Marco Pipolo

Developing Integrated Decontamination Methods Involving Chemical Treatment (Fenton's Reaction and Ozonation) and Biofiltration by Invasive Asian Clams for Challenging Effluents

Dissertação de Mestrado Integrado em Engenharia Química, orientada pela Doutora Raquel J. Costa e pelo Doutor Rui C. Cardoso Martins, apresentada ao Departamento de Engenharia Química da Faculdade de Ciências e Tecnologias da Universidade de Coimbra

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"O destino não é uma questão de sorte, é uma questão de escolha.
Não é algo para se esperar, é algo para se conquistar."

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Abstract

The presence of persistent organic contaminants, often emerging molecules, is an increasing problem in different types of water (even in drinking water supplies). Looking for appropriate water treatment solutions that are not only more efficient than current decontamination methods, but also more respectful for the environment is one of the most active research lines of our time. This work is aligned with such strategy, addressing two topics: (i) the treatment of winery effluent and (ii) the removal of parabens from water. While being fairly independent, the two working topics share a common research approach – ultimately integrated decontamination methods involving chemical treatment and biofiltration by the damaging invasive Asian clam are intended. The first project consisted of a pilot scale study of combined Fenton's reaction and biofiltration applied to treat winery effluent. A clams' biofilter of 0.408 m^2 was designed and used to process 80 L of Fenton-treated wastewater. The results showed that high percentage of COD was removed from the effluent, with the final water quality being comparable with that resulting from the standard process in this field.

The aim of the second project was the development of ozonation as a treatment approach for parabens, eventually to be combined with biofiltration. The results reported here indicate that is possible to reduce the percentage of COD of an artificial effluent rich in parabens using ozonation. Moreover, this process was also proven to reduce the toxicity of the model effluent.

This thesis opens up the prospect for alternative processes involving a symbiosis between nature and chemistry to reduce the environmental impact of chemical processes in waste water treatment.

Abstract (Italian Version)

La presenza di contaminanti organici persistenti, che spesso sono contaminanti emergenti, è un problema in diversi tipi di acque (anche nell'acqua potabile). Cercare una soluzione a questo problema che sia non solo la più efficiente rispetto ai metodi utilizzati oggi per la decontaminazione, ma anche che rispetti di più l'ambiente è uno degli obiettivi del nostro tempo. Questo lavoro di tesi riguarda due temi: (i) il trattamento di acque reflue derivanti da attività di vinificazione e (ii) la rimozione di parabeni dall'acqua. Pur essendo abbastanza indipendenti, i due lavori condividono un approccio comune di ricerca - metodi di decontaminazione integrati che coinvolgono il trattamento chimico e biofiltrazione da parte dell'invasiva Asian Clam. Il primo lavoro è uno studio su scala pilota di un processo combinato tra reazione Fenton e biofiltrazione per il trattamento di effluenti vinicoli. Un biofiltro di 0.408 m^2 è stato utilizzato per filtrare 80 L di acque reflue vinicole dopo la reazione Fenton. I risultati mostrano che un'alta percentuale di COD è stata rimossa dall'effluente vinicolo e la qualità finale dell'acqua è comparabile con i processi standard generalmente usati in questo campo.

Lo scopo del secondo lavoro è lo sviluppo dell'ozonation come trattamento per i parabeni ed eventualmente la sua combinazione con la biofiltrazione. I risultati ottenuti indicano che, sfruttando questo processo, è possibile ridurre la percentuale di COD di un effluente artificiale ricco di parabeni. Si dimostra inoltre, una riduzione di tossicità dell'effluente modello.

Questa tesi apre prospettive di processi alternativi che comportano una simbiosi tra natura e chimica per ridurre l'impatto ambientale dei processi chimici nel settore del trattamento di acque reflue.

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1. Introduction

Making the world cleaner is a challenge as important as doing it by spending resources as efficiently as possible.

