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COUPLED PHOTOCHEMICAL-BIOLOGICAL SYSTEM TO TREAT BIORECALCITRANT WASTEWATERS

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Chapter 1: Introduction

The need of fresh ***water*** in our time has become a problem of important concern. Many sources have been exhausted and other sources are likely to be polluted due to the industrial activity, of special impact among the developed or developing countries. According to the United Nations World Health Organization (WHO), lack of adequate sanitary facilities and poor hygienic practices are common throughout the developing countries, due to political, economical and climatic reasons (*WHO, 2005*). The lowest levels of service coverage are to be found in Asia and Africa where more than half of the rural populations are excluded from any measurable progress in this area. Globally, 2400 million people, most of them in developing countries, do not have access to improved sanitation facilities. Data collected over ten years show that little progress has been made in reducing this number.

Many different ***chemicals*** are discharged into the aquatic environment. Some of them are not only toxic but also partly or even barely biodegradable. Therefore, they are not easily removed by biological means. The need to restore water for new uses makes purification of ***wastewater*** practically essential to achieve a desired degree of quality. To this end, other suitable wastewater ***treatment technologies*** have to be studied. Recently, an important class of technologies named ***Advanced Oxidation Processes*** (AOPs) have emerged as suitable for accelerating the oxidation and destruction of a wide range of organic contaminants in polluted water (*Glaze et al., 1987*).

1.1.- Social and Legal Framework

Water concern severely rises at the beginning of the 21st century and menaces to have bad consequences for many sectors of our society. The most pessimistic predictions, state that water would be reason of war in a non-far future.

Industrialization has entailed a significant increase of water needs, not only for industrial purposes but also for domestic uses. Furthermore, Earth population has almost doubled in the last 30 years. Sources of fresh water are scarce and insufficient. It occurs more often the situation that vast areas of Earth, in developed countries as well, suffer lack and shortage of water due to Climate Change. Furthermore, areas with a certain level of population density suffer desertification. World Health Organization reports that it is indispensable to secure access to clean water and to adequate sanitation facilities for all people, in order to eradicate all kinds of diseases.

Thus, the need to reuse water arises, and becomes essential. It is indispensable to achieve a certain degree of quality for each different purpose, such as purification of drinking water, recycle water for industrial or agricultural needs, or treat water to be poured back to rivers after its use, among others functions.

It is undeniable that a great effort has been carried out around treatment procedures and technologies. From simply dilution to the environment in the industrial revolution, to end-of-pipe treatment technologies and finally the application of the "Clean Processes" philosophy, which have entailed the emergence of new concepts such as Recycling, Reuse or Reduce. Thank to Research & Development centres, more specific, better, faster and accurate treatment technologies are available. Moreover, it is possible to control more factors and new quality control parameters have been identified.

An example of these control possibilities is the Toxic Release Inventory (TRI) Program of the United States Environmental Protection Agency (EPA). Table 1.1-1 shows data of on-site surface water discharges of phenol-like pollutants that have been monitored for different years. It can be pointed out that discharges have been reduced for almost all pollutants.

In developed countries, society is more conscious of environmental problems and puts pressure on governments to improve legislation. Moreover, environmental disasters usually have strong political consequences every time that occur. Consequently, authorities tend to write laws that are

more restrictive in order to reduce risks on many procedures and processes, such as chemical and industrial activities, but also to increase drinking water quality or to protect the environment.

Table 1.1-1: Annual release of toxic phenol-like pollutants in the United States. On-site surface water discharges for facilities in all industries (*EPA, 1988; EPA, 2000; EPA, 2005*).

Compound	Emission 1988 (ton.year^{-1})	Emission 2000 (ton.year^{-1})	Emission 2005 (ton.year^{-1})	2005 Overall Ranking[a]
phenol	117.6	21.3	45.8	21
2,4-dinitrophenol	44.8	10.6	0.20	143
catechol	145.4	8.3	18.1	32
hydroquinone	3.3	1.9	4.9	66
quinone	0.06	0.64	0.0	362
pentachlorophenol	1.1	0.31	0.24	134
p-cresol	0.52	0.18	0.12	157
Chlorophenols	0.12	0.045	0.025	192
2-nitrophenol	0.0005	0.025	0.027	191
2,4,5-trichlorophenol	0.0	0.023	1.6	97
2,4-dichlorophenol	0.049	0.023	0.012	205
m-cresol	0.13	0.019	0.247	132
2,4,6-trichlorophenol	0.023	0.013	0.6	109
4-nitrophenol	0.00	0.007	0.212	139
o-cresol	0.20	0.006	0.056	177

[a]Ranking among all reported chemicals in a selected year

In Europe, environmental and consequently water-related policies are promoted by the European Parliament. The European Union (EU) has established a Community framework for water protection and management. The framework Directive provides, among other things, for the identification of European waters and their characteristics, based on individual river basin districts, and the adoption of management plans and programmes of measures appropriate for each body of water.

The most recent update is the European Water Framework Directive (WFD) 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy (*EU, 2000*). This directive replaces, harmonises and further develops the Community controls under Council Directive 76/464/EEC. The framework directive identifies different specific uses of water, such as drinking water, bathing water, water suitable for fish-breeding, shellfish water quality and urban wastewater treatment. Due to their volume, discharges of urban wastewater are the second most serious cause of pollution of waters in the Union.

Based on the Directive 2000/60/EC, and amending some of its articles, other regulations have been published. One of the most important is the Decision 2455/2001/EC (*EU, 2001*), which establishes the list of priority substances in the field of water policy. It may be pointed that among the priority substances, there are substituted phenols or non-biodegradable chlorinated species (NBCS), such as herbicides and pesticides. The priority substances are identified using a procedure for compiling data produced by a method suggested in the Directive 2000/60/EC: the COMMPS procedure: Combined Monitoring-based and Modelling-based Priority Setting. The list will be reviewed and adapted by the European Commission at the latest four years after the entry into force of the Water Framework Directive, and then at least every four years. The review will take account of any information, which comes to its attention.

More recently, pharmaceuticals and personal care products (PPCPs) and especially endocrine disrupting chemicals (EDCs) are considered as emerging contaminants, which are still unregulated or in process of regularisation (*Barcelo, 2003*). Probably they are going to appear in the next Directives.

Special care must be taken of the levels of quality of water intended for human consumption. Directive 98/83/EC (*EU, 1998*) establish a list of chemical parameters and values that water must accomplish to be supplied. For example, total concentration of pesticides or polycyclic aromatic hydrocarbons (PAHs) cannot be higher than 0.5 and 0.1 $\mu g.L^{-1}$ respectively.

Recent texts add new aspects about the protection of the aquatic environment and groundwater against pollution. Directive 2006/11/EC (*EU, 2006b*) regulate the pollution caused by certain dangerous substances discharged into the aquatic environment, whereas Directive 2006/118/EC (*EU, 2006c*) aims for the protection of groundwater against pollution and deterioration. The European Union emits also more specific regulation, regarding the substances' nature. For example, the Council Decision 2006/507/EC (*EU, 2006a*) regulates the elimination and minimisation of production, use and release of Persistent Organic Pollutants (POPs).

To meet the internal goals set by EU legislative framework, pollutant sources have to be identified and appropriate environmental technologies have to be developed and implemented (*Gernjak, 2006*). Advanced Oxidation Processes (AOPs), the combination of AOPs and biological treatment and the application of solar radiation as a driving force of the AOPs have been identified by the European Commission (EC) (*EC, 2003; EC, 2005*).

1.2.- Advanced Oxidation Processes

Due to the toxic characteristics of non-biodegradable organic pollutants, e.g. NBCS or POPs, a wastewater polluted by these compounds may not be treated by a conventional biological process. In addition, separation technologies do not really eliminate the problem, since they transfer the pollution to another phase or a more concentrated effluent.

A family of processes that may be appropriate for treating organic pollutants are the so-called Advanced Oxidation Processes (AOPs) or Advanced Oxidation Technologies (AOTs).

AOPs are promising methods for the remediation of contaminated wastewaters containing non-biodegradable organic pollutants (*Ollis and Al-Ekabi, 1993*). They are based on the in situ generation of highly potent chemical oxidants such as the ***hydroxyl radical*** ($HO^{\bullet}$), a powerful non-selective chemical oxidant, which has a strong oxidation potential and acts very rapidly with most organic compounds. This capability of exploiting the high reactivity of radicals in driving oxidation processes is suitable for achieving the complete abatement and mineralization of the pollutant through even less contaminant species (*Malato et al., 2002*).

In Table 1.2-1 some reduction potentials of well-known oxidants in acidic media are tabled, in which stands out the high potential of the hydroxyl radical over other oxidants.

Table 1.2-1: Standard oxidation potentials against Standard Hydrogen Electrode (SHE) of some oxidants in acidic media (*Hunsberger, 1977*).

Oxidant	E^o (V)
Fluorine	3.03
Hydroxyl radical	2.80
Atomic oxygen	2.42
Ozone	2.07
Hydrogen peroxide (H_2O_2)	1.77
Potassium permanganate ($KMnO_4$)	1.67
Hypobromous acid (HBrO)	1.59
Chlorine dioxide (ClO_2)	1.50
Hypochlorous acid (HClO)	1.49
Chlorine (Cl_2)	1.36
Bromine (Br_2)	1.09

Many systems are classified under this wide definition of AOPs. Among them, Fenton (and its related processes), ***Photo-Fenton***, Photocatalysis, Ozonation and Wet Oxidation can be mentioned. Most of the AOPs use a combination of strong oxidants, e.g. O_3 and H_2O_2, with

catalysts, e.g. transition metals ions or photocatalysts, and irradiation, e.g. ultraviolet (UV), visible (Vis), or ultrasound (US).

The AOPs may be classified depending on the source to generate the oxidizing species. This commonly used classification is shown in Figure 1.2-1, where the main Advanced Oxidation Processes can be observed: Photolysis, AOPs based on ozone, AOPs based on hydrogen peroxide, Hot AOPs, Photocatalysis, electro-chemical oxidation, Ultrasound technologies and electron beam oxidation. This work is focused on a technique based on hydrogen peroxide. Its name is ***Photo-Fenton***, and results from irradiating Fenton's reagent with UV or visible light.

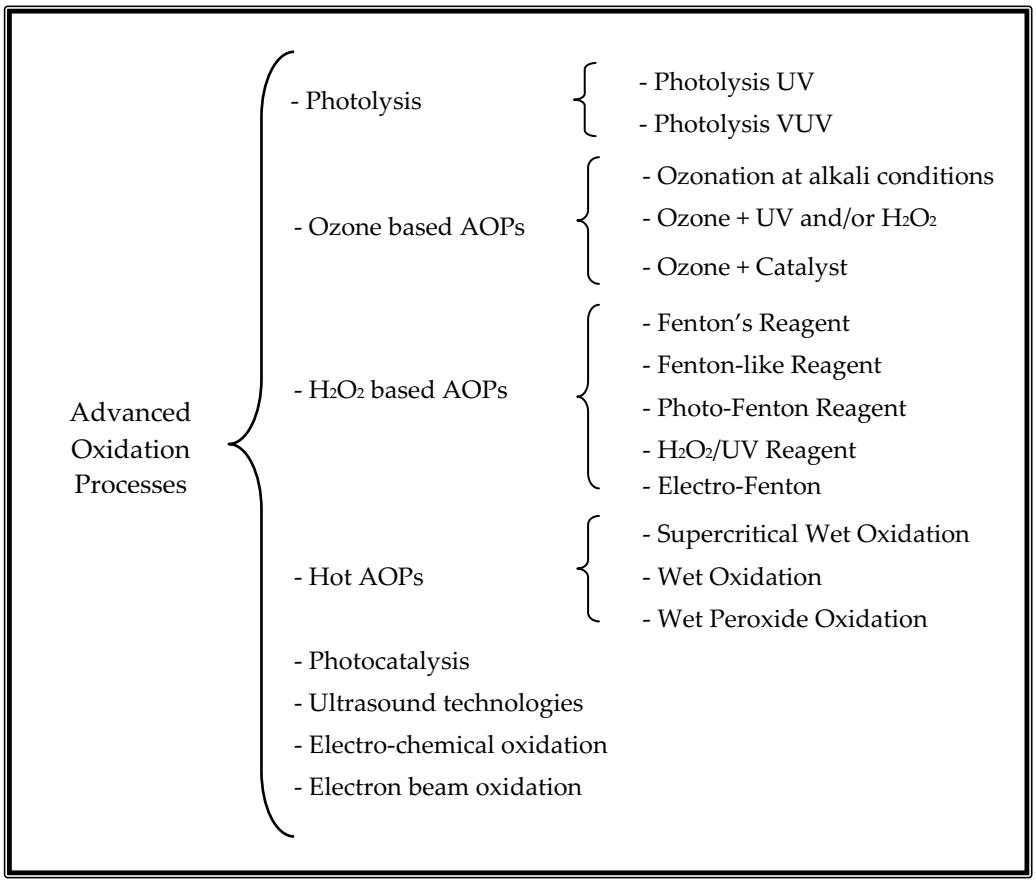


Figure 1.2-1: Classification of Advanced Oxidation Processes.

1.2.1.- Overview of different AOPs

Next, some of the AOPs are explained in order to give a brief idea of the different kind of processes of this family.

Photolysis as an AOP

Among the photolysis technologies, it is possible to distinguish the Ultraviolet photolysis and the Vacuum-ultraviolet (VUV) photolysis. Direct photolysis involves the interaction of light with molecules -in addition to water- to bring about their dissociation into fragments (*Braun et al., 1993*). Thus, in every process that UV radiation is used as an energy source, photolysis might occur, or at least, there is relation between organic matter and UV light.

VUV photolysis consists of reactions carried out under UV irradiation which spectral domain is around 140-200 nm. Under these operating conditions two degradation processes may take place: the direct photolysis of organics and the formation of oxidizing radicals, such as the hydroxyl ($HO^{\bullet}$) radical (*Gonzalez et al., 2004*).

