplicate and as inoculums was used activated sludge coming from two different treatment plants: the first located in Cuma and known also as wastewater treatment plant of Napoli Ovest, and the second one in Minturno.

Each test was performed in bench scale reactors where bi-distilled water and activated sludge have been added to a liquid bulk containing elements at trace level and nutrients (Urano and Kato, 1986) up to have a final volume of 250 ml.

The composition of the liquid bulk used for the tests is described in detail as follows:

- 5 ml of a mixture containing potassium phosphate dibasic,  $K_2HPO_4$  ( $25.75 \cdot 10^{-3} \text{ g m}^{-3}$ ), potassium dihydrogen phosphate,  $KH_2PO_4$  ( $8.5 \cdot 10^{-3} \text{ g m}^{-3}$ ), sodium hydrogen phosphate hydrate,  $Na_2HPO_4 \cdot 12H_2O$  ( $44.6 \cdot 10^{-3} \text{ g m}^{-3}$ ), ammonium chloride,  $NH_4Cl$  ( $1.7 \cdot 10^{-3} \text{ g m}^{-3}$ );
- 0.5 ml of an aqueous solution of magnesium phosphate heptahydrate,  $MgSO_4 \cdot 7H_2O$  ( $22.5 \cdot 10^{-3} \text{ g m}^{-3}$ ), 0.5 ml of calcium chloride,  $CaCl_2$  ( $27.5 \cdot 10^{-3} \text{ g m}^{-3}$ );
- 1 ml of ferric chloride hexahydrate,  $FeCl_3 \cdot 6H_2O$  ( $0.25 \cdot 10^{-3} \text{ g m}^{-3}$ ).

The amount of bi-distilled water and sludge added to the liquid bulk were as much as to have in the reactor a concentration of solids equal to  $3000 \text{ g m}^{-3}$ . The reactors were mixed and aerated at  $25^\circ\text{C}$ . The pH of the solution was set to a value next to 7 by adding HCl or NaOH. The consumption of substrate was monitored as long as it was completely degraded, after that the agitation system was switched off to let the sample settle. At the end of the sedimentation process, an aliquot of supernatant was collected and an equal volume of a solution containing inorganic nutrients and substrate was added enough to get back the system to the initial conditions. From that moment were set the equipments of agitation and aeration going again.

This procedure was followed for all the xenobiotic compounds considered and repeated for each of them a number of times ranged between 7 and 9, in order to acclimate the microorganisms; such a condition is observed in the graph  $S_0/S$  vs  $t$  thank to the overlapping of two curves corresponding to two consecutive loads of substrate (Figure 1).

## 2.2 Results

The data coming from the experimental tests were processed (as couples of points  $\ln(S_0/S)-t$ ) by the linear regression tool and for each of the xenobiotic compounds considered was evaluated the kinetic constant of the biodegradation process (Figure 2) corresponding to the slope of the regression line.

