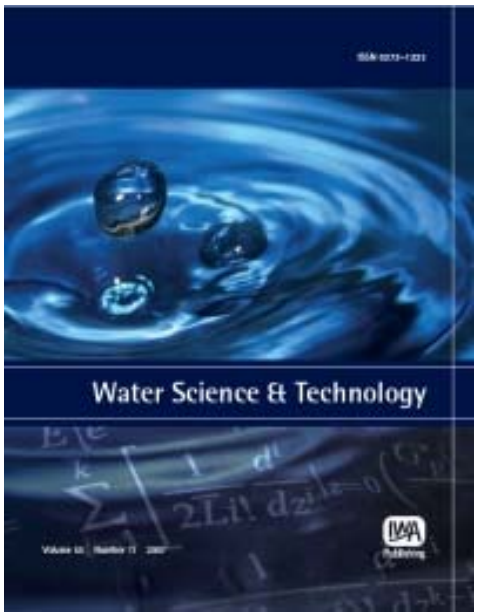

Impact of some herbicides on the biomass activity in biological treatment plants and biodegradability enhancement by a photo-Fenton process

T. B. Benzaquén, M. T. Benzzo, M. A. Isla and O. M. Alfano

ABSTRACT

In recent years, the use of agrochemicals has increased because they are essential for profitable agricultural production. Herbicides are heavily demanded compounds and among these, the most marketed are 2,4-D, atrazine and acetochlor. They have characteristics that can cause problems to humans and the environment. Therefore, it is necessary to design systems that can reduce these compounds to harmless molecules. This work aims at evaluating the possibility of incorporating these herbicides into degradable effluents in a biological treatment system, without reducing its efficiency. For this purpose, studies of organic matter degradability in the presence of these agrochemicals were performed. A synthetic effluent based on glucose and mineral salts was inoculated with microorganisms. Glucose consumption and biomass concentration were assessed. Subsequently, preliminary studies were performed to test the viability of degradation of the most harmful compound with an advanced oxidation process (AOP). The results showed that the incorporation of these herbicides into degradable effluents in a biological treatment system has a negative impact on microorganisms. Therefore, the application of an AOP, such as the Fenton or photo-Fenton processes, prior to a biological treatment was found to degrade these substances to simpler and less toxic molecules.

Key words | advanced oxidation process, biological treatment, herbicides

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NOMENCLATURE

Gl	Glucose concentration, mg L^{-1}.
VSS	Volatile Suspended Solids concentration, mg L^{-1}.
R	Hydrogen peroxide to atrazine molar ratio, dimensionless.
q_w	Net radiative flux at the reactor wall, Einstein $cm^{-2}\,s^{-1}$.
X	Biomass or microorganisms concentration, mgVSS L^{-1}.
$\overline{X}$	Mean value of the biomass concentration, mgVSS L^{-1}.
S	Limiting substrate concentration, mgGl L^{-1}.
r_x	Growth rate of microorganisms, mgVSS $L^{-1}\,h^{-1}$.
r_s	Rate of glucose uptake by microorganisms, mgGl $L^{-1}\,h^{-1}$.
$Y_{x/s}$	Biomass yield, mgVSS $mgGl^{-1}$.
K_s	Half saturation constant, mgGl L^{-1}.
k_s	Specific substrate removal rate, L $mgVSS^{-1}\,h^{-1}$.
k_s'	Modified removal rate, h^{-1}.
t	Time, h.

Greek Letters

λ	wavelength, nm.
μ	specific growth rate of biomass, h^{-1}.

Subscripts

Fe^{3+}	relative to ferric ion.
At	relative to atrazine.

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INTRODUCTION

Nowadays, worldwide, the most frequent method of pest control in agriculture is the use of agrochemicals. Intensive agriculture has grown exponentially and the use of agrochemicals is one of the most important actors that have brought about the current yield productivity. However, their use does involve risk, because most have inherent properties that make them dangerous to human health and the environment, if not used properly.