Ozone based Advanced Oxidation Processes

Ozone is a very powerful oxidizing agent, which is able to participate in a great number of reactions with organic and inorganic compounds. Among the most common oxidizing agents, ozone is only surpassed in oxidation power by fluorine, hydroxyl radicals and atomic oxygen (see Table 1.2-1). Ozonation chemistry is complex; it is characterized by driving the oxidation in two mechanisms; the direct reaction with the dissolved molecular ozone (O_3) and the indirect reactions with the radical species ($HO^{\bullet}$, $HO_2^{\bullet}$), that are formed when ozone decomposes in water (*Hoigne and Bader, 1975; Hoigne and Bader, 1976*). The combination of both pathways for the removal of a compound will depend on the nature of this, the pH of the medium and the ozone dose (*Beltran et al., 1997*).

The ozonation of dissolved compounds in water can only constitute an AOP by itself when the hydroxyl radicals are the oxidizing agents of the process. At pH 10, for example, the half-life of ozone in water may be less than one minute. Thus, ozonation is considered an AOP when carried out at alkali conditions (above pH= 9). At these conditions, three molecules of ozone produce two $HO^{\bullet}$ radicals according to Reaction 1.2-1 (*Gottschalk et al., 2000*).

$$3O_3 + HO^- + H^+ \rightarrow 2HO^{\bullet} + 4O_2$$ Reaction 1.2-1

Ozone treatment may be enhanced by the addition of hydrogen peroxide and/or UV radiation (*Peyton and Glaze, 1988*) or the presence of metal cations, such as ferrous and ferric ions or by alumina.

Hydrogen peroxide based Advanced Oxidation Processes

Hydrogen peroxide is a safe, efficient and easy to use chemical oxidant suitable for a wide usage on contamination prevention. However, since hydrogen peroxide itself is not an excellent oxidant for many organic pollutants, it must be combined with other substances, such as metal salts or ozone, or an application of energy, as UV light to produce the desired degradation results. Figure 1.2-2 shows the ultraviolet absorption spectrum for H_2O_2, which indicates that the absorption is certainly active over the entire UV spectrum.

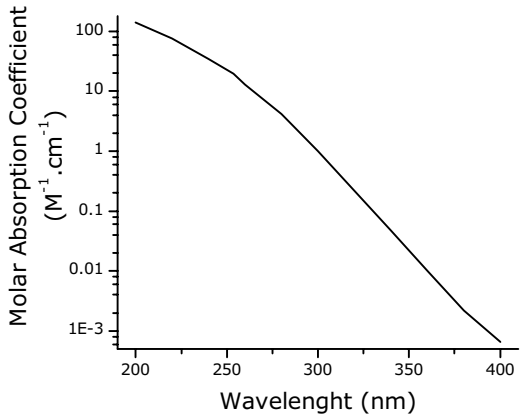


Figure 1.2-2: H_2O_2 Ultraviolet Absorption Spectrum.
http://www.h2o2.com

Among these techniques, are noteworthy the UV/H_2O_2 process, the Fenton process, the Photo-Fenton process and all the related processes (Fenton-like for example). Fenton and photo-Fenton will be largely commented below.

Regarding the UV/H_2O_2, it is a process which involves the generation of HO• radical through UV photolysis of H_2O_2 with a yield of two radicals formed per photon absorbed by 254 nm radiation (*Parsons, 2004*).

Hot Advanced Oxidation Processes

Among these technologies Supercritical Water Oxidation (SCWO), Subcritical Oxidation or Wet Oxidation (WO) and Wet Peroxide Oxidation (WPO) can be mentioned. These processes differ

from the rest of the AOPs not only in terms of operating conditions but also in the concentration of the pollutants present in the wastewater. They are used mainly for concentrated wastewaters in order to allow auto thermal operation, and thus a reduction in the operating costs. SCWO takes place above the critical point of water (T>375 ºC and P>22.1 MPa) and WO engages with oxidation at a temperature range of 125–300 ºC and pressures of 0.5–20 MPa.

Wet Oxidation involves the liquid phase oxidation of organic or oxidizable inorganic components at elevated temperatures and pressures using a gaseous source of oxygen. When using H_2O_2 instead of oxygen or air as the oxidizing agent, the process is named Wet Peroxide Oxidation, and in this case the operating conditions of temperature and pressure are lower (*Garcia-Molina et al., 2005*), hence the running costs might be lower. WO process efficiency may be improved by interaction with catalysts, being then named Catalytic Wet Oxidation (*Silva et al., 2004*).

Photocatalysis for oxidation of pollutants

Photocatalytic degradation has proven to be a promising method for degrading refractory chlorinated aromatics (*Calza et al., 1997; Davis and Green, 1999; Wei, 1999*), with the advantage of carrying out the treatment under relatively mild reaction conditions (*Rao et al., 2003*). Differently from the conventional catalytic methods, the catalyst is in this case activated by photons instead of a thermal activation. A largely used combination in this technology is the TiO_2/UV light system, where Titania is under the form of anatase. When illuminated with light of energy higher than the band gap, electrons and holes are formed in a semiconductor and are capable of initiating chemical reactions (*Herrmann, 1999*).

High energy AOPs: Ultrasound and Electron beam technologies

The origin of ultrasonically induced oxidation is the generation of cavitation bubbles. Each bubble acts as a reactor with supercritical operating conditions. In aqueous systems at an ultrasonic frequency of 20 kHz a cavitation bubble is supposed to reach 4000 ºK of temperature and 1000 bar of pressure. Ultrasound is useful for water decontamination, surface cleaning, soil washing, control of air-borne contamination or sewage treatment (*Parsons, 2004*)

The electron beam process uses the Coulomb interaction of accelerated electrons with atoms or molecules of gases, liquids or solids. By these interaction ions, thermalised electrons, excited states and radicals are formed. The free radicals react with organic matter. For example, for disinfection purposes, they react with cell membranes, enzymes and nucleic acids to destroy microorganisms (*Martin et al., 2005*).

1.2.2.- Application Range of AOPs

A suggestion of application of different oxidation processes is shown in Figure 1.2-3. The figure shows a technology map, which indicates a possible application range of each oxidation processes. The map outlines the areas where technologies are most effective according to the authors. The boundaries should be used only as a guide, since there are many particular cases. According to the map, at high TOC, hot AOPs are important technologies, since only air or oxygen as relatively low cost oxidants, are able to keep operating costs down. On the other hand, at high flow rates biological treatment plants are viable, since they do not entail technological difficulties when are scaled-up. The area of importance in the present work is at low flow rates with medium and low organic loads. AOPs are important technologies at these conditions.

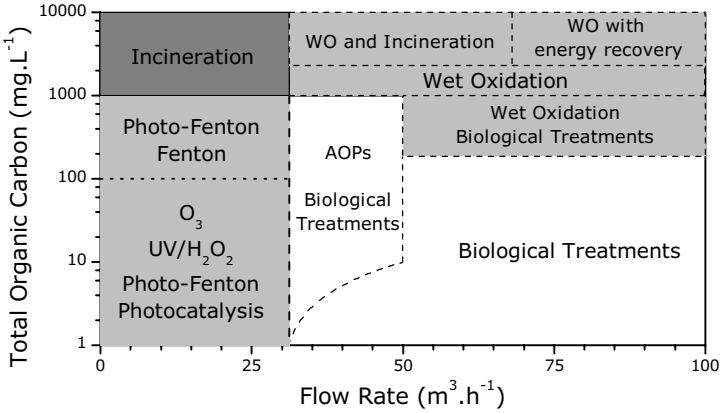


Figure 1.2-3: Technology map. Application range of different oxidation technologies. Adapted from *Hancock* (*1999*) and *García-Molina* (*2006*).

1.3.- Photo-Fenton Process

Photo-Fenton (Ph-F) process, or photo-assisted Fenton process is an efficient method for wastewater treatment (*Bauer and Fallmann, 1997*). Ph-F is known to be able to improve the efficiency of Fenton reaction (Reaction 1.3-1) -also named dark-Fenton in order to distinguish better both processes- by means of the interaction of light radiation and Fenton's reagent (Reaction 1.3-2). Thus, the Ph-F process is mostly based on the Fenton mechanism, with a combination of reactions in which light contributes.

The Fenton reaction, Reaction 1.3-1, was first reported by H. J. H. Fenton in 1894 (*Fenton, 1894*). He reported that H_2O_2 could be activated by Fe^{2+} salts to oxidize tartaric acid. The key reaction

occurs between a Fe^{2+}-aquo-complex and H_2O_2 but the reaction can be described differently. In 1934, *Haber and Weiss* (*1934*) proposed that the active oxidant generated by the Fenton reaction is the ***hydroxyl radical*** ($HO^{\bullet}$), one of the most powerful oxidants known (see Table 1.2-1), to provide what is now referred to as the "classical" or "free radical" chain reaction. This mechanism has been expanded and revised during the 20th century until 1975, when Walling published an influential account (*Walling, 1975*). Later, others would propose that high-valent (Fe^{4+} or Fe^{5+}) oxoiron complexes might also participate in Fenton chemistry (*Bossmann et al., 1998*).

$$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + HO^- + HO^{\bullet}$$ Reaction 1.3-1

$$Fe^{3+} + H_2O \xrightarrow{h\nu} Fe^{2+} + H^+ + HO^{\bullet}$$ Reaction 1.3-2

1.3.1.- Classic reaction mechanism

As it is classically described, hydroxyl radicals are produced by Fenton reaction by decomposition of ***hydrogen peroxide*** when reacting with ***ferrous ions*** (*Walling, 1975*) (Reaction 1.3-1). Irradiation with sunlight or an artificial ***light*** source of wavelength 180-400 nm (*Wadley and Waite, 2004*) or even in the visible spectra (*Oliveros et al., 1997*), increases the rate of contaminant degradation mainly by stimulating the reduction of Fe^{3+} to Fe^{2+} (*Wadley and Waite, 2004*) (Reaction 1.3-2). This is the so-called ***photo-Fenton reaction*** (*Parsons, 2004*). Therefore, the concentration of hydroxyl radicals becomes higher (*Benitez et al., 2000*). In consequence, it promotes a faster degradation of the pollutant. In theory, by combination of Fenton and photo-Fenton reactions two moles of $HO^{\bullet}$ should be produced per mole of H_2O_2 consumed (*Parsons, 2004*). Nevertheless this reaction yield is hardly ever reached.

As it is generally accepted, hydroxyl radicals formed by the complex reaction mechanism, oxidize organic species initiating a radical chain oxidation (Reaction 1.3-3), generating organic-radical species, which undergo oxidation (Reaction 1.3-4 and Reaction 1.3-5) up to mineralization (Reaction 1.3-6). In Reaction 1.3-6, Cl^- is released in the case of oxidation of chlorinated compounds. Other species, such as sulphur or nitrogen may be found in non-biodegradable organic pollutants.

$$HO^{\bullet} + R_iH \rightarrow H_2O + R_i^{\bullet}$$ Reaction 1.3-3

$$R_i^{\bullet} + H_2O_2 \rightarrow ROH + HO^{\bullet}$$ Reaction 1.3-4

$$R_i^{\bullet} + O_2 \rightarrow ROO^{\bullet}$$ Reaction 1.3-5

$$Intermediates + HO^{\bullet} \rightarrow CO_2 + H_2O + Cl^-$$ Reaction 1.3-6

The organic free radicals produced may be oxidized during the process by Fe^{3+} (Reaction 1.3-7), reduced by Fe^{2+} (Reaction 1.3-8), or dimerised according to Reaction 1.3-9 (*Tang and Tassos, 1997; Walling, 1975*). Reaction of dimerisation agrees with the fact that polymerization occurs during the Fenton and photo-Fenton processes (*Bianco and Gehlen, 2002*).

$$R^{\bullet} + Fe^{3+} \rightarrow R^{+} + Fe^{2+} \qquad \text{Reaction 1.3-7}$$

$$R^{\bullet} + Fe^{2+} \rightarrow R^{-} + Fe^{3+} \qquad \text{Reaction 1.3-8}$$

$$2R_i^{\bullet} \rightarrow \text{product (dimer)} \qquad \text{Reaction 1.3-9}$$

The mechanistic pathway of the Fenton and Ph-F processes according to the classic interpretation is even more complex. Reaction 1.3-10 to Reaction 1.3-12 simplify the main scavenging effects that may occur during the process (*Pera-Titus et al., 2004*). Among them, Reaction 1.3-11 and Reaction 1.3-12 are of special interest, due to the participation of H_2O_2 and Fe^{2+}. The doses of these compounds (H_2O_2 and Fe^{2+}) must be optimized taking into account these scavenging effects, in order to diminish the negative effect.

$$HO^{\bullet} + HO^{\bullet} \rightarrow H_2O_2 \qquad \text{Reaction 1.3-10}$$

$$HO^{\bullet} + H_2O_2 \rightarrow HO_2^{\bullet} + H_2O \qquad \text{Reaction 1.3-11}$$

$$Fe^{2+} + HO^{\bullet} \rightarrow Fe^{3+} + HO^{-} \qquad \text{Reaction 1.3-12}$$

1.3.2.- Iron(IV) reaction mechanism

A modern interpretation of Fenton (and photo-Fenton) mechanism, assumes that other oxidizing intermediates such as highly valent iron-complexes (Fe^{4+}) are formed during oxidation of Fe^{2+} to Fe^{3+} (Reaction 1.3-13 and Reaction 1.3-14) (*Bossmann et al., 1998; Pignatello et al., 1999; Rios-Enriquez et al., 2004*).

$$Fe_{aq}^{2+} + H_2O_2 \rightarrow \left[Fe^{2+} - H_2O_2\right] \rightarrow Fe_{aq}^{4+} \qquad \text{Reaction 1.3-13}$$

$$Fe_{aq}^{4+} + H_2O \rightarrow Fe_{aq}^{3+} + HO^{\bullet} + HO^{-} \qquad \text{Reaction 1.3-14}$$

Thermodynamic calculations have demonstrated that the reaction between Fe^{2+}_{aq} and H_2O_2 as it is suggested in the classic mechanism cannot take place (*Goldstein et al., 1993; Masarwa et al., 1988*). In contrast, the formation of a hydrated iron(II)-H_2O_2 complex is thermodynamically favoured (*Bossmann et al., 1998*). The mechanism of the thermal Fenton process is described by *Bossmann et al. (1998)* by the following reactions.