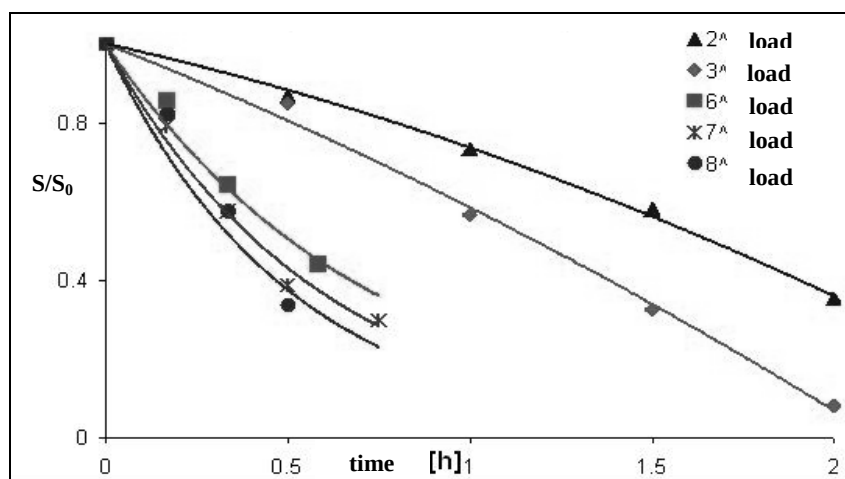


Figure 1 Consumption of substrate varying time and loads

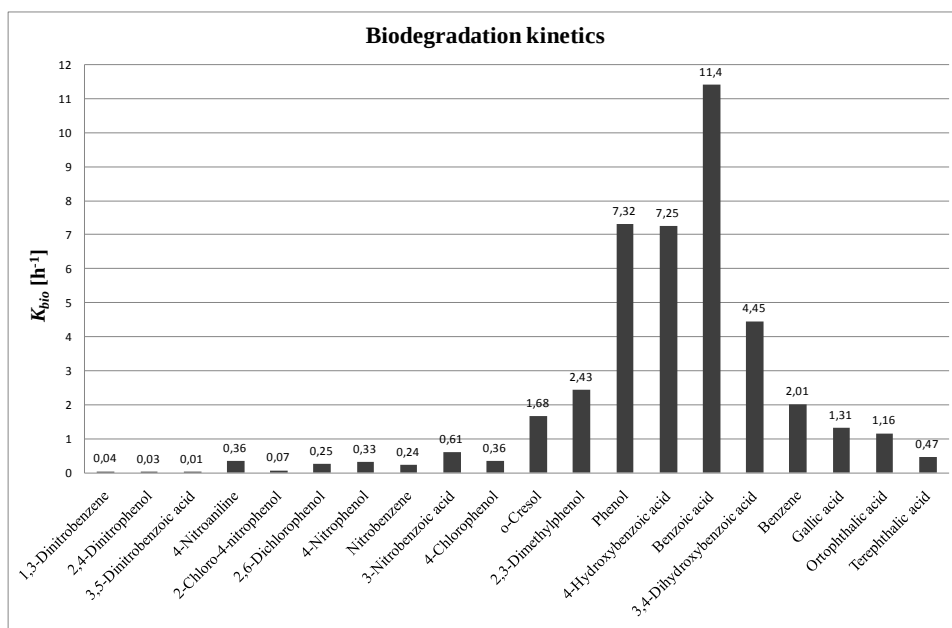


Figure 2 Biodegradation kinetics of twenty xenobiotic compounds

In order to assess the influence of the type of activated sludge on the value of  $K_{bio}$ , the experimental batch tests described above were repeated for 5 of the xenobiotic compounds considered before (i.e. phenol, o-cresol, terephthalic acid, benzoic acid and 4-nitrophenol) using activated sludge taken from the same treatment plant but at different times of year as well as at same time of year but from two different treatment plants.

The results obtained by these tests showed that the time of year marginally affects the biodegradation rate of the all 5 compounds considered whereas the different origin of the sludge causes a high variability on the biodegradation rate, in particular the datum related to the benzoic acid showed a variability around 100%.

### 3 EVALUATION OF THE PERFORMANCE OF CONVENTIONAL AND INNOVATIVE TREATMENT SYSTEMS IN REMOVING XENOBIOTIC

To perform the experimental activities, two pilot plants were started up, respectively consistent of a conventional activated sludge plant and a MBR plant.

#### 3.1 Materials and methods

The conventional activated sludge plant was used to carry out a large number of tests, characterizing by a constant flow, equal to  $38.4 \text{ l d}^{-1}$ , of synthetic sewage prepared mixing a fixed doses of sodium acetate, ammonium chloride, potassium phosphate, sodium bicarbonate, magnesium sulphate, calcium chloride and ferric chloride, close to the characteristics of municipal wastewaters in terms of pH, COD and ammonia concentrations. In this flow were dissolved various doses of para-nitrophenol (compound commonly used in many manufacturing processes for the synthesis of pesticides, some drugs, insecticides and herbicides), enough to have concentrations ranged among  $2 \text{ g m}^{-3}$  and  $700 \text{ g m}^{-3}$ .

In detail, the pilot plant was fed according to an experimental plan arranged to have a succession of 12 steps of load (Table 1), each characterized by a different concentration of para-nitrophenol. This activity was aimed at assessing, for each load step, the characteristics of biodegradability of para-nitrophenol as well as the effects that the presence of this compound could have on the biological degradation of the main biodegradable pollutants present in a municipal wastewater (i.e. organic matter and ammonia).