Persistent contaminants, often emerging molecules, and the need to sustainably remove them from contaminated waters are serious problems and have increasingly been acknowledged as public health and quality of life questions.

The concentrations of different contaminants found in groundwater, surface waterbodies and within Waste Water Treatment (WWT) plants are alarming, even more so in big metropolis. Some of those contaminants are particularly problematic for a number of reasons, for example because they are especially toxic at low concentrations, or they are especially persistent and difficult to remove from water, or they cannot be processed by traditional treatment processes. Challenging contaminants are transversal to a range of activity sectors, from the basic chemical to the pharmaceutical and food industries.

This thesis focuses on two challenging pollutants in particular: (i) phenolic-rich winery effluent and (ii) parabens, a class of emerging contaminants that have been reaching natural waters. One of the major issue with the winery effluent is its seasonal character, i.e. its composition varies greatly throughout the year according to the wine production cycle. As it is very toxic, mainly because of its phenolic contents, it cannot be directly discharged for treatment in municipal WWT plants, especially at the peaks of the winery activity when its contaminant charge is extremely high. Parabens are common in the pharmaceutical and cosmetic industries, where they are used mainly as preservatives. The efficiency of conventional biological processes used in WWT plants in processing and degrading parabens is very low, and hence they have increasingly been found in natural waterbodies. Water contamination by parabens is very concerning because of their endocrine-disrupting potential, including their ability to mimic oestrogen. As current water treatment methods fail, the first goal of this thesis was to contribute to the development of improved, environmentally-benign and economically viable practices to deal with winery effluents and parabens.

While two independent problems and fairly distinct contamination scenarios were addressed, a common research approach was followed – integrated decontamination methods involving chemical treatment and biofiltration by the Asian clam were envisaged for both pollutants. In other words, a key connection point between the two studies comprising this thesis is the use of clam biofilters in a symbiosis with chemical processes. From this perspective, another goal of the thesis was to contribute to the management of the invasive Asian clam *Corbicula fluminea*, a major threat to freshwater biodiversity and a costly

biofouling pest. The Convention on Biological Diversity recommends four hierarchical action axes for the effective management of invasive species: prevention, early detection, eradication and long-term mitigation axes. Benefits can certainly be drawn by adding a fifth axis – application – to these four. Once invasive populations are fully established in an ecosystem, eradication becomes extremely difficult, and in most situations the invasion process cannot be reverted. Under these circumstances, there is interest in finding applications for the pests, and thus, to some extent, offset their damaging effects. This thesis intends to contribute for the improvement of Asian clam management by exploring the application axis. *C. fluminea* has higher filtration capacity (up to 500 ml/h/individual), and it has been shown to be fairly tolerant to and bioaccumulate a range of water contaminants. Therefore these bivalves seem to be promising biofilters to intercept wastewater discharges, reducing pollutant contents and toxicity.

Within the framework above, two projects were carried out under the scope of this thesis.

The first project explored the possibility of combining Fenton's reaction and biofiltration by the clams to treat winery effluent. Building on previous in-house laboratory data, this study was conducted at the pilot scale, using effluent from real winery practice.

The second project was concerned with the combination of ozonation with biofiltration by *C. fluminea* as a treatment method for parabens. The development of such an integrated decontamination approach is still at an incipient stage. This thesis only addressed the development of the chemical treatment component, the first step towards the combined remediation tool. In this context, the aim of the study was to get an insight into the effect of ozonation on parabens to eventually find optimal chemical treatment conditions. Subsequent biofiltration of ozonation-treated paraben effluent could not be studied due to time constraints, but preliminary toxicity tests to assess the tolerance of the *C. fluminea* to the contaminants were carried out.