One of the most dangerous factors of environmental pollution generated by the use of these products is the disposal of wastes, such as those originated from empty pesticide containers that have not been properly rinsed. It is known that these containers keep inside about 2% of total product. It still remains an unsolved problem in many countries. Discarded, unrinsed and improperly rinsed empty containers are a health risk to the people who handle them, and they can contaminate the environment (Huston & Pignatello 1999).

In the market for agrochemicals herbicides prevail and within the most used worldwide can be found: 2, 4-D, atrazine and acetochlor. These compounds are classified as 'moderately toxic', and can be transferred to the aquatic environmental systems as a result of their moderate water solubility.

Atrazine, 2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine, is a member of the *s*-triazine group of herbicides. For decades, atrazine has been widely used all over the world to control pre- and post-emergence of a variety of broadleaf and grass weeds in corn cultures (USEPA 2001). The persistence of atrazine in the soil despite its moderate water solubility is a key factor influencing atrazine potential to contaminate the soil, the surface and the groundwater. It is also known that this pesticide has endocrine disrupting capabilities, and is classified as a possible human carcinogen by the USEPA. 2,4-dichlorophenoxyacetic acid (2,4-D) is a selective herbicide of the phenoxyacetic acid group. It is applied to control broadleafed weeds. It has been reported that 2,4-D may cause a considerable health risk (WHO 2003; Balague *et al.* 2002). And it is also known that 2,4-D persists in the soil and contaminates the surface and the underlying groundwater. Even after a long period of not using it, various amounts of 2,4-D were detected in surface and groundwater (Lagana *et al.* 2002). The chloroacetamide herbicide acetochlor (2-chloro-*N*-(ethoxymethyl)-*N*-2(2-ethyl-6-methylphenyl)-acetamide) is adopted for pre-emergence control of annual grasses and small seeded broadleaf weeds in corn and soybean. Acetochlor is a rather mobile pollutant of the soil, posing a potential danger to the aquatic environment (Lengyel & Földényi 2003).

The prevalence of these compounds in the environment has stimulated investigations into the degradation of hazardous substances in water and contaminated soil. Today, it is known that the conventional biological treatments are used to treat wastewater containing high organic load, mainly because its cost is comparatively lower than other processes. Nevertheless, when these biodegradable effluents are combined with effluents that have toxic chemicals such as herbicides, these treatments are not satisfactory because the high toxicity of these substances can have a negative impact on the activity or viability of the microbial biota, thus reducing the efficiency of treatment (Parra *et al.* 2000; Sarria *et al.* 2003; Mantzavinos & Psillakis 2004; Muñoz & Guieysee 2006). Based on this problem, advanced oxidation processes have been recently used to increase the biodegradability of recalcitrant pollutants (Engwall *et al.* 1999; Lapertot *et al.* 2007; Oller *et al.* 2007; Farré *et al.* 2008; González *et al.* 2008; Ballesteros *et al.* 2009).

This paper aims to assess whether wastewater with herbicides such as 2,4-D, acetochlor or atrazine, can be combined with other bio-degradable effluent without causing a negative impact on the biological activity of microorganisms during biological treatment. Preliminary studies were also performed by means of Fenton and photo-Fenton processes to evaluate the degradation of the most harmful compound to obtain simpler, less toxic and therefore more easily biodegradable molecules.

MATERIALS AND METHODS

Reagents

The herbicides tested were: 2,4-D ($C_8H_5Cl_2O_3$, 98%, Sigma-Aldrich), atrazine, ($C_8H_{14}ClN_5$, $\geq$ 98%, technical grade, Sigma-Aldrich), atrazine (90%, commercial formulation, SYNGENTA), and acetochlor ($C_{14}H_{20}ClNO_2$, 90%, commercial formulation, DOW AGROSCIENCES S.A.). In each case, the concentration of each herbicide was 50 ppm.