**Table1.** Experimental plan to feed the activated sludge reactor

| Step of load | Concentration of 4-NP estimated<br>( $\text{g m}^{-3}$ ) | Concentration of 4-NP measured<br>( $\text{g m}^{-3}$ ) |
|--------------|----------------------------------------------------------|---------------------------------------------------------|
| 1            | 2                                                        | 2.195                                                   |
| 2            | 3.5                                                      | 3.556                                                   |
| 3            | 4                                                        | 3.895                                                   |
| 4            | 6                                                        | 6.152                                                   |
| 5            | 7                                                        | 6.625                                                   |
| 6            | 10                                                       | 9.556                                                   |
| 7            | 14                                                       | 14.277                                                  |
| 8            | 30                                                       | 29.675                                                  |
| 9            | 60                                                       | 61.098                                                  |
| 10           | 150                                                      | 152.89                                                  |
| 11           | 300                                                      | 302.45                                                  |
| 12           | 700                                                      | 698.2                                                   |

The MBR pilot plant consists of four main elements:

- a storage tank containing synthetic sewage;
- an anoxic reactor to perform the denitrification process equipped with a probe to measure pH and temperature and having a sensor that evaluating constantly the level of water in the anoxic reactor controls the start up of a peristaltic pump that feeds the following reactor;
- an aerobic reactor where the biological process takes place under aerobic conditions; such a reactor contains the filter element made of an hollow fiber membrane, model ZW10, Zenon, and is equipped with two probes to measure respectively, the oxygen concentration and the pH;
- a tank where the permeate is stored.

Depression and over pressure on the membrane surface caused respectively by the extraction of the permeate and the backwashing operation are constantly monitored by a meter in the aim of checking that the membrane is working properly anytime.

The periodic removal of the excess sludge is done, when necessary, by opening the valve located at the bottom of the tank.

In all reactors the temperature is monitored by a metal coil in which a fluid at a temperature set by a heater, flows. A dedicated software stores all data measured by the instruments, reports any malfunctions and enables to change online the operational conditions.

The MBR reactor was fed with synthetic sewage contaminated by para-nitrophenol in a concentration of  $60 \text{ g m}^{-3}$  in order to assess the effect of this compound on the efficiency of the treatment as well as the feasibility of the MBR plant in removing such a xenobiotic compound.

The experimental activity lasted 5 months and a half and was consisted of 3 phases:

- the first phase lasted about 3 months and was needed to reach the steady state condition of the MBR system;
- the second phase which required a time of about 2 months and a half was focused on the assessment of the MBR performance reactor when it is fed with para-nitrophenol;
- the third phase lasted one week and was aimed at testing the performance of the MBR system when it is no more fed with para-nitrophenol.

### **3.2 Results**

The influent and the effluent of the activated sludge pilot plant were constantly monitored. Among all chemical and physical parameters taken into account, particularly significant has been the evaluations of COD, ammonia, nitrite and nitrate (only in the effluent) and, of course, para-nitrophenol, which have highlighted a high efficiency of the system, whose performances in terms of organic matter removal and nitrification of ammonia have never been lower than 70-80% (Table 2).

With regard to para-nitrophenol, it was observed a significant removal efficiency as well; moreover it was possible to model the degradation kinetics of this compound using the Michelis-Menten law for a single substrate where the maximum rate of degradation and the value of the semi-saturation constant are respectively equal to  $0.012 \text{ h}^{-1}$  and  $31 \text{ g m}^{-3}$ .

The experimental results coming from the activated sludge pilot plant have shown that the performance of the system drops when para-nitrophenol is present in sewage with a concentration of  $60 \text{ g m}^{-3}$ . Indeed, observing the graphs in Figures 3 and 4, can be

clearly noticed that, even in the presence of a constant value of  $400 \text{ g m}^{-3}$  in the influent, the concentration of COD in the effluent is significantly higher when the treatment plant is fed with para-nitrophenol, even if it is lower than  $100 \text{ g m}^{-3}$ , which represents the limit threshold for the reuse of treated wastewater according to Italian regulation DM 185/2003.

With regard to the feasibility of the MBR in the para-nitrophenol removal, are currently available only a few results and they show an efficiency of about 70-90%.

**Table 2.** Performance of the activated sludge pilot plant for the COD removal

| Step of load | Efficiency for the COD removal(%) |
|--------------|-----------------------------------|
| 1            | 78.10                             |
| 2            | 75.86                             |
| 3            | 81.41                             |
| 4            | 74.06                             |
| 5            | 79.35                             |
| 6            | 78.29                             |
| 7            | 75.08                             |
| 8            | 75.74                             |
| 9            | 78.06                             |
| 10           | 74.03                             |
| 11           | 77.29                             |
| 12           | 78.74                             |