Initial reactions: formation of the Fe_{aq}^{4+} complex:

$$\left[Fe(OH)(H_2O)_5\right]^+ + H_2O_2 \leftrightarrow \left[Fe(OH)(H_2O_2)(H_2O)_4\right]^+ + H_2O \qquad \text{Reaction 1.3-15}$$

$$\left[Fe(OH)(H_2O_2)(H_2O)_4\right]^+ \rightarrow \left[Fe(OH)_3(H_2O)_3\right]^+ + H_2O \qquad \text{Reaction 1.3-16}$$

The hydrated iron(II) complex (Fe^{2+}_{aq}) interacts with H_2O_2 (Reaction 1.3-15) in a ligand exchange reaction which produces the hydrated iron(II)-H_2O_2 complex. By this exchange mechanism, a steady-state concentration of iron(II) bound to H_2O_2 is reached. The formation of the intermediate iron(IV) complex (Reaction 1.3-16) occurs through a two-electron-transfer reaction. The existence of unusually charged metal complexes, Fe^{4+}_{aq}, as well as Fe^{5+}_{aq} and Fe^{6+}_{aq}, has been proven (*Rush and Bielski, 1986*).

Propagation reactions:

$$\left[Fe(OH)(H_2O_2)(H_2O)_4\right]^+ \rightarrow \left[Fe(OH)_3(H_2O)_4\right]^+ \qquad \text{Reaction 1.3-17}$$

$$\left[Fe(OH)_3(H_2O)_4\right]^+ + H_2O \rightarrow \left[Fe(OH)(H_2O)_5\right]^{2+} + HO^{\bullet} + HO^- \qquad \text{Reaction 1.3-18}$$

The intermediate iron(IV) complex may react further (Reaction 1.3-17) leading to the formation of a free hydroxyl radical and Fe^{3+}_{aq} (Reaction 1.3-18). These reactions are not of importance as long as the organic matter content is high enough to react with the Fe_{aq}^{4+} complex.

Termination and recycling:

Thermal reduction (Fenton)

$$\left[Fe(OH)(H_2O)_5\right]^+ + H_2O \leftrightarrow \left[Fe(OH)(HO_2)(H_2O)_4\right]^+ + H_3O^+ \qquad \text{Reaction 1.3-19}$$

$$\left[Fe(OH)(HO_2)(H_2O)_4\right]^+ + H_2O \leftrightarrow \left[Fe(OH)(H_2O)_5\right]^+ + HO_2^{\bullet} \qquad \text{Reaction 1.3-20}$$

$$\left[Fe(OH)(H_2O)_5\right]^{2+} + H_2O + HO_2^{\bullet} \rightarrow \left[Fe(OH)(H_2O)_5\right]^+ + O_2 + H_3O^+ \qquad \text{Reaction 1.3-21}$$

Photochemical reduction (Photo-Fenton)

$$\left[Fe(OH)(HO_2)(H_2O)_4\right]^{+*} + H_2O \leftrightarrow \left[Fe(OH)(H_2O)_5\right]^+ + HO_2^{\bullet} \qquad \text{Reaction 1.3-22}$$

Reaction 1.3-19 to Reaction 1.3-21 describe the reduction of Fe^{3+}_{aq} to Fe^{2+}_{aq} by H_2O_2 during thermal Fenton, and consequently the recycling of Fe^{2+}_{aq} to the beginning of the mechanism (Reaction 1.3-15). Concerning the photoenhanced process (Reaction 1.3-22), i.e. Photo-Fenton, it has been shown that the UV irradiation accelerates the recycling of Fe^{2+}_{aq} (*Goldstein et al., 1993*), that might be due to photoinduced oxidation of the ligands of various iron(III) complexes. This process is faster than the reduction of iron(III) to iron(II) as described in Reaction 1.3-20.

Another photoinduced mechanism is the reduction of the electronically excited Fe^{3+}_{aq} by dissolved organic matter. As long as organic matter concentration is high, the photooxidation of water (Reaction 1.3-22) does not play any significant role.

Organic matter degradation:

Once the oxidative complex is initiated, the degradation of the organic matter occurs as described in the following reaction (Reaction 1.3-23).

$$\left[Fe(OH)_3(H_2O)_3\right]^+ + C_xH_yO_z + H_3O^+ \rightarrow \left[Fe(OH)(H_2O)_5\right]^{2+} + C_xH_yO_z^{+\bullet} + H_2O \qquad \text{Reaction 1.3-23}$$

The oxidative degradation of organic matter may go further, as long as the complex formation between iron(II) and hydrogen peroxide (Reaction 1.3-15) is not obstructed. It is remarkable that the Fe^{3+}_{aq} complex obtained in Reaction 1.3-23, is reduced in Reaction 1.3-21 and continues the mechanism cycle.

Although it has been shown an evidence for the formation of the hydroxyl radical during the photolysis of Fe^{3+}_{aq} complex (*Bossmann et al., 1998*), the direct photooxidation of iron(III)-coordinated organic ligands is significantly higher as long as organic compounds are present in solution.

The mechanism also explains the scavenging effects on Fe^{2+}_{aq} and H_2O_2. The so-called back-reaction (Reaction 1.3-24) consumes Fe^{2+}_{aq}; the thermal Fenton reaction (Reaction 1.3-15) leads to depletion of both Fe^{2+}_{aq} and H_2O_2; and finally the reaction of Fe^{4+}_{aq} (generated in Reaction 1.3-16) with Fe^{2+}_{aq} (Reaction 1.3-25).

$$Fe^{2+}_{aq} + OH^{\bullet} + H^+ \rightarrow Fe^{3+}_{aq} + H_2O \qquad \text{Reaction 1.3-24}$$

$$Fe^{4+}_{aq} + Fe^{2+}_{aq} \rightarrow 2Fe^{3+}_{aq} \qquad \text{Reaction 1.3-25}$$

Reaction pathways, which may be of importance for the understanding of the mechanism of the Fenton reaction, are shown in Figure 1.3-1. All the reactions described previously are depicted, following the cycle of formation of complexes, propagation and termination, as well as the oxidation of organic matter and the photoinduced pathways. The cycle explains the mechanism as described by the "Fe^{4+} interpretation". Nevertheless, the figure is as well useful to depict most of the classic mechanism.

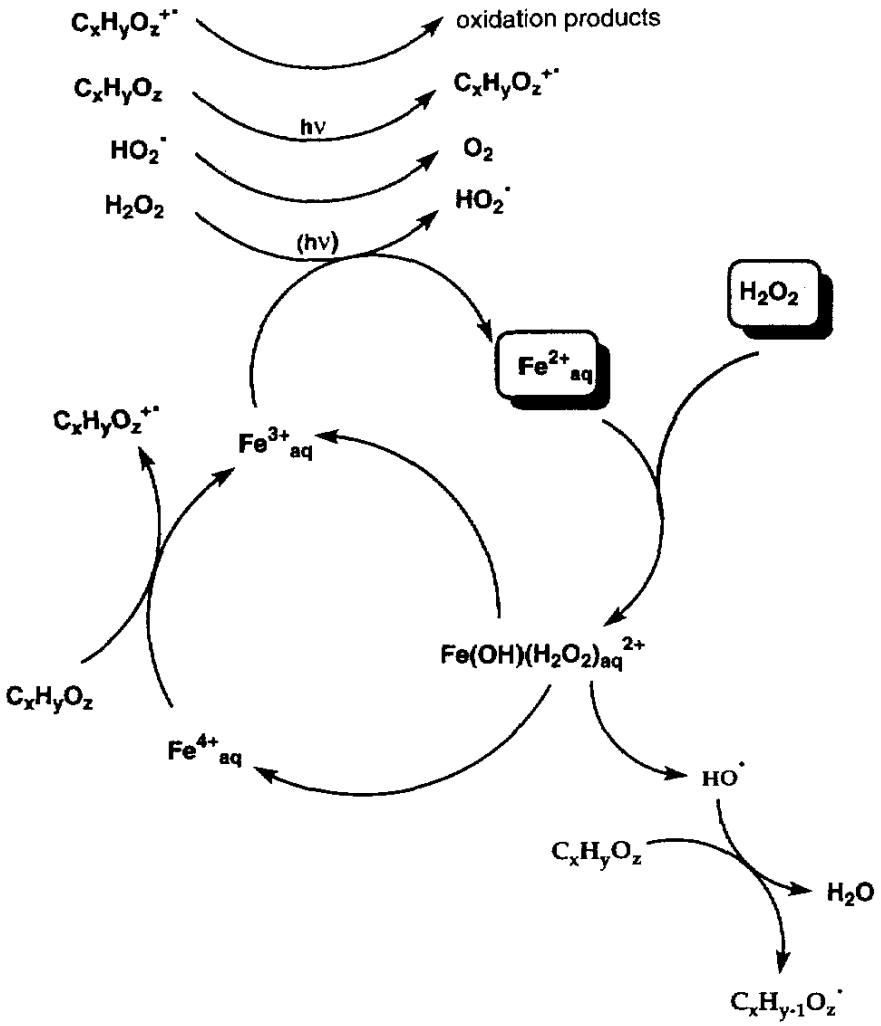


Figure 1.3-1: Mechanistic scheme of reactions involved in the thermal and in the photochemically enhanced Fenton reaction (*Lei et al., 1998*).

Table 1.3-1 shows the reduction potentials for the different species that might appear during the reaction. The high value of the $HO^{\bullet}_{aq}/H_2O_{aq}$ couple is remarkable in front of the values of complex couples. However, it seems that the production of hydroxyl radicals is too slow to compete with direct electron transfer between the substrate and a hydrated higher-valent iron species.

Table 1.3-1: Reduction Potentials of Fe^{2+}_{aq}, Fe^{3+}_{aq}, H_2O_2, and the Reactive Intermediates $HO^{\bullet}$ and Fe^{4+}_{aq}, against SHE (*Bossmann et al., 1998*).

Redox couple	E^0 (V)
$HO^{\bullet}_{aq}/H_2O_{aq}$	2.59 (pH=0)
$HO^{\bullet}_{aq}/HO^{-}_{aq}$	1.64 (pH=14)
$Fe^{3+}_{aq}/Fe{=}O^{2+}$ (porphyrine chelate)	≈0.9 (pH=0)
$Fe^{3+}_{aq}/Fe{=}O^{2+}$ (porphyrine chelate)	≈1.3 (pH=7)
$Fe^{3+}_{aq}/Fe^{4+}_{aq}$	≈1.8 (pH=0)
$Fe^{3+}_{aq}/Fe^{4+}_{aq}$	≈1.4 (pH=7)
$Fe^{2+}_{aq}/Fe^{3+}_{aq}$	0.771 (pH=0-3)
H_2O_2/H_2O	1.776 (pH=0)*a*
H_2O_2/H_2O	0.878 (pH=14)*a*
H_2O_2/O_2	0.682 (pH=0)*a*
H_2O_2/O_2	-0.076 (pH=14)*a*

1.3.3.- Various aspects of Photo-Fenton process

Many photochemical reactions are possible in Photo-Fenton systems. Diverse aspects such as the emission spectrum of the light source, concentration and absorbance of photoactive species and quantum efficiencies, affect the contribution of a given reaction of the complex Photo-Fenton mechanism (*Pignatello et al., 2006*). Another effect that it has to be considered is the influence of some organic species on the reactivity of iron, and consequently on the rate or course of the reaction.

Hydroxyl radicals, or the oxidizing agents, oxidize the organic matter in different pathways. In the case of aromatic pollutants, hydroxyl radicals may add to the aromatic or heterocyclic rings (hydroxylation). The ring system usually is hydroxylated before it is broken up during the oxidation process (*Gernjak, 2006*), as it shows Figure 1.3-2 (*Neyens and Baeyens, 2003*), which describes the oxidation pathway of a benzene molecule.

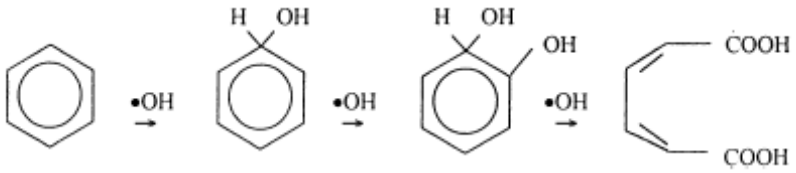


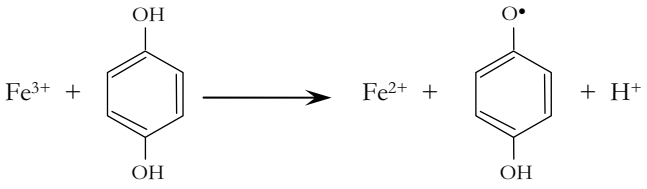
Figure 1.3-2: Aromatics degradation scheme

Substances containing quinone and hydroquinone structures are frequently found as intermediates of Photo-Fenton degradation of aromatic pollutants, produced by reactions equivalent to the scheme in Figure 1.3-2.

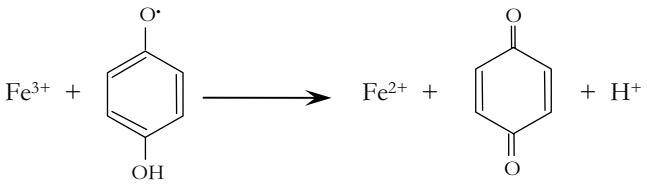
As stated by *Chen and Pignatello* (*1997*) quinones may act as catalysts (in Fenton and Ph-F) and photocatalysts (in Ph-F) in the presence of Fe^{3+} and H_2O_2 and may cause a reduction even above wavelengths where Fe^{3+} and H_2O_2 are not photolyzed, which might enhance the degradation rate.