The synthetic effluent studied for the biodegradation, was generated with distilled water, dextrose anhydrous glucose (+) as organic source ($C_6H_{12}O_6$, Cicarelli), and nutrients in mineral salts as magnesium sulfate heptahydrate ($MgSO_4.7H_20$, Cicarelli), anhydrous potassium phosphate

monobasic (KH_2PO_4, Anedra), and salts of different composition (N 12%; P 5%; K 14%; Ca 3.6%; Mg 1.2%; S 6%). The total volume of the medium used in each experimental run was 600 mL. The inoculums were carried out with freeze-dried bacteria, taken from a commercial consortium called *Bi-Chem SM 700 of Sybrom Chemical*, recommended for septic tanks, lagoons, activated sludge, distillation filters and digesters to grade down proteins, starches, cellulose, detergents and fats. The consortium was acclimated before each experiment over a period of approximately 20 h in a medium identical to that used in each subsequent run.

Photo-oxidation experiments were performed employing ferric sulfate ($FeSO_4.nH_2O$, Carlo Erba, RPE), reagent-grade hydrogen peroxide (30% w/v, Ciccarelli p.a.) and concentrated sulfuric acid for pH adjustment. The photo-treated solutions were neutralized by NaOH (reagent-grade, Mallinckrodt).

Experimental setup and procedure

Biological reactor

Biological treatment with activated sludge was carried out in a laboratory-scale reactor operated in a batch mode. This reactor was mounted inside a fume hood on an orbital shaker, in order to ensure at all times a homogeneous suspension of microorganisms. Figure 1(a) shows a schematic representation of the experimental setup. Dissolved oxygen (DO) required by microorganisms to maintain active growth continuously, was provided by means of diffusers connected to an aeration pump. Runs lasted 12 h, and liquid samples were taken at equal time intervals (every 2 h). The glucose (Gl) and biomass concentrations were determined for each sample. The biomass concentration was assimilated to the volatile suspended solids (VSS) concentration. All samples were taken and analysed in duplicate. The following physicochemical parameters were kept constant during the experimental runs: temperature (25 °C), pH (6–7), DO (8–9 ppm) and agitation (165 rpm). Firstly, a run was carried out on herbicides-free medium. This blank run was used as a control for subsequent runs.

Photoreactor

Photo-Fenton tests were carried out in an isothermal, well-stirred batch recycling reactor. The scheme of the experimental device is presented in Figure 1(b). The flat-plate reactor was irradiated from both sides with two tubular lamps placed at the focal axis of two cylindrical reflectors of parabolic cross section. The radiant energy was supplied by two black light UV-lamps. The main characteristics and dimensions of the reactor, lamps and reflectors are shown in Table 1. The system included a borosilicate glass storing tank, which was equipped with a liquid sampling valve, a thermometer and a pH control. Also, the experimental setup had an all-glass heat exchanger connected to a thermostatic bath, to keep the temperature constant during the reaction (25 °C), and a centrifugal pump to achieve a high recirculating flow rate of the aqueous solution.

The experimental procedure started when the herbicide solution was added to the storage tank; then the iron sulfate solution was added and the pH adjusted to 2.8–3. Afterwards, an initial amount of hydrogen peroxide solution was added and the liquid was well mixed by recirculation. Afterwards, the first sample was withdrawn and immediately, in the case of photo-Fenton runs, the reactor cover