To mitigate the effects that chemical processes and human activities have on the environment and on public health must be one of the duties of a Chemical Engineer. This thesis aims to be a contribution for the global improvement of water quality using sustainable process. Overall, this investigation was driven by:

– environmental purposes, since the target contaminants are not effectively processed by conventional waste water treatment, and thus aquatic life is currently under continuous exposure to them , which can trigger low dose effects;

– ecological concerns, as the study represents an attempt to improve the management of a costly and ecologically damaging bivalve pest;

– public health issues, like cancer connected to the presence of pollutants in the water;

– social motivations, given that pollution is a growing concern for the society;

– economic motivations, as the cost-effective use of an invasive pest is sought.

This thesis is organized in five chapters. After this introductory chapter, where the relevance, motivation and objectives of the study have been presented, chapter 2 provides some theoretical background and a state-of-the art that are essential to comprehend and discuss the work developed. Chapters 3 and 4 are the core of the thesis, addressing the two working projects as discussed above. Chapter 3 deals with the combination of Fenton's reaction and biofiltration for the treatment of winery effluent. Chapter 4 is dedicated to the development of integrated ozonation/biofiltration as a remediation tool for water contaminated by parabens. By reporting the experiments conducted, these two chapters have the same structure, including data on the most important chemicals that have been used, the description of the experimental procedures for all the trials and analytical methods, and the experimental results and respective discussion. Finally, in chapter five, general conclusions are drawn, important remarks are done, and some recommendations for future research are given.

2. Background and state-of-the-art

This chapter provides the support necessary for the comprehension of the core of the thesis. In accordance with the work carried out, it is divided in three major parts. The first part focuses on the two contaminants addressed – winery effluent and parabens – with reference to their nature and the current treatment practices and challenges. Then, the chemical treatment processes employed – Fenton's reaction and ozonation – are discussed. In the last part of the chapter, the Asian clam *C. fluminea*, used as the basis for biofiltration, is presented.

2.1 Two challenging contaminants

2.1.1 Winery effluent

The nature of the contaminant. Wine production requires the implementation of a biotechnological sequence involving several operations. Wine is a product obtained from the fermentation of fresh grapes, (crushed or not) or grape must. The production of white wine is normally conduced in a clarified must, which is obtained after grape stem removal, pressing of the grape berries and subsequent clarification. Red wine is usually produced by the fermentation of a non clarified must, prepared after grape stem removal and crushing of grape clusters. Musts can also be fermented in the presence of grape stems. After fermentation, wines must be clarified and stabilized, chemically and microbiologically, prior to bottling. Although few products are added to the must and/or wine, several residues are rejected, either as liquid or solid waste, throughout the process.

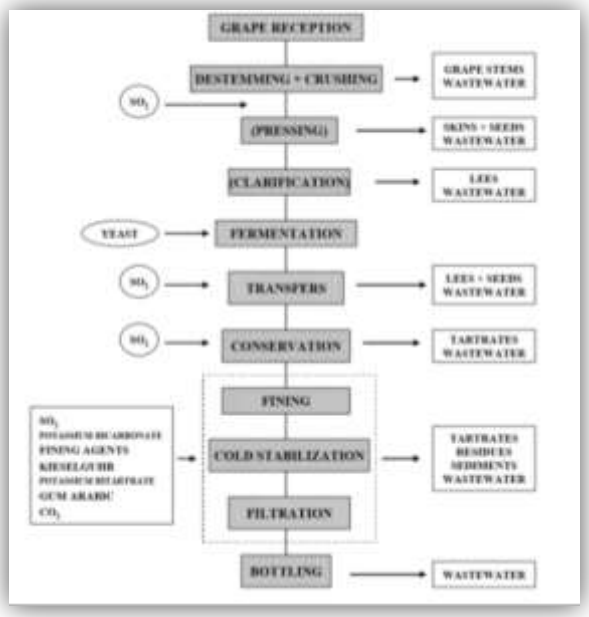

Figure 1- Technological process adopted at ACPB wine-cellar (Brito, 2007).