In dark-Fenton, quinone (also named benzoquinone) and hydroquinone may provide an alternative, quicker pathway for the regeneration of Fe^{2+} through another compound named semiquinone (*Chen and Pignatello, 1997*), which is further recycled to quinone by different mechanisms. Thereby, each molecule can reduce several ferric iron ions in a catalytic cycle. Anyway, this catalytic cycle is finally interrupted, since by further hydroxylation the ring finally opens, and leads to mineralization of the molecule, as it occurs in Figure 1.3-2 with a benzene molecule.

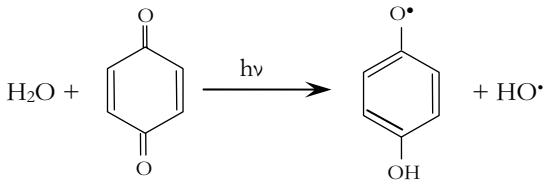
In Ph-F process, occurs an additional reaction that participates in the quinone-catalytic cycle, which is the photolysis of quinone to semiquinone. As a side product, even a hydroxyl radical is generated. Reaction 1.3-26 to Reaction 1.3-28 show the quinone-catalytic cycle as explained by *Chen and Pignatello* (*1997*) that may occur during Fenton (first and second reactions) and Ph-F (also the third reaction).



Reaction 1.3-26



Reaction 1.3-27



Reaction 1.3-28

Effect of Fenton's reagents

Many parameters might affect the efficiency or degradation rate of photo-Fenton. As seen above, during the description of reaction mechanisms, H_2O_2 and Fe^{2+} doses might affect the efficiency of the process (*Torrades et al., 2003*). In fact, these parameters are taken into account in the experimental study of the process.

According to the mechanisms, there are adverse reactions participated by H_2O_2 or iron. Thus, an excess of reagents may cause an increase of these negative reactions kinetics.

Effect of pH on the process

Another parameter that might produce a negative influence on the process is pH (*Kavitha and Palanivelu, 2004; Pignatello et al., 2006*). As it is generally accepted, photo-Fenton reaction is optimum at pH 2.8 (*Pignatello, 1992*) where approximately half of the Fe(III) is present as Fe^{3+} ion and half as $Fe(OH)^{2+}$ ion, which is the photo-active species. Below this pH, the concentration of $Fe(OH)^{2+}$ declines and at higher pH, Fe(III) precipitates as oxyhydroxides. Figure 1.3-3 and Figure 1.3-4 show the different nature of iron in water depending on pH. As shown in the figures, at pH 2.8, the photoactive $Fe(OH)^{2+}$ present a maximum concentration.

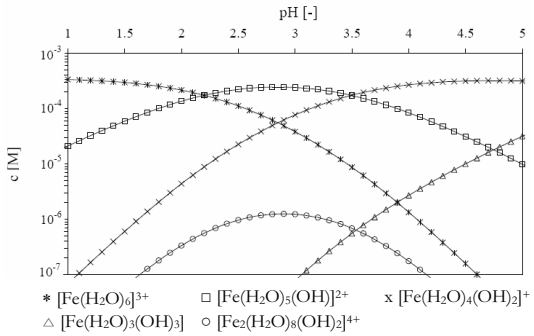


Figure 1.3-3: Ferric iron species present in aqueous solution at different pH at a concentration of 20 mg.L^{-1} (*Gernjak, 2006*).

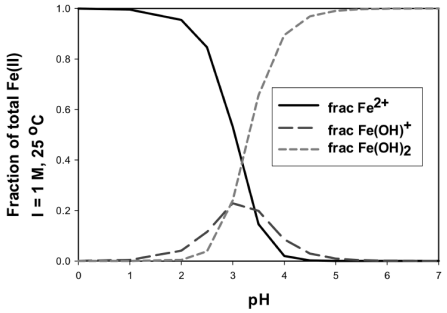


Figure 1.3-4: Speciation of Fe^{2+} in water as a function of pH at 1 *M* ionic strength (*Pignatello et al., 2006*).

Interestingly, some authors state that Fe^{2+} and its oxalate, citrate, and phosphate complexes react with H_20_2 efficiently to produce $HO^{\bullet}$ in water at pH values ranging from 3 to 8. (*Zepp et al., 1992*). This fact agrees with some authors' conclusions (*Huston and Pignatello, 1996*), who state that the use of ferrioxalate as a source of Fe^{2+} for the Photo-Fenton process, increase their possibilities since it produces additional degradation pathways, by generation of other (than hydroxyl) radical species.

Effect of Temperature

The influence of temperature on this technology has been described in the literature. Obviously, temperature affects the reaction kinetics, but depending on the mechanism, may affect (or not) the process's overall efficiency, since it may accelerate the adverse reactions more than the positive ones. Interestingly, *Lee and Yoon* (*2004*) observes a dependency on $HO^{\bullet}$ production rate, but *Freitas et al.* (*2005*) do not report any significant influence. Therefore, one of the parameters under evaluation in the experimental study of Photo-Fenton is the effect of temperature on removal results and biodegradability enhancement.

Effect of inorganic anions:

A supposed scavenging effect of Cl^- (*Maciel et al., 2004*) and other ions (*De Laat et al., 2004*) on Fenton and in some cases Photo-Fenton, has been reported. *De Laat et al.* (*2004*) point out that inorganic anions, such as Cl^-, SO_4^{2-}, $H_2PO_4^-/HPO_4^{2-}$, etc. may produce significant effects on the overall reaction rates in the Fenton process. The anions may basically produce four effects: complexation reactions with the ferrous or ferric cations; precipitation reactions, which lead to a decrease of the active dissolved Fe^{3+}; scavenging of hydroxyl radicals (or the oxidizing agent) and formation of less reactive inorganic radicals; and oxidation reaction involving these inorganic radicals.

The predominant species present in solution at the pH range used for the Fenton and photo-Fenton processes are dichlorine anion ($Cl_2^{\bullet-}$) and sulphate ($SO_4^{\bullet-}$) radicals. These species may oxidize Fe^{2+} and H_2O_2 with rate constants of the same order of magnitude than those with $^{\bullet}OH$. However, the second-order rate constants for their reactions with most of the organics demonstrate that their reactivity is lower than the reactivity of the hydroxyl radical. The supposed scavenging effects of the anionic species are shown by Reaction 1.3-29 and Reaction 1.3-30.

$$HO^{\bullet} + A^{n-} \rightarrow A^{\bullet(n-1)-} + HO^- \qquad \text{Reaction 1.3-29}$$

$$Fe(II) + A^{\bullet(n-1)-} \rightarrow Fe(III) + A^{n-} \qquad \text{Reaction 1.3-30}$$

where Fe(II) and Fe(III) represents all the ferrous and ferric species respectively, A^{n-} is an inorganic anion, and $A^{\bullet(n-1)-}$ is an inorganic radical.

Regarding Photo-Fenton, there are no definitive conclusions about the effect of salinity. Only *Maciel et al.* (*2004*) reports moderate effects of chlorine. Supplementary explanation on this field is found in a specific introduction in Section 3.6, in which an experimental study is carried out in order to elucidate the effect of chloride on Photo-Fenton efficiency.

1.4.- Solar driven Photo-Fenton.

It has been observed that the sun can be a very useful light source for driving Photo-Fenton process in the wavelength range above 300 nm (*Bauer and Fallmann, 1997; Chen and Pignatello, 1997; Pignatello, 1992*). Figure 1.4-1 shows a solar radiation spectrum. The most important part is the spectrum from a wavelength above 300 nm and up to 500 nm approximately.

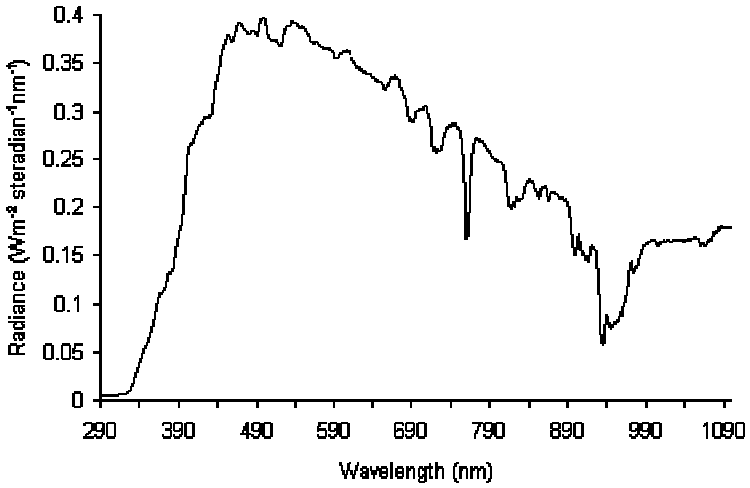


Figure 1.4-1: Global irradiance solar spectrum

Compared to artificial-light driven Photo-Fenton, one of the characteristics likely to be of more importance in solar-based Photo-Fenton is the economy and simplicity of the process. There is no need to put in contact electric devices with liquid, which may involve significant technological difficulties and risks.

Several solar reactors have been tested. Probably, the so-called Compound Parabolic Collector (CPC) provides the most efficient light collection for low concentrating systems (*Muschaweck et al., 2000*). They have no tracking system, i.e. they have no mobile parts, and the design permits to collect almost all the radiation (direct and diffuse) incident at the CPC area, and is available for the process in the reactor, in this case the photochemical process. A diagram describing the reflection properties of a CPC is shown in Figure 1.4-2. Another aspect of importance regarding the process industrialization is that CPC can be easily up-scaled due to the simple engineering concepts involved (*Gernjak, 2006*).

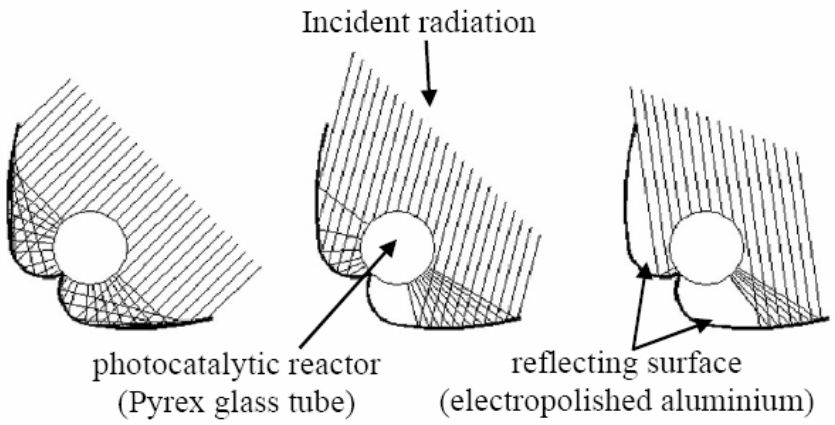


Figure 1.4-2: Reflection properties of a Compound Parabolic Collector (CPC) (*Blanco, 2002*).

Focussing on the evolution of solar Photo-Fenton process, some authors (*Sagawe et al., 2001*) suggest a feasible combination of photochemical utilization of solar energy for the photo-Fenton process with its thermal use. Thus, photons in the wavelength region 300-500 nm can induce the important photochemical conversion of Fe^{3+} to Fe^{2+}, while all photons (λ>300 nm) can, in principle, provide thermal energy to increase the process temperature, and consequently, according to these authors, result in optimal reaction conditions.

1.5.- Biological treatment of wastewater

Biological treatment of wastewater, groundwater, and aqueous hazardous wastes is often the most economical alternative when compared with other treatment options. The ability of a compound to undergo biological degradation is dependent on a variety of factors, such as concentration, chemical structure and substituents of the target compound. The pH or the presence of inhibitory compounds can also affect the biological degradation (*Contreras et al., 2003*). Although many organic molecules are readily degraded, many other synthetic and naturally occurring organic molecules are biorecalcitrant.

There are five major groups of biological processes: aerobic, anoxic, anaerobic, combined (aerobic, anoxic, and anaerobic processes), and pond processes (lagoon processes). The individual processes are further subdivided, depending on whether treatment is accomplished in suspended-growth systems, attached-growth systems, or combinations thereof (*Metcalf and Eddy, 1991*).

Aerobic processes are biological processes that occur in the presence of oxygen (*Metcalf and Eddy, 1991*). There exist different operating configurations as said above (suspended, attached and combined). The most common one in the suspended-growth group is the activated sludge process, which mostly works in continuous operating mode. As described by *Metcalf and Eddy* (*1991*), in this processes, organic waste is introduced into a reactor where an aerobic bacterial culture is maintained in suspension by the use of diffused or mechanical aeration. The reactor content is known as mixed liquor. After a specified period, the mixture is passed into a settling tank, where the cells are separated from the treated wastewater.

The same process can be carried out in batch operating mode. In this case, both the settling and the supernatant separation processes are done in the same tank. The biomass in the reactor can increase or decrease, but as the process goes along, the growth rate is balanced by the death rate and a steady state is reached. This reactor configuration is known as ***Sequencing Batch Reactor*** (SBR). Several studies have shown that discontinuous processes such as the SBR present some advantages for biodegrading xenobiotic compounds. The periodic operation imposes selective pressures that can select a defined population able to degrade problematic compounds (*Buitron et al., 2004*). Figure 1.5-1 shows the sequence of operations that are carried out during a SBR cycle. The duration of each step may vary depending on the treatment purposes (e.g. carbon degradation or nitrogen removal).