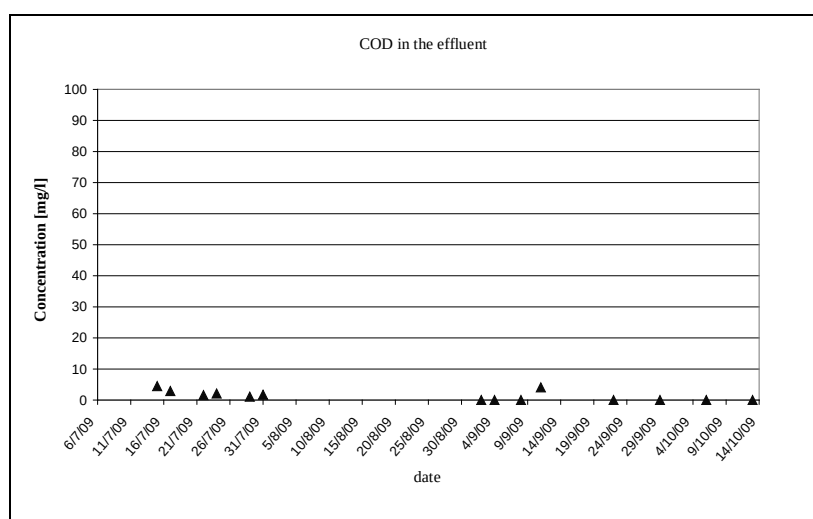


**Figure 3** COD in the effluent when the influent has no content of para-nitrophenol

#### 4 PREDICTIVE MODEL TO EVALUATE THE BIODEGRADATION KINETICS

A mathematical expression able to predict the biodegradability of organic substances containing only a single benzene ring based on their specific molecular structure was developed.

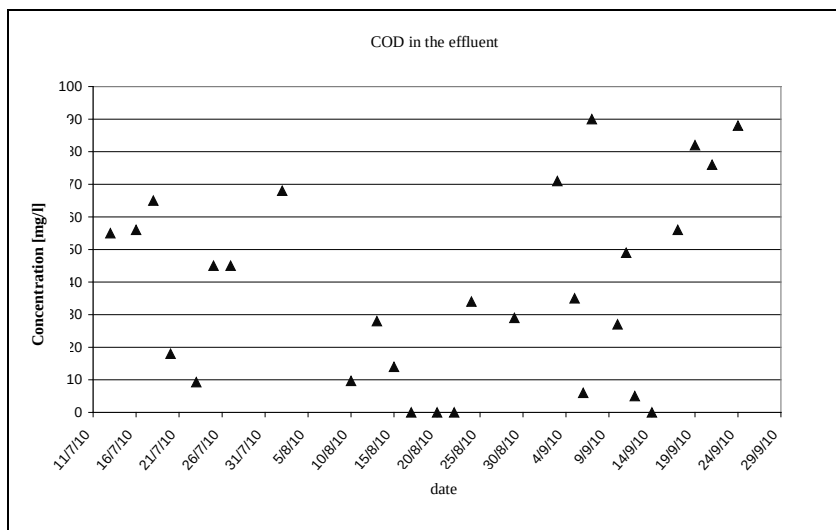


Figure 4 COD in the effluent when the influent has a content of para-nitrophenol of  $60 \text{ g m}^{-3}$

#### 4.1 Materials and methods

The approach used is in agreement with the tradition (Okey and Stensel, 1996; Karelson and Lobanov, 1996) of the QSARs (Quantitative Structure Activity Relationships), and in particular the methods (Raymond *et al.*, 2001) known as QSBRs (Quantitative Structure Biodegradability Relationships), for which the molecular activity taken into account is the biodegradability.

These methods have the peculiarity to identify a large number of molecular descriptors, such as, for example, the weight, the size and the charge of molecules, the chemical formula of the molecule in the presence of different functional groups such as hydroxyl  $\text{OH}^-$ , the amine  $\text{NH}_2^-$ , the nitric  $\text{NO}_2^-$ , the methyl  $\text{CH}_3^-$ , the carboxyl  $\text{COOH}^-$ , or the presence of certain ions such as chloride,  $\text{Cl}^-$ , etc ... and to show a dependence between them and the biodegradability of many organic compounds.

For the construction of such mathematical expressions are often used methods of multiple linear and non-linear regression (Loreti, 1998), and experimental data are used for their calibration. In particular in this work has been used the methodology of multiple linear regression to write a mathematical expression and the data experimentally obtained and reported in the first paragraph of this paper to calibrate the mathematical expression.

The multiple linear regression equation has the following expression:

$$\log K_{bio} = b_0 + b_1 X_1 + b_2 X_2 + \dots + b_n X_n + \varepsilon \quad (9)$$

where

$K_{bio} [\text{T}^{-1}]$  is the biodegradation rate per unit of biomass involved in the process of biodegradation of a specific chemical compound;

$b_i$  are the regression coefficients;

$X_i$  are the molecular descriptors taken into account.