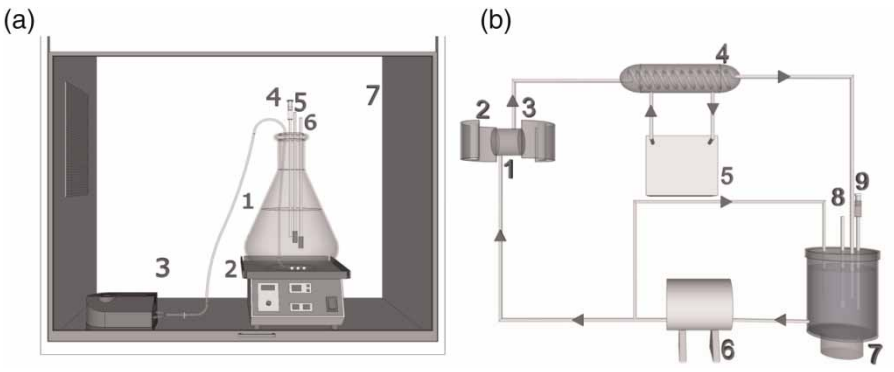

Figure 1 | Flow sheet of the experimental device. (a) *Biological reactor*: 1–reactor, 2–orbital shaker with temperature control, 3–aeration pump, 4–liquid sampling, 5–pH control, 6–DO control, and 7–fume hood. (b) *Photoreactor*: 1–photoreactor, 2–UV-lamp, 3–parabolic reflector, 4–heat exchanger, 5–thermostatic bath, 6–pump, 7–storage tank, 8–thermometer, and 9–liquid sampling.

Table 1 | Reactor, lamp, and reflector characteristics and dimensions

Reactor	
Irradiated volume	69.94 cm^3
Diameter	4.40 cm
Length	4.60 cm
Total liquid volume	3,000 cm^3
Net radiative flux at the reactor wall (q_w)	8.63×10^{-9} Einstein $cm^{-2}\ s^{-1}$
Lamp Philips TL K 40W/09 N	
Nominal power	40 W
Output power: 315 nm $\leq \lambda \leq$ 400 nm (peak at 365 nm)	6.6 W
Diameter	3.8 cm
Nominal arc length	61 cm
Reflector	
Parabola characteristic constant	2.75 cm
Distance vertex of parabolic reflector reactor	6.0 cm
Length	50 cm

removed to start the reaction. Experimental runs were carried out during 200 min, and the liquid sampling operation was repeated at equal time intervals.

A set of experimental runs for three hydrogen peroxide to atrazine molar ratios ($R = 35$, 105 and 210), $C^0_{Fe3+} =$ 10 ppm and with and without radiation was performed.

Analytical methods

For the biological experiments glucose concentration was determined by the enzymatic colorimetric method (Lott & Turner 1975). For the determination of the VSS, analytical methods included in the *Standard Methods for the Examination of Water and Wastewater* (APHA 1995), Technique 2540-E, were used. This, in turn, requires the determination of total suspended solids (TSS), Technique 2540-D from the same source.

Atrazine concentration was measured using reverse phase liquid chromatography (flow rate 1 mL min^{-1}) with a UV detector (detection wavelength equal to 221 nm) in a HPLC–UV (Waters, Model Code 5CH) with C-18 column (X-Terra® RP) and employing acetonitrile/water (50/50) as the mobile phase. Hydrogen peroxide concentration was analysed with a modified iodimetric technique (Allen *et al.* 1952) using a UV–VIS CARY 100 BIO spectrophotometer at 350 nm and ferrous ions with absorbance measurements of the Fe(II)–phenantroline complex at 510 nm.

RESULTS AND DISCUSSION

Biological experiments

The results are summarized in Figure 2, where the evolution of glucose concentration for each experimental run is depicted. Note that in all cases there is a lag-phase of approximately 2 h, corresponding to a pre-inoculums acclimatization period. Afterwards, when each one of the herbicides were added to the medium, there was a marked decrease in the glucose consumption rate.

Comparison of specific rate of glucose degradation

To analyse the effect of the herbicides concentration on the biotreatment performance, the biodegradation kinetics were modelled. Generally, the kinetic models are based on empirical equations, which relate the biomass growth with the substrate uptake, glucose in this particular case. The most general case of kinetic representation is the biomass increase in a time period; thus, the equation describing the kinetics for the cases that do not present either decay or substrate uptake for maintenance, is given by:

$$r_x = \frac{dX}{dt} = \mu X \tag{1}$$

and

$$r_s = \frac{dS}{dt} = -\frac{1}{Y_{x/s}} r_x \tag{2}$$

where X is the biomass or microorganisms concentration (mgVSS L^{-1}), S the limiting substrate concentration (mgGl L^{-1}), r_x the growth rate of microorganisms (mgVSS