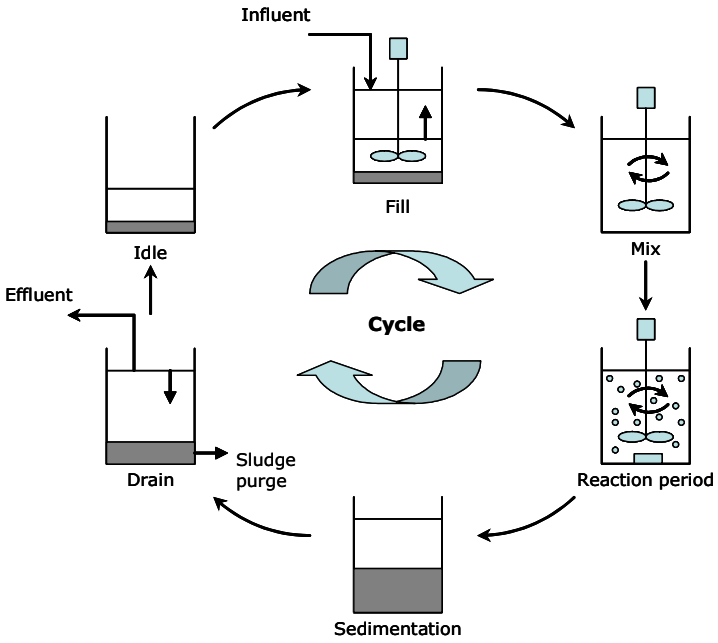


Figure 1.5-1: Biologic Sequencing Batch Reactor scheme.

Among SBRs, different operating configurations may be distinguished too: suspended, attached, combined and even in fluidized-bed. For its identification, the attached growth SBR is named ***Sequencing Batch Biofilter Reactor*** (SBBR). Its main feature is the fixed bed of biomass. It may be found as biofilm in some works. Both water and gas pollution remediations have been reported as applications for the SBBR. In the case of a biofilter, as biomass is attached to a support, it is not necessary to perform sedimentation or settling stage. Moreover, instead of a mechanical mixing, the liquid may be recirculated from the top to the bottom of the reactor.

More specific introduction concerning biofilters is explained below, in Chapters 4 and 5.

1.6.- Coupling an AOP with a biological treatment

AOPs involve relative high costs if compared to biological treatments. However, the use of AOPs is more suitable or even indispensable when the solution to be treated is not easily biodegradable or when the amount of organic matter is low. One economically viable option to treat wastewater containing non-biodegradable pollutants consists of combining an AOP, for instance Ph-F, and a biological post-treatment. In this case the chemical step is used to enhance the biodegradability of the wastewater, so that it can be more easily treated biologically (*Sarria et al., 2003*). Photo-Fenton has been suggested to be feasible and promising to treat wastewaters containing non-biodegradable chlorinated species (Chlorophenols), being used as a pre-treatment method to increase the biodegradability (*Fallmann et al., 1999*).

As shown in Figure 1.6-1 the strategy of wastewater treatment (*Sarria et al., 2002*) can be planned considering the biodegradability of the wastewater to be treated. If it shows no biodegradability and toxicity, a pre-treatment step, for example an AOP technique such as Ph-F, is required before the biological treatment.

In order to measure the biodegradability enhancement, or the biodegradation possibilities, there exist different parameters, measurements and ratios. It is, for example, possible to measure biodegradability by means of a respirometric test (*Amat et al., 2005*) or an inhibition test by means of measuring oxygen uptake rate (OUR). The Zahn-Wellens test has been reported as well as a good method (*Sarria et al., 2002*). Other biodegradability measures including substrate destruction, EC_{50} toxicity measurements, cell growth counts and intracellular ATP levels (Adenosine 5'-triphosphate) have been also used in many works (*Scott and Ollis, 1995*). It is also possible to perform a direct test in a biological reactor.

Among the different existing parameters to study the biodegradability enhancement and consequently the integration possibilities, the ratio between the Biochemical Oxygen Demand at 5 days (BOD_5) and the Chemical Oxygen Demand (COD) is used in many works (*Arslan-Alaton and Gurses, 2004; Chamarro et al., 2001; Park et al., 2001; Yeber et al., 1999*). The BOD_5/COD ratio points out which part of the matter is capable of being readily decomposed by biological means, especially by bacterial action, among the total amount of oxygen required to convert the matter to carbon dioxide and water. For example, *Metcalf & Eddy* (*1991*) suggest that a municipal wastewater can be considered biodegradable if the attained ratio is >0.4.

This ratio allows the estimation of the biodegradability of organic water pollutants. It also provides useful information for water monitoring concerning its self-purification process, for example in the case of wastewater being released to the environment. In this case, an identification guide may be as follows:

BOD_5/COD > 0.59: easily and entirely biodegradable organic content
BOD_5/COD ≈ 0.1-0.59: incomplete biodegradation
BOD_5/COD < 0.1: persistent organic pollutants, not biodegradable

CODs are often higher than BOD values (by a factor of two or more) because many biologically resistant compounds are easily oxidized under the harsh conditions of potassium dichromate oxidation applied for COD measurement (*Oppenländer, 2002*). The analytical methods to measure COD and BOD_5 are well standardized. Furthermore, as compared with other methods, the measure does not require very complicated systems.

BOD_5 is normally related to readily biodegradable substances, and COD may be a measure of all organic matter present in the medium. In fact, these basic definitions are one of the reasons for the election of this ratio to measure the coupling possibilities. COD and BOD_5 can be considered pseudo-compounds, so that it is possible to model the biodegradability occurrence during the process by means of these parameters.

There is also another parameter of importance in order to measure pollution or degradation. Total Organic Carbon (TOC) is a measure of all carbon related to organic substances. Thus, TOC supplies very basic information for biodegradation purposes: is the amount of carbon that the bacterial population can use to obtain energy or for growth purposes.

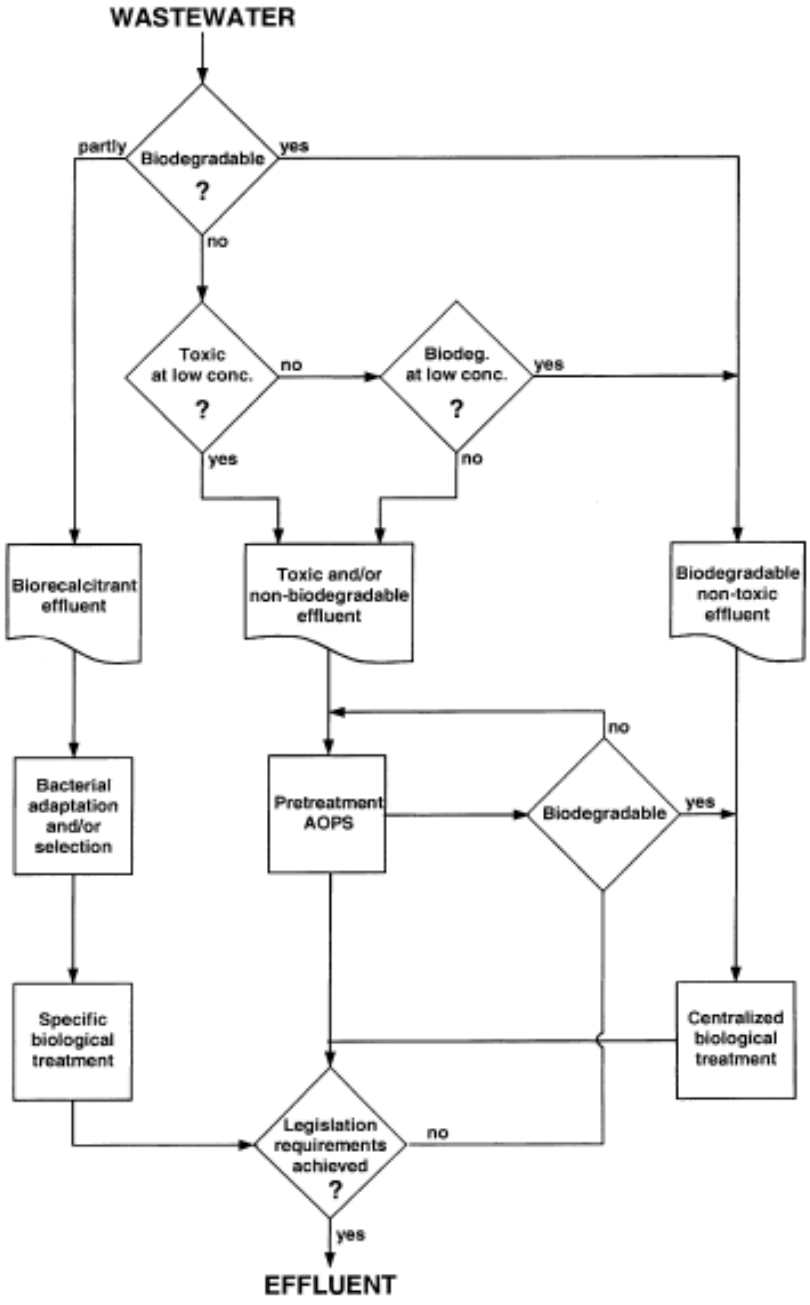


Figure 1.6-1: General strategy of wastewater treatment (*Sarria et al., 2002*), considering AOPs and biological treatment.

1.7.- Experimental Design

Experimentation is used to know and to understand better a process or a system. The development of techniques that increase the experimentation efficiency implies saving of time and money. Moreover, details that are non-observable in a classical experimentation methodology can be elucidated.

Many processes are optimized assuming that various treatment parameters do not interact. However, the response is a result of interactive influences of the different variables (*Mason, 1989*). An experimental design methodology is based on multivariate methods which allow more efficient data collection and easier data treatment than the technique consisting of changing the level (value) of one variable at a time, while maintaining the other variables at fixed levels (*Gob et al., 1999*) by performing a minimum set of well chosen experiments (*Perez et al., 2001*).

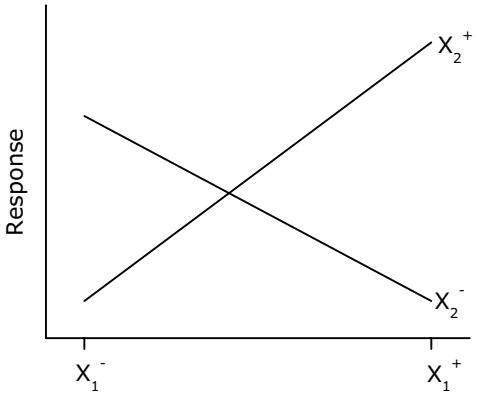


Figure 1.7-1: Interaction between variables X_1 and X_2 over the response

The major drawback of the one-factor-at-a-time methodology is that the possible interaction among the studied variables is not considered. Interaction is defined as the influence that one variable might have on the other variable's effect over the studied response. Figure 1.7-1 shows the existence of interaction of X_1 and X_2 over the response, that is to say that the response as a function of X_1 depends on the value of X_2.

A factorial design is an experimentation strategy suitable to study a process which responses might be affected by different variables. It consists of modifying simultaneously all the variables within the experimental domain, instead of modifying one at a time. The factorial design uses the experimental data in order to obtain the maximum information. Therefore, it is possible to

distinguish between the essential and the non-essential variables, and to know in which direction the optimum is situated. A factorial design can be also used as an optimization tool, by means of functions that connect the variables with the responses.

To carry out the optimization, and to study the effect of each variable and their interaction on different responses, a Response Surface Methodology (RSM) may be used. The response surface plots can be used to study qualitatively the effect of different operating conditions, locate the optimum, and estimate a mathematical equation that describes the system. In order to obtain good quality response surfaces, a complete experimental design must be elucidated. The so-called Central Composite Design (CCD) is a model that leads to generate experimental designs. The CCD is a combination of a simple factorial design at two levels, a star-design and the centre point of experimentation. Further information about experimental designs and CCD may be found later (Section 2.4).

1.8.- An overview of 4-chlorophenol

Model compounds are generally used in research because their characteristics are well known and show typical properties of a wide group of compounds. NBCS can be found in many industrial and agricultural applications. As their name indicates, their structure contains chlorine, and in many cases are aromatics. Among them, several species are based on phenolic structures.

Chlorophenols are chemical derivates of phenol, which contain from one to five chlorine atoms. 4-chlorophenol (4-CP) is a well known model compound, used in many cases to study the efficiency of a wastewater treatment (*Boncz et al., 1997; Garcia-Molina et al., 2005; Krutzler et al., 1999; Perez et al., 2001; Torrades et al., 2003*). Its structure, a phenolic ring with a chlorine atom (Figure 1.8-1), makes it suitable to observe the mechanism of an oxidizing process and compare the results to those which would be attained with other chlorinated biorecalcitrant organic molecules, such as pesticides or herbicides. Chlorine is largely present in these kinds of compounds. In fact, more than 85% of all pharmaceuticals and more than half the products of the chemical industry depend on chlorine chemistry (*Eurochlor, 2007*).

The analysis of 4-chlorophenol and its degradation intermediates in water phase is simple by the common analytical tools, such as chromatography for example.

1.8.1.- Properties of 4-chlorophenol

4-CP is known as a toxic and non-biodegradable organic compound and is widely used for the production of dyes, drugs, and fungicide (*Theurich et al., 1996*). Chlorophenols in general show no biodegradability ($BOD_5/COD < 0.01$, laboratory results) and they release chloride ion when degraded. Due to its chlorinated nature, 4-CP (and Chlorophenols in general) may persist in the environment (*Takeuchi et al., 2000*) and it is considered to act as uncoupler of oxidative phosphorylation (*Terada, 1990*).

As it occurs with all Chlorophenols, 4-CP is characterized by producing disagreeable taste and odour to drinking water at concentrations below 0.1 $\mu g.L^{-1}$ and adverse effects on the environment. It is a white needle-like crystalline solid at ambient temperature with a boiling point well above the boiling point of water. In Table 1.8-1 some physical and toxicity properties and hazardous effects of 4-chlorophenol are given.