$\varepsilon$  is the error associated with the estimate.

The molecular descriptors considered are in the order as follows:

the nitric group  $\text{NO}_2^-$ ;  
the hydroxyl group  $\text{OH}^-$ ;  
the carboxyl group  $\text{COOH}^-$ ;  
the chloride ion  $\text{Cl}^-$ ;  
the methyl group  $\text{CH}_3^-$ ;  
the molecular weight MW.

The values of the regression coefficients were calculated by the method of least squares.

In matrix form the expression to obtain the column vector of regression coefficients ( $b$ ) is as follows:

$$b = (X^T X)^{-1} X^T Y \quad (10)$$

where  $X$  and  $Y$  are respectively the matrix of molecular descriptors and the column vector of known terms, that are the logarithms of  $K_{bio}$ .

With the apex is indicated the transposed matrix, whereas with the exponent  $^{-1}$ , the inverse.

For each value of  $b$  the error associated with the formula of standard deviation has been estimated and this value is graphically represented by the length of the error bar shown in the diagram in Figure 5, which shows a comparison between the experimental data (diamond) and those obtained at the end of the calibration process of the proposed mathematical expression (round).

## 4.2 Results

By means of the multiple linear regression method, using the experimental data, has been obtained the following mathematical expression:

$$\log K_{bio} = 0.7731 - 1.1449 \cdot \text{NO}_2^- - 0.0715 \cdot \text{OH}^- - 0.1538 \cdot \text{COOH}^- + \dots \\ - 0.7290 \cdot \text{Cl}^- - 0.2116 \cdot \text{CH}_3^- - 0.1430 \cdot \text{MW} \quad (11)$$

Taking as reference the biodegradability of the molecule of benzene, in Figure 5 it is possible to notice how the presence of functional groups such as  $\text{OH}^-$ ,  $\text{COOH}^-$  and  $\text{CH}_3^-$  increase the biodegradability of the chemical compounds, whereas the presence of the nitric group  $\text{NO}_2^-$  and the ion chloride  $\text{Cl}^-$  is responsible for a decreasing. Moreover, for the functional groups that enhance the biodegradability of the benzoic acid ring, as well as for those that get it worse, the presence of more elements belonging to the same functional group results in a reduction of the biodegradability.

## 5 CONCLUSION

The activities described in this article have concerned on the one hand, the estimate of the constants of biodegradation relating to 20 xenobiotic compounds and, on the other, the assessment of their removal by biological treatment systems; the values of the constants of biodegradation can be used to design properly the biological process

involved in their removal from contaminated water, whereas with regard to the performance of conventional activated sludge treatment plants, it was verified that they are minimally affected by the presence in the influent of para-nitrophenol, an intermediate compound in the synthesis of paracetamol and finally, was evaluated the feasibility and the usefulness of the MBR technology to treat wastewaters contaminated by para-nitrophenol.

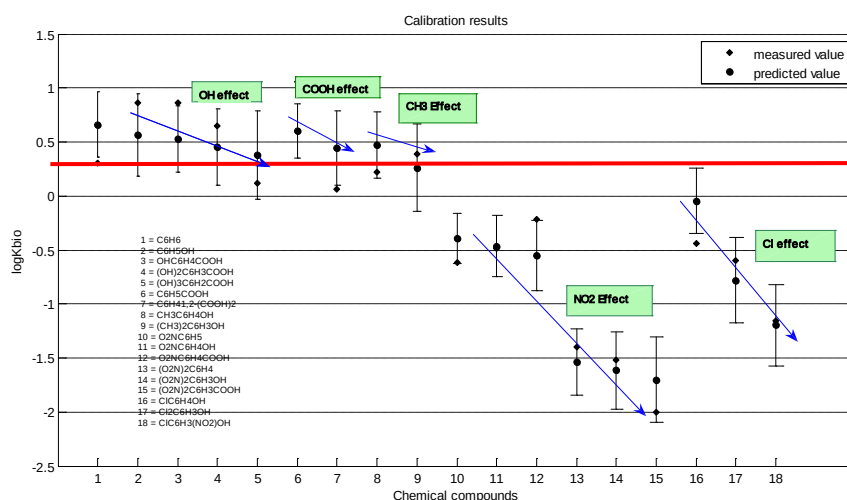


Figure 5 – Results of the calibration process

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