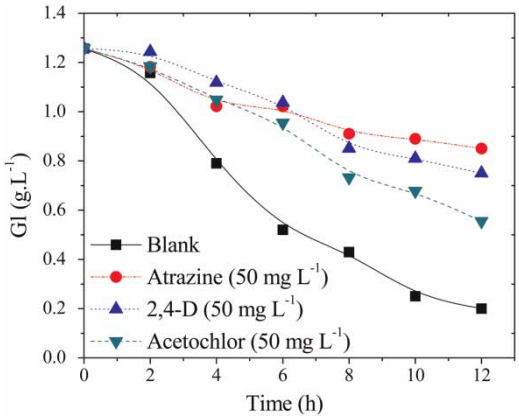

Figure 2 | Glucose concentration against time for different experimental runs.

$L^{-1}\,h^{-1}$), r_s the rate of glucose uptake by microorganisms (mgGl $L^{-1}\,h^{-1}$), μ the specific growth rate of biomass (h^{-1}), and $Y_{x/s}$ the biomass yield (mgVSS $mgGl^{-1}$).

One of the most widespread models is the Monod's model (Monod 1949). When S is several times greater than the half saturation constant (K_s), Monod's equation reduces to the called 'first order approximation to Monod's Model':

$$\mu = k_x S \tag{3}$$

Taking into account that the biomass does not suffer considerable variations in percentage terms during the course of the biological degradation, a second assumption can be made: a mean value of the biomass concentration is considered ($X = \bar{X}$). Thus,

$$k_s' = k_s \overline{X} \tag{4}$$

where k_s is the specific substrate removal rate (L.mgVSS^{-1} h^{-1}) and k_s' is the modified removal rate (h^{-1}). Then, solving the integral with the initial condition $S = S_0$ at $t = 0$, the result is:

$$-\ln\frac{S}{S_0} = k_s' t \tag{5}$$

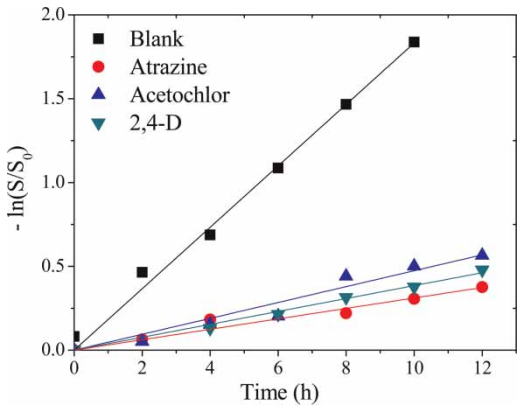

Figure 3 | Experimental data fit using the first order approximation to Monod's Model with $\bar{X}$.

As can be seen in Figure 3, this simple kinetic model allows a reasonable adjustment of the experimental data for each of the studied herbicides. The average values obtained from the biomass ($\bar{X}$) in each run, together with the calculated values of k_s' and k_s, are shown in Table 2. The percentages of decrease in the specific rate of glucose uptake, k_s, obtained by the different herbicides with respect to the corresponding specific rate obtained in the 'blank' run, are also shown in the fifth column of Table 2.

Fenton and photo-Fenton experiments

Atrazine was the herbicide that caused the greatest inhibition of the biological activity (measured in terms of specific rate of glucose consumption) of the aerobic biomass typically found in the activated sludge. Based on these results, preliminary studies were conducted to investigate the feasibility of the atrazine degradation by Fenton and photo-Fenton processes in order to remove it from effluents of biological treatment plants.

Figure 4 illustrates typical experimental results of the atrazine relative concentration (C_{At}/C_{At}^0) as a function of time, for irradiated and non-irradiated experiments. To study the effects produced by the addition of hydrogen peroxide on the pollutant degradation for the photo-Fenton process, a set of experimental runs for three atrazine to hydrogen peroxide molar ratios ($R = 35$, 105 and 210) and a constant value of the ferric iron initial concentration (10 ppm) was performed; the corresponding H_2O_2/Fe molar ratios for these experimental runs are 45.4, 135.7 and 271.5, respectively. On the other hand, to compare the Fenton and photo-Fenton degradation rates, the following operating conditions were adopted: $R = 35$ and $C_{Fe3+}^0 = 10$ ppm.