Table 1.8-1: Properties of 4-chlorophenol

Physical properties (*ILO, 1999*)	
Boiling point (ºC)	220
Meting Point (ºC)	43
Relative density (water = 1)	1.3
Solubility in water, g/100 mL at 20 ºC	2.7
Vapour Pressure (kPa)	13 at 20 ºC
Toxicity properties (*NIOSH, 1983*)	
LD_{50} Oral ($mg.kg^{-1}$)	261
LD_{50} Percutaneous ($mg.kg^{-1}$)	1390
Hazardous Effects (*CESARS, 1989*)	
Unpleasant and penetrating odour	
Toxic by skin adsorption, ingestion or inhalation	
Tissue irritant	
When heated to decomposition, it emits highly toxic fumes	

As the rest of the Chlorophenols, 4-CP is flammable but does not actually burn; rather it decomposes on heating to form toxic, volatile, chlorinated gases. It is a weak acid and a versatile intermediate in chemical synthesis because both the hydroxyl group and the aromatic ring can react by electrophilic and nucleophilic substitution.

1.8.2.- Origins and uses of 4-chlorophenol

4-Chlorophenol is manufactured by direct chlorination of phenol (*Ullmann's, 1991*) and its introduction into the environment is a result of many origins. Manufacturing plants; discharges from factories producing higher chlorinated phenols or phenoxy herbicides, which are produced using 4-CP as raw material; through the degradation of other chemicals, such as

phenoloxyalkanoic acids; as a result of the chlorination of humic matter or natural carboxylic acids during the chlorination of municipal drinking water (*Exon, 1984*).

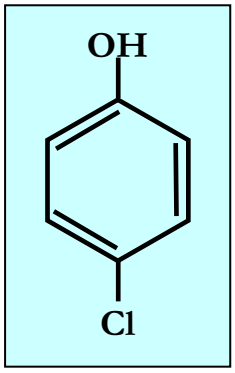


Figure 1.8-1: 4-chlorophenol molecule structure

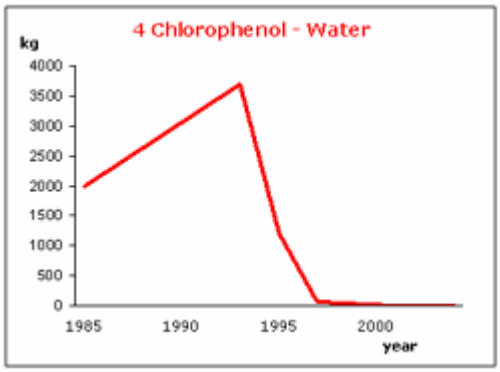


Figure 1.8-2: Emissions of 4-chlorophenol to water since 1985 collected by EPER (*Eurochlor, 2006*).

There also exist indirect entries of 4-CP to the aquatic environment. In paper mills, during the bleaching of paper, 4-CP is formed as by product. It is reported that 4-CP is formed due to the microbial breakdown of agricultural herbicides. Chlorophenols, and in particular 4-CP, have been extensively used for wood preservation, which has often led to environmental contamination (*Melin et al., 1998*).

Data on emissions to air and water for 22 chlorinated substances have been collected since 1985 by Euro-Chlor. This data includes all the chlorinated products in the European Pollutant Emission Register (EPER) list. Figure 1.8-2 shows the evolution of 4-chlorophenol emission to water since 1985. In average among all the pollutants in the list, between 1985 and 2004 there has been 99 % reduction in the total tonnage released to water and 93 % in that released to air. A target of a further 75 % reduction to water and 50% to air has been set for 2010 compared to the 2001 baseline.

All the chlorophenols have been used as biocides. Monochlorophenols have been used as antiseptics (*HSDB, 1998*) although in this role they have largely been replaced by other chemicals (*WHO, 1989*). The principal use of Monochlorophenols has been as intermediates for the production of higher chlorinated phenols (*WHO, 1989*). Specifically, 4-CP has been used as a disinfectant for home, hospital, and farm uses (*WHO, 1989*), as an antiseptic in root canal treatment (*Gurney and Lautenschlager, 1982*) and are also used to prevent growth of microorganisms in the manufacture of some industrial products.

Among these, the main uses of 4-CP are the following (*ATSDR, 1999; EPA, 1980*):

- Extraction of sulphur and nitrogen from coal
- As an intermediate in the synthesis of dyes and drugs
- As a denaturant in alcohol
- Solvent in the refining of oils
- Production of the herbicide 2,4-D, the germicide 4-CP-o-cresol and 2,4-DCP
- Wood preservation

1.9.- Objectives

The aim of the present work is to study engineering aspects of the combination of two processes, a photochemical and a biological, for the treatment of wastewater containing non-biodegradable organic pollutants. The photochemical process is the so-called Photo-Fenton process and the biological treatment is a Sequencing Batch Biofilter Reactor (SBBR), which is a fixed bed biomass reactor. This kind of reactor is supposed to be more resistant to changes and to toxic conditions.

The experimental study is carried out by studying different aspects of the degradation and mineralization possibilities of the integrated process over a model wastewater, which is a solution of 200 $mg.L^{-1}$ of a model pollutant, 4-chlorophenol, with deionised water.

The first studies concern the identification of parameters that produce a significant influence on degradation capabilities, as well as costs. By a Response Surface Methodology (RSM), it is intended to describe mathematically these different effects, in order to plan a strategy of integration. As some products of Photo-Fenton have later to be fed into a biological reactor, a characterization of products is carried out, being the parameters that indicate the biodegradability of importance, and among them, the ratio BOD_5/COD the most important. Moreover, different engineering aspects such as up-scaling, control and modelling are endeavoured. A last study concerns the effect of salinity on Ph-F process, since some common industrial wastewaters that contain non-biodegradable pollutants, contain significant amounts of NaCl too.

In all the Ph-F experiments, the initial pH is 2.8, since it is generally accepted to be optimal. During the experiments, no control over the pH is applied, since it is not desirable to add other species into the system.

Then, the integration of the processes is intended. The SBBR is going to be tested in order to find the operating conditions that allow mineralizing as much as possible spending the minimum reagents' amounts in the Ph-F. In order to fix a numerical objective, more than 90 % of mineralization is aimed.

The last part aims for the characterization of the SBBR in order to perform a future optimization of the system. The objective is to describe which conditions allow to reach the maximum degradation rates and to find which parameters can aid in the control of the process. The supposed resistance to toxicity of a SBBR is going to be proved.

Chapter 2: Experimental and methods

2.1.- Experimental

2.1.1.- Laboratory-scale Photo-Fenton experiments: Device and Procedures

The device consists of a 2.2 L cylindrical jacketed reservoir, with 3 UV 8W fluorescent tubes placed inside (Philips F8T5/BLB; 340-400 nm with a maximum at 370 nm). The vessel is covered with aluminium foil to avoid losses of light and to avoid the external influences. The device is also equipped with a magnetic stirrer. Photon flow arriving in the system is estimated to be constant throughout an experimental phase by means of an actinometry (see Section 2.3.7). A scheme of the installation is shown in Figure 2.1-1.

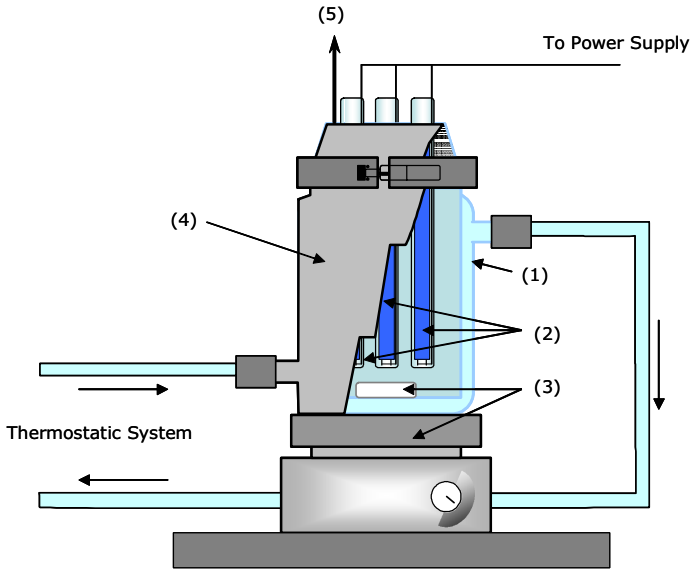


Figure 2.1-1: Photo-Fenton reactor.
(1) Jacketed vessel (2) UV Lamps (3) Stirring system
(4) Aluminium Foil (5) Sample extraction

The target solution consists of 2 L of 200 $mg.L^{-1}$ (1.56 mM) 4-chlorophenol (4-CP) aqueous solution. A volume of a concentrated 4-CP solution is acidified with sulphuric acid at pH approximately 3 to avoid iron precipitation. Then the necessary amounts of $FeSO_4.7H_2O$ (as a source of Fe^{2+}) and NaCl in the study of the effect of Salinity (Section 3.6) are added and the volume is brought to 2 L. The solution is poured into the reactor and mixed thoroughly. Then the pH is fixed to 2.8. Temperature is fixed at the desired value by a thermostatic bath. UV fluorescent lamps are switched on in order to reach stable irradiation. After 5 minutes, the necessary amount of H_2O_2 is added into the reactor under magnetic-stirring. The system is let to react until all the H_2O_2 is consumed. Through the process, samples are withdrawn in timed

intervals and immediately quenched with some drops of $NaHSO_3$ 40% w/v solution for measuring the Total Organic Carbon (TOC) or in the same volume of Methanol for the analysis by HPLC. Samples are filtered with Millipore Millex-GV PVDF 0.22 μm filters.

2.1.2.- Pre-industrial-scale Photo-Fenton Experiments: Device and Procedures

The pre-industrial-scale device is placed in the facilities of Plataforma Solar de Almería, Almería-Spain. The reactor consists of a continuously stirred tank, a centrifugal recirculation pump, a solar collector and connecting tubing and valves (Figure 2.1-2). Several on-line sensors and devices for heating and cooling of the process fluid are installed in the connecting tubing. The tank is a 20 L round-bottom Pyrex flask. Piping and valves are made of polypropylene. A pump (Bominox SIM-1051, 370 W, 380 V AC) with a three-phase frequency regulator is used to manipulate the flow rate. The temperature control is done by separating heating and cooling devices. With these systems, temperature of the experiment can be controlled within 10° to 60°C. In this work, all tests are performed at constant temperature (27 °C). The solar reactor is composed of 4 compound parabolic collector units (concentration factor = 1) with an area of 1.04 m^2 (total area 4.16 m^2), mounted on a fixed platform tilted 37º (latitude of Almería (Spain)) facing south. Each unit has five borosilicate-glass tubes of 1.8 mm thick and 50 mm outer diameter. The total illuminated volume inside the absorber tubes is 44.6 L. For performing tests adequately, the collector is covered with special "hand-made" aluminium sheets and removed when the experiment starts.

The solution to be treated consists of 82 L of 200 $mg.L^{-1}$ (1.56 mM) 4-chlorophenol (4-CP) aqueous solution. A volume of a concentrated 4-CP solution is fed into the reactor and diluted to 82 L. The solution is led to mix thoroughly. After that, the solution is acidified with sulphuric acid, and the necessary amount of $FeSO_4.7H_2O$ (as a source of Fe^{2+}) is added. Finally, the pH is fixed to 2.8.

In a first set of experiments, the entire amount of H_2O_2 is added at the beginning of the experiment, and is let to react until peroxide is totally consumed. Another method of operation is the Addition Experiment. In this case, a dose of hydrogen peroxide of 50 $mg.L^{-1}$ is fed into the reactor. When H_2O_2 is completely abated, a sample is withdrawn and another load of hydrogen peroxide of 50 $mg.L^{-1}$ is supplied. The process is carried out subsequently up to a total amount of 550 $mg.L^{-1}$ of H_2O_2 has been supplied.

Samples are collected at predetermined times, and quenched with some drops of $NaHSO_3$ 40% w/v solution for measuring Total Organic Carbon (TOC) content, and mixed with the same

volume of methanol for the analysis by HPLC. In both cases, samples are filtered (Millipore 0.20 μm Nylon Filters). Analysis of COD is carried out following the procedures of Standard Methods as in the laboratory experiments. Analysis of H_2O_2 concentration is carried out by titration (see Section 2.3.6).

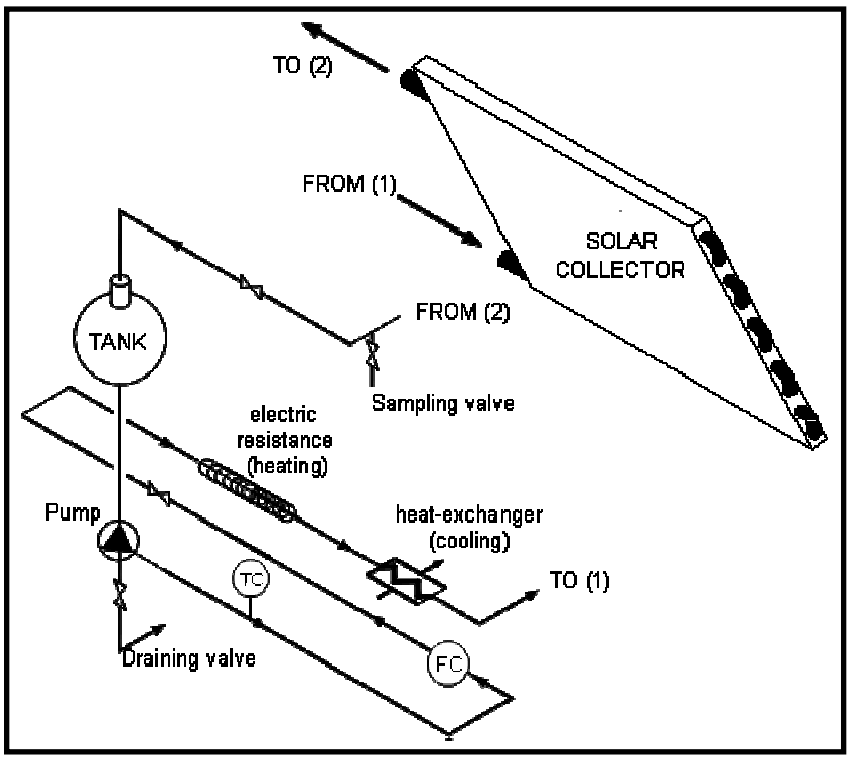


Figure 2.1-2: Solar Pre-industrial-scale device

In order to compare experiments with different sun conditions, time is normalized to an expression called "30 W time" (t_{30W}) (*Malato et al., 2003*). This gives an idea of the energy reaching any surface in the same position with regard to the sun. With Equation 2.1-1, combination of the data from several days' experiments and their comparison with other photocatalytic experiments is possible where t_n is the experimental time for each sample, UV is the average solar ultraviolet radiation measured during Δt_n, and t_{30W} is a "normalized illumination time". In this case, time refers to a constant solar UV power of 30W.m^{-2} (typical solar UV power on a perfectly sunny day around noon).

$$t_{30W,n} = t_{30W,n-1} + \Delta t_n \frac{UV}{30}\frac{V_i}{V_T}; \Delta t_n = t_n - t_{n-1} \qquad \text{Equation 2.1-1}$$

2.1.3.- Preparation of the feed for the biological reactor

The effluent that results from the photo-Fenton treatment must be prepared before being fed into the biological reactor. The procedure for the preparation of this solution is similar to the preparation for the BOD_5 analysis.

The pH of the Ph-F treated solution is around 3, which is not suitable for a biological reactor. Firstly, the solution is neutralized to pH around 7 by means of NaOH and buffered with sodium dihydrogen phosphate solution 1.5 N (6 mL of buffer per litre of feed). Next, 2 mL of the following solutions per litre of feed are added; ammonium chloride 0.71 N, magnesium sulphate 0.41 N, ferric chloride 0.018 N and calcium chloride 0.25 N. The obtained solution is kept in the fridge to avoid the formation of bacterial culture. Previously to being fed into the biological reactor is placed outside the fridge until it reaches room temperature and is stirred to be homogenous.

2.1.4.- Fixed-bed biological reactor: the Biofilter. Device and Procedures

The biological reactor, i.e., the biofilter, is a 2.5 L glass jacketed column filled with porous material to serve as support to the microorganisms. In the present work, the porous material is volcanic stone. This kind of material gives a suitable attachment for the bacteria to be easily settled, and it involves low capital costs. The volcanic stones have a high specific area. Thus, it is possible to reach a high biomass concentration and therefore it is supposed to have high degradation capacities.

The vessel is a jacketed cylinder and follows a height/diameter ratio of 3 to 1. The volcanic stones occupy a volume of 0.9 L, so that the reactor holds 1.6 L of liquid. The device is equipped with feeding, recirculation and draw-off pumps (Cole-Parmer). Air is fed at a flow rate of 2 $L.min^{-1}$ during research described in Chapter 4, and is fed at 0.1 $L.min^{-1}$ in the study described in Chapter 5. It is proved non-limiting for the degradation. An electrovalve is used as check valve in order to hold back the liquid when air is stopped. Power supply to feeding, recirculation and draw-off pumps as well as to air compressor and check valve is controlled by a time-based control system (Siemens LOGO! 230 RC). Temperature at the reactor is controlled by a thermostatic bath (Haake DC10-K10) at 27 ºC. The device is depicted in Figure 2.1-3.

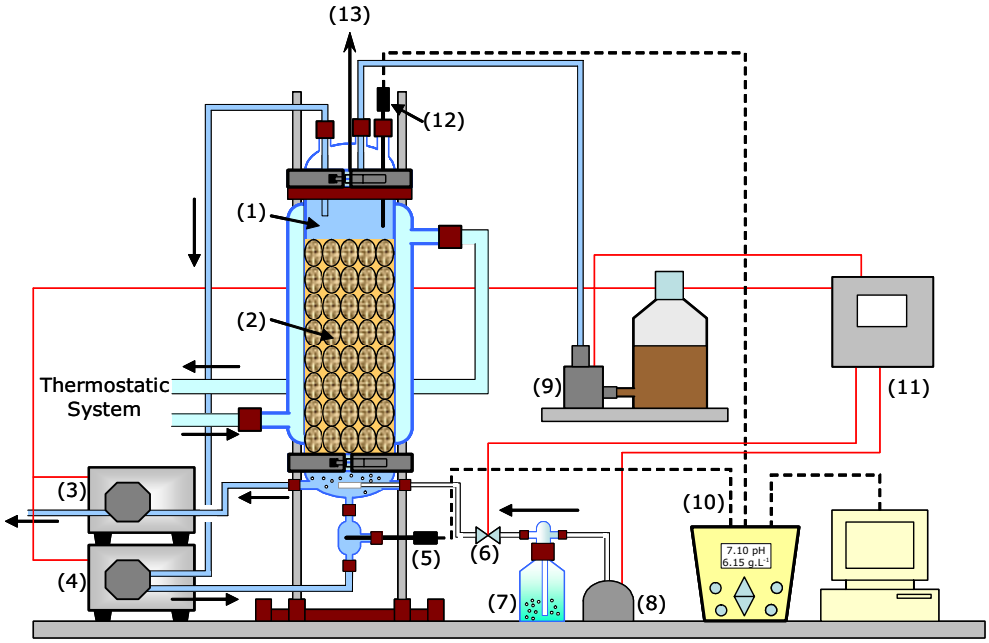


Figure 2.1-3: Sequencing Batch Biofilter Reactor (SBBR) scheme.

(1) Jacketed reactor	(2) Volcanic Stones	(3) Draw-off pump	(4) Recirculation pump
(5) DO probe	(6) Check valve	(7) Humidifier	(8) Air compressor
(9) Feeding pump	(10) Data logger	(11) Control system	(12) pH probe
(13) Sample extraction			

Samples are withdrawn from the top of the column and Total Organic Carbon (TOC), Chemical Oxygen Demand (COD), Total Suspended Solids (TSS) and Total Volatile Suspended Solids (TVSS) are analyzed. Dissolved Oxygen (DO) and pH are monitored and data is logged by means of a multiparametric bench meter VWR Symphony SB90M5. This device allows the measurement of 5 parameters at the same time.

To carry out the measurement of the Oxygen Uptake Rate (OUR), the time-based control system is programmed in order to cut off the air supply for short periods. During these periods, the decrease in dissolved oxygen is monitored.

The reactor works in sequencing batch-operating mode. 1.6 L of pre-treated and prepared (see chapter 2.1.2) wastewater are fed into the reactor and let react until the content is changed for a new refill. That is a cycle.

The cycle ends as follows. The recirculation and air supply are stopped. Although the device is a fixed bed reactor there is always some biomass mixed with the liquid content, mainly in the first

cycles. The draw-off pump is switched on and approximately 85 % of the total volume is taken out. Next, the same amount of newly prepared pre-treated wastewater (already neutralized, buffered and with micronutrients), is poured into the reactor by means of the feeding pump. Finally, the recirculation pump and the air supply are switched on. Along the biological treatment, some deposit was observed on the recirculation tubes. However, it did not involve any problem, since it did not obstruct the flow.

The biomass used as inoculum comes from an urban wastewater treatment plant (Gavà, Barcelona, Spain), extracted from the secondary digesters recirculation. The reactor in the long experimental periods is covered with aluminium foil in order to avoid the growth of algae in the thermostatic system.

2.1.5.- Additional devices

In order to check the stripping and adsorption influences on the bioreactor performance, two 1.5 L vessels with the same diameter as the bioreactor are used. In the adsorption test, the liquid and stone volumes used are in the same proportion than in the biofilter (0.3 stones/liquid volume ratio) and the content is stirred during the experiment. In the stripping test, the same airflow rate as in the biofilter is supplied (2 L/min). Both reactors are depicted in Figure 2.1-4. The results of these tests are explained in sections 4.2.1 and 4.2.1.2.

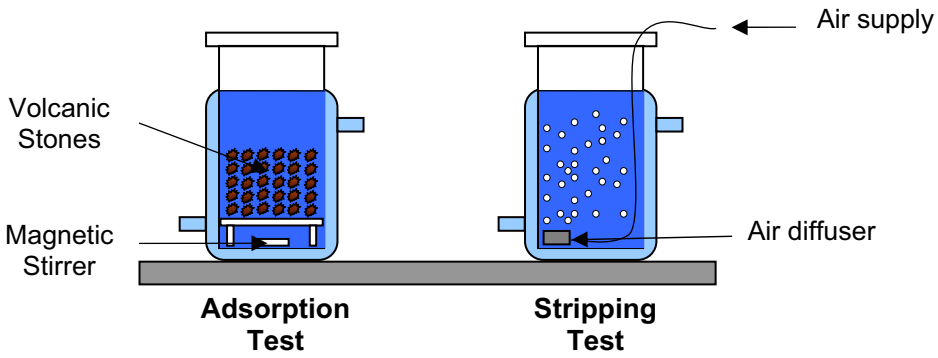


Figure 2.1-4: Adsorption (left) and Stripping (right) tests devices.

2.2.- Reagents and chemicals

The reactants used in this study are supplied by the following companies. 4-CP, is supplied by Merck and $FeSO_4.7H_2O$ and H_2O_2 (30% w/v) are supplied by Panreac Quimica, S.A. (Spain). The chemicals to prepare the BOD_5 and COD analysis solutions are supplied also by Panreac Quimica, S.A. All of them are analytical grade reagents.

2.3.- Analytical methods

2.3.1.- High Performance Liquid Chromatography (HPLC)

To study the degradation of 4-chlorophenol and follow the intermediates generated, it is necessary to determine its concentration during time. The selected method is the High Performance Liquid Chromatography (HPLC) in reverse phase.

The HPLC is supplied by Waters Corporation (Massachusetts, USA). It consists of:

- Waters Degasser
- Controller Waters 600 (pumping system)
- Autosampler Waters 717
- Oven for columns and temperature controller Waters
- Photodiode array detector Waters 996
- Millenium Software (year 2003-2005) and Empower Software (years 2005-2007)

The column is supplied by Tecknokroma S. Coop. C. Ltd (Barcelona, Spain), and presented the following features:

- Packing: SPHERISORB ODS2
- Particle Size: 5 μm
- Length: 25 cm
- Inner diameter: 0.46 cm

The mobile phase is a mixture acetonitrile and water (40:60 in volume), acidified at pH 3 by the addition of phosphoric acid, and isocratically delivered (constant composition and flow rate) by the pump system at a flow rate of 1 $mL.min^{-1}$. The injected volume of sample is 10 μL. Temperature is set at 25 ºC. Under these conditions, 4-chlorophenol has a retention time of 7.5 min approximately. The calibration is carried out by means of 4-chlorophenol standards. The determination of 4-chlorophenol concentration is carried out by integration of the peak observed by the UV detector at a wavelength of 287 nm.

2.3.2.- Total Organic Carbon (TOC)

To assess the amount of organically bounded carbon, the organic molecules must be broke down to single carbon units and converted to a single molecular form that can be measured quantitatively. TOC analyzers convert organic carbon to carbon dioxide (CO_2) by means of heat, and oxygen, over a catalyst. By an infrared detector, CO_2 is analyzed. The device is calibrated by a solution of potassium hydrogen phthalate.

Samples are analyzed for TOC by means of a Shimadzu TOC-V_{CSN} TOC analyzer. The measure of TOC is of importance, because it shows the level of mineralization of the species present in the solution, which is a way to measure the efficiency of the process.

2.3.3.- Chemical Oxygen Demand (COD)

Chemical oxygen demand (COD) is used as a measure of oxygen requirement of a sample that is susceptible to oxidation by strong chemical oxidant. In the present case, the oxidant is dichromate in acidic medium.

2.3.3.1.- High Range Method

To analyze COD in the range from 100 to 340 $mg.L^{-1}$ (the maximum expected value), the Standard Methods 5220D procedures are followed (*SM, 1985*). To this end, 2.5 mL of sample are added to a mixture of potassium dichromate and sulphuric acid.

After the addition, the vials are placed in a digester, which is a metal block where temperature is maintained at a desired value. In this case, temperature is fixed at 150 ºC and samples are let in the digester for 2 hours. After that time, samples are let to cool down and then, their absorbance is analyzed by means of a spectrophotometer at 600 nm (Hach Odyssey, Hach Company, Colorado, USA). By this procedure, the generated Cr^{3+} is measured, which amount is directly connected to the amount of oxygen demand.

2.3.3.2.- Low Range Method

Low Range Method allows measuring COD below 100 $mg.L^{-1}$. It differs to the high range in two aspects; potassium dichromate digestion solution is 10 times less concentrated and absorbance is measured at 420 nm, since in this case the remaining Cr^{6+} is analyzed. The method is standardized by the American Society for Testing and Materials (ASTM). Specifically is the Method ASTM D 1252 – 06, Test Method B (*ASTM, 2006*).

2.3.4.- Biochemical Oxygen Demand (BOD)

The Biochemical Oxygen Demand (BOD) is related to the amount of biodegradable organic matter in a water sample. During oxidative degradation of organic matter, the aerobic microorganisms consume the dissolved oxygen present in water. BOD is expressed as weight of consumed oxygen per unit volume of aqueous solution, during a defined period at a defined temperature. In this case, the BOD is measured during 5 days at 20 ºC.

For the evaluation of BOD_5 (Biochemical Oxygen Demand within 5 days) the WTW OxiTop measuring system (Weilheim, Germany) thermostated at 20 ºC is used. The measure is done following the Standard Methods 5210D procedures (*SM, 1985*), which is a respirometric method.

The Oxitop is a manometric respirometer, which relates the oxygen uptake to the change in pressure caused by oxygen consumption while maintaining a constant volume. The sample volume depends on the expected BOD range. A higher BOD requires less volume.