Notice that the non-irradiated reaction rate is always lower than the corresponding irradiated reaction rate; for example, for a reaction time equal to 120 min, the photo-Fenton system produces an organic pollutant conversion 51% greater than that obtained with the Fenton system.

Table 2 | Average values of biomass concentration and computed values of k_s', k_s, with their respective standard errors, and the percentage of k_s decrease for each agrochemical

Experimental runs	$\bar{X}$ (gVSS L^{-1})	k_s' (h^{-1})	k_s (L.gVSS^{-1} h^{-1})	Decrease k_s (%)
Blank	1.48	0.1832 (±0.00308)	0.1238 (±0.00208)	–
2,4-D	1.49	0.0385 (±0.00082)	0.0258 (±0.00055)	79.1
Atrazine	1.52	0.0304 (±0.00062)	0.0199 (±0.00041)	83.9
Acetochlor	1.49	0.0474 (±0.00257)	0.0317 (±0.00173)	74.4

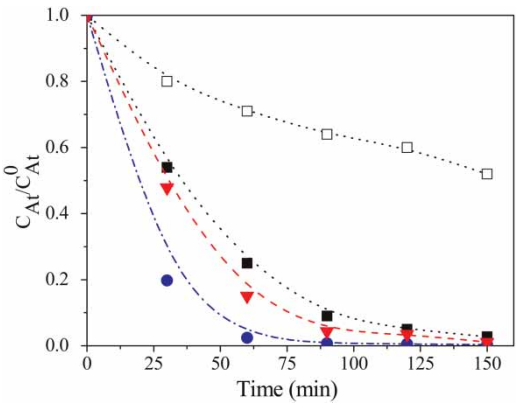

Figure 4 | Atrazine relative concentration as a function of time for Fenton and photo-Fenton processes at pH = 3, with $C^0_{Fe3+} = 10$ ppm, and different atrazine to hydrogen peroxide molar ratios (R). Fenton processes: $R = 35$ (···, □); photo-Fenton processes: $R = 35$ (···, ■), $R = 105$ (- - -, ▼) and $R = 210$ (··· , •).

Besides, for the photo-Fenton treatment, it can be observed that increasing the atrazine to hydrogen peroxide molar ratio (R) from 35 to 210, increases the atrazine conversion.

Additional information about the effect of the hydrogen peroxide and ferric ion initial concentrations on the degradation and mineralization processes of atrazine under non-irradiated (Fenton) and irradiated (photo-Fenton) conditions can be found in Benzaquén *et al.* (2012).

CONCLUSIONS

It was found that the inoculated bacteria were able to metabolize almost all the glucose in the agrochemicals-free medium in about 12 h, while in this period of time a lower glucose consumption was observed when the tested agrochemicals (2,4-D, atrazine and acetochlor) were added to the medium. In all cases, the herbicide addition has led to a decrease in the specific glucose degradation rate.

The biological results could be satisfactorily adjusted with the 'First-Order Approximation to Monod's Model', from which it was possible to calculate the specific rate of glucose uptake for each agrochemical. When these results were compared with the agrochemicals-free assay, a negative effect on the specific glucose uptake rate was observed.

At the same time, it was observed that atrazine had the most important inhibition effect on the microorganism performance, as this herbicide diminished the value of the specific rate of glucose uptake of about 84%.

Fenton and photo-Fenton processes have been demonstrated to degrade atrazine in water samples. As expected, we also found that the use of UV-radiation significantly improved the effectiveness of the Fenton system.

These results suggest the convenience of eliminating these pollutants before entering a biological treatment plant, employing a chemical oxidation treatment in order to reduce them to simpler and less toxic molecules. In particular, Fenton or photo-Fenton processes can be attractive technologies to degrade atrazine.

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