In this case, and due to that the expected BOD_5 value is in the smallest range, the analysis volume used is 432 mL. 425 mL of sample are neutralized to pH 7 and are buffered by sodium dihydrogen phosphate 1.5 N solution (2.595 mL). Then 0.865 mL of NH_4Cl (0.71 N), $MgSO_4$ (0.41 N), $CaCl_2$ (0.25 N) and $FeCl_3$ (0.018 N) solutions are added to the sample. Next, 0.650 mL of prepared biomass is added. The mixture is fed into a standardized 500 mL glass bottle. Two NaOH tablets are put into the cap basket. The bottle is sealed with the Oxitop, which is activated to start the measurement, and is placed inside a chamber in which the temperature is stabilized at 20 ºC. During the analysis, the NaOH tablets consume the CO_2 produced due to bacterial activity, and the decrease in pressure is measured by the Oxitop.

2.3.5.- Acute Toxicity measurement by Microtox

Microtox® is a standardised toxicity test system. The Procedure employs the bioluminescent marine bacterium (*Vibrio fischeri*) as the test organism to assess the toxicity of water, soil or sediment samples. The bacteria are exposed to a range of concentrations of the material being tested. The reduction in intensity of light emitted from the bacteria is measured by transmittance. The change in light output and concentration of the toxicant produce a dose/response relationship. The results are normalised and the EC_{50} value is calculated.

The EC_{50} is defined as the median Effective Concentration (EC_{50}). It is a calculated toxicity value representing the sample concentration (%) estimated to cause 50 % response by the exposed test

organisms (concentration or percentage of sample producing 50 % reduction in light). The Microtox Acute Toxicity Test is a 15 minutes exposure metabolic inhibition test.

The device is a Microtox M500 toxicity Analyzer (Azur Environmental, Delaware, USA). The Microtox Acute Test has achieved official "Standards Status" in several countries including an ASTM Standard (D-5660-95) in the USA or a DIN 38412 in Germany. The final ISO Draft (11348-3) entitled "Water Quality-Determination of the Inhibitory Effect of Water Samples on the Light Emission of *Vibrio fischeri* (Luminescent Bacteria) Test" has been approved.

2.3.6.- Analysis of H_2O_2 (iodometric titration)

This analytical method is widely used to measure the concentration of hydrogen peroxide in Advanced Oxidation Processes based on this reagent, in order to control its consumption. Through the Photo-Fenton process, hydrogen peroxide is the only reagent that is consumed and which depletion ends the catalytic cycle.

A considerable number of analytical methods are based on reaction of iodine. In an iodometry, an oxidant substance is mixed with and excess of iodide (I^-). Due to the oxidation, a certain amount of iodine is formed, which by complexation with the excess of iodide leads to tri-iodide (I_3^-) (Reaction 2.3-1). Finally, tri-iodide is titrated with a thiosulphate standard solution, using starch as indicator (Reaction 2.3-2).

$$H_2O_2 + 3KI + 2H^+ \rightarrow I_3^- + 2H_2O \qquad \text{Reaction 2.3-1}$$

$$I_3^- + 2S_2O_3^{2-} \rightarrow 3I^- + S_4O_6^{2-} \qquad \text{Reaction 2.3-2}$$

This method is used in the experiments carried out at the *Plataforma Solar de Almería*, Almería, Spain.

2.3.7.- Actinometry

Actinometry is a common method to determine the intensity of a radiation source. In the present case, the actinometrical system used is the photochemical decomposition of oxalic acid in presence of uranyl nitrate (*Kuhn et al., 2004; Vicente and Esplugas, 1983*). The decomposition reaction of oxalic acid, in a pH range between 3 and 7, and a conversion of oxalic acid lower than 20% is the following:

$$(UO_2)^{2+} + H_2C_2O_4 \xrightarrow{h\upsilon} (UO_2)^{2+} + CO_2 + CO + H_2O \qquad \text{Reaction 2.3-3}$$

By the knowledge of the actinometry and the lamp characteristics, the radiation intensity can be calculated.

2.3.8.- Total Suspended Solids (TSS)

Suspended solids refer to non-filterable matter suspended in water and wastewater. "Total solids" is the term applied to the material residue left in a vessel after evaporation of a sample and its subsequent drying in an oven at a defined temperature. Total solids involve "***total suspended solids***", which is the portion of total solids retained by a filter (of 2.0 μm -or smaller- nominal pore size), and "total dissolved solids", which is the portion of matter that passes through the filter.

In the analysis of TSS (Standard Methods 2540D), a well-mixed sample is filtered through a weighed standard glass-fiber filter and the residue retained on the filter is dried to a constant weight at 103 to 105 ºC. The increase in weight of the filter represents the total suspended solids.

TSS are calculated as shows Equation 2.3-1.

$$mg\ TSS.L^{-1} = \frac{(A\text{-}B)\times 1000}{sample\ volume,\ mL} \qquad \text{Equation 2.3-1}$$

Where A is the weight of filter + the dried sample (mg) and B is the weight of filter (mg).

2.3.9.- Total Volatile Suspended Solids (TVSS)

The residue from the previous test (TSS) is ignited to constant weight at 550 ºC (Standard Methods 2540E). The remaining solids represent the fixed total, dissolved, or suspended solids while the weight lost on ignition is the volatile solids fraction. The determination is useful for the control of the operation of a wastewater treatment plant because it offers a rough approximation of the amount of organic matter present in the solid fraction of wastewater, activated sludge, and industrial wastes.

The TVSS are calculated as the lost of weight (Equation 2.3-2):

$$mg\ TVSS.L^{-1} = \frac{(A\text{-}C)\times 1000}{sample\ volume,\ mL} \qquad \text{Equation 2.3-2}$$

Where A is the weight of residue + filter before ignition (mg) and C is the weight of residue + filter after ignition (mg).

2.3.10.- Chloride concentration measurement

Chloride concentration is measured by means of an Ion Selective Electrode (ISE). A Crison Ag/AgCl ISE is connected to a bench meter. Electric Potential must be measured against a reference electrode, which contains 1M KNO_3 solution. Ion strength must be adjusted in order to measure chloride concentration properly. In this case, the Ion Strenght Adjustor (ISA) is a solution of $NaNO_3$ (5M). 1 % in volume of ISA is added to 15 mL of sample. ISE and reference electrodes are submerged into the sample, and potential is measured under stirring.

2.3.11.- Scanning Electron Microscopy for Biofilm Samples

The Scanning Electron Microscope (SEM) is a microscope that uses electrons rather than light to form an image. The SEM has a large depth of field, which allows a large amount of the sample to be in focus at one time. The SEM also produces images of high resolution, which means that closely spaced features can be examined at a high magnification. Preparation of the samples is relatively easy since most SEMs only require the sample to be conductive. The combination of higher magnification, larger depth of focus, greater resolution, and ease of sample observation makes the SEM one of the most heavily used instruments in research areas today.

Two different images may be obtained. Secondary Electron Image (SEI) is a direct image, and gives an idea of the topography of the sample. Backscattered Electron Image (BEI) is an indirect image of the chemical nature of the sample. Their contrast is related to the density of the external layer of the sample.

In this case, samples to be measured are the volcanic stones placed in the biological reactor. The sample must be prepared before its observation. The best results, are obtained when the following procedure is carried out. An entire stone, is withdrawn from the reactor, and is collected into a vial, with liquid from the bioreactor as well. The stone is not let directly to the atmosphere at any time. Then, half of the liquid is taken out and pure ethanol is added to the vial. The mixture is let for some minutes. Then, again, half of the liquid is withdrawn, and more ethanol is poured in the vial. This procedure is repeated 5 or 6 times, since it is considered that the sample is in pure ethanol. Then, the sample is dried in a CO_2 atmosphere chamber and a thin layer of carbon is applied in order to make the sample conductive.

The drying process, application of the carbon layer and imaging of the sample is carried out in the facilities of the Univeristy of Barcelona's Scientific-Technical Services (SCT-UB, Serveis Cientificotècnics UB).

2.3.12.- Characterization of the microbial diversity in the SBBR

To identify bacterial strains present in the SBBR the following procedure is carried out.

DNA Extraction and Quantification

The DNA is estracted by using the QBiogene FastDNA SpinKit for Soil. This method employs physical disruption of the cells (bead-beating) followed by physical binding the DNA to a silica matrix, washing with an Ethanol based solution, and eluting in purified water. Extraction will be verified by agarose gel electrophoresis and quantified by spectrophotometry.

Polymerase Chain Reaction (PCR):

PCR is used in order to amplify the 16S rDNA genes of the DNA that has been extracted. The amplification is near full-length and is carried out using the primers 8F and 1492R (Taq polymerase from Eppedendorf). The primers sequences are:

8F: 5' –AGAGTTTGATCCTGGCTCAG-3'

1492R: 5'-GGWTACCTTGTTACGACTT-3'

"Shot-Gun" Cloning:

Shot-gun Cloning is one method of obtaining 16S rRNA gene sequence information from the microbes present in a sample. Now, the PCR products are ligated (or attached) randomly into a plasmid vector. 4-TOPO plasmid provided by Invitrogen is used for delivering the PCR product into the *E. coli* cells. These cells have been treated chemically so that they take up DNA readily from their environment. This process is called *transformation*. Once the cells are transformed with a plasmid containing a random PCR product insert, they are then spread out on Petri dishes. When spread properly, one colony originates from one cell which was transformed with a single plasmid with a single insert.

PCR of Clones and DGGE bands for sequencing:

The inserts of the clones are now retrieved for DNA sequencing and identification using primers specific to the plasmid vector. Primers M13F and M13R are used. Because these primers are specific to the cloned vector and are not "universal" primers, then contamination is not as much of a concern as with previous PCRs using universal primers.

Purification and analysis:

A QiaPrep Spin© (Qiagen) Purification Kit is used to clean the 96 clone plasmids. This process is performed 5 times at room temperature using 24 clones each time, due to the 30 spots limit

capacity of the centrifuge used (Eppendorf 5417R Centrifuge). Firstly, the centrifugal tubes where the plasmids are contained are removed from a storage freezer and are shaken in a vortex mixer to homogenize the samples. At this point, the plasmids that have precipitated due to the low temperatures of the freezer get re-dissolved in the liquid, turning it cloudy. With a Pasteur Pipette, 1.5 µl are taken from each centrifuge tube and introduced in a 2ml eppendorf tube. Samples are centrifuged at 4000 rpm, compacting the plasmid to a pellet. The supernatants are discarded. With the aid of a micropipette, 250µl of Buffer P1 (Qiagen) solution are added. Then, each eppendorf is agitated until the pellet is completely re-suspended, and afterwards 250µl of Buffer Lysis P2 (Qiagen) solution are added. All eppendorf tubes are inverted 5 times to homogenize the buffer lysis with the plasmids, obtaining a more transparent solution. Afterwards, 350µl of Buffer N3 (Qiagen) are added to stop the lysis, and eppendorfs are gently inverted 5 times obtaining a white precipitate. Then, the eppendorfs are centrifuged at 4000 rpm during 10 minutes, and the supernatants formed are introduced in new eppendorfs with filter and filtered again for 1 minute to collect the DNA on the top of the filter. The filters are removed from the eppendorf tube and are putted on new eppendorf tubes (obviously without filter) where 750 µl of Buffer PE are added to clean the DNA. These eppendorfs are centrifuged for 1 minute and again, the liquid is discarded and the filter with the clean DNA is saved. The filters that collect the DNA are moved to a new eppendorf and they are centrifuged again for 1 minute to dry the DNA. Afterwards, 33µl of Milli-Q water are added to the pellet and the tubes are let to rest for one minute. They are centrifuged for another minute at 4000 rpm with the eppendorf's lids open, being careful positioning them in order not to get the lids broken when centrifuging. At the end of this process, the plasmids should be clean.

These procedures were carried out at the Univeristy of Barcelona's Scientific-Technical Services (SCT-UB, Serveis Cientificotècnics UB).

2.4.- Experimental design and data analysis

A Central Composite Design is a fractional factorial Experimental Design. It generates an experimental design of 17 operating conditions. Each variable is studied at 5 levels, and the central point is done at least in triplicate to evaluate the error (Figure 2.4-1). The levels are the different values in which each variable is tested. The central point is the one with the central value for all the variables. Response Surface Methodology (RSM) explores the relationships between several explanatory variables and one or more response variables. The method was introduced by (*Box and Wilson, 1951*). The main idea of RSM is to use a set of well-designed experiments to obtain an optimal response. If the variables are studied at least at 3 levels, the response surface can be then mathematically written as a quadratic function (Equation 2.4-1), with the effects of each variable and their interactions.

$$\hat{y} = b_0 + b_1 x_1 + b_2 x_2 + b_3 x_3 + b_{12} x_1 x_2 + b_{13} x_1 x_3 + b_{23} x_2 x_3 + b_{11} x_1^2 + b_{22} x_2^2 + b_{33} x_3^2$$

Equation 2.4-1

In the equation, $\hat{y}$ is the response, for example TOC removal, b_i are the effect of the i variable, b_{ij} are the interaction of variables i and j and x_i are the variables (for example, $[H_2O_2]_0$, $[Fe^{2+}]_0$ and temperature). Response surfaces, equations and statistical treatment of the experimental results are carried out by means of STATGRAPHICS Plus 4.1 (Statistical Graphics Corp., USA).

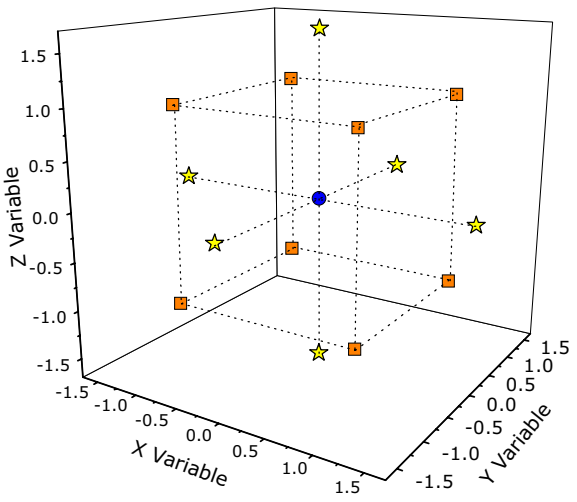


Figure 2.4-1: Experimental Design. Central Composite Design with 3 variables.