Figure 1 represents a schematic process, applied at ACPB, to produce green wines. For these wines, the ordinary winemaking process is followed, but ageing is avoided in order to preserve the original freshness and fruity characteristics.

Winemaking is a seasonal process with high activity in autumn, which corresponds to vintages and fermentations. The process also occurs during spring on the occasion of transfers and filtrations, while performed to a lesser extent during the winter and summer months.

Winery effluents contain four types of principal pollutants:

– sub-product residues: seeds, stems, lees, skins, tartar and sludge;

– lost brut products: musts and wines accidentally lost during washings;

– products used to treat the wines: finishing agents, filtration earths;

– cleaning and disinfection products, used to wash materials and soil.

Must and wine constituents are present in wastewaters, at variable proportions: sugars, esters, ethanol, glycerol, organic acids, and a numerous population of bacteria and yeasts. These tend to be easily biodegradable elements, except for polyphenols, which not only make effluent biodegradation more difficult, but also require an adapted flora. Table 1 shows some examples of winery wastewaters having different compositions.

The effluents from wine production have large variability, in both quantity and quality, making evaluation of daily pollution complex. Generally, the production of 1 m^3 of wine generates a pollution load equivalent to that of 100 persons (Antonio G. Brito, Brewery and Winery Wastewater Treatment: Some Foca Points Of Design and Operation 2007). The amount and quality of the effluent produced throughout the year also varies greatly following the cycle of wine production above.

The washing operations carried out during the different wine production steps are at the origin of the rejection of fully charged wastewaters. These winemaking operations can be distributed as follows:

– vintage preparation: washing and disinfection of materials;

– grape reception: washing of reception materials, cleaning the floors (with or without addition of cleaning products);

– vinifications: rising of fermentation and clarification vats, cleaning the floors (with or without addition of cleaning products);

– transfers: rising vats after transfers, cleaning the floors (with or without addition of cleaning products);

– filtrations: rinsing kieselguhr and earth filters.

Table 1- Examples of effluent compositions (typical or range values) from four different wineries (Antonio G. Brito, Brewery and Winery Wastewater Treatment: Some Foca Points Of Design and Operation 2007).

	Wine cellar			
	ACPB	A	B	C
Production (m^3 /year)	250	730	3000	6000
pH	5.7	4.9	4.7	4.0 - 4.3
COD (mg/L)	1200-10266	5200	12150	9240 - 17900
BOD (mg/L)	130-5320	2500	8100	5540 - 11340
Total N (mg/L)	12 – 93	61	48.2	74 - 260
Total P (mg/L)	23	25	5.5	16 to 68

Treatment practices and challenges. The spatiotemporal dynamics and the combination of high amounts of organic and inorganic compounds in winery wastewater make its treatment very challenging.

Table 2 provides an overview of the management methodologies that may be used for the depuration of winery wastewater. Several treatment systems, both physico-chemical and biological, have been assayed to reduce the organic load of this type of effluent. Some of such technologies are based on membrane bioreactors (MBRs), sequencing batch reactor (SBR), upflow anaerobic sludge blanket (UASB), anaerobic sequencing batch reactor (ASBR) or jet loop reactors (JLR). Most of the methods that have been suggested to treat winery wastewater have some common characteristics: they are relatively expensive, not applicable in all situations, and not always able to deal with fluctuations in the hydraulic and pollution loads.

The main disadvantage of traditional biological processes for this sort of effluent is related to the acclimation time required by the microorganisms. The high variations of the effluent load and composition reduce the efficiency of such systems. Moreover, the presence of bio-refractory substances in the effluent (such as phenolic compounds) compromises the biological action over the wastewater. In this context, the application of a preliminary advanced oxidation process (AOP) may improve the effluent's biodegradability. Amongst the AOPs, Fenton's reaction appears as an interesting alternative. Table 3 compares Fenton and Photo-Fenton processes when applied to winery wastewaters. Photo-Fenton system is more efficient than Fenton's reaction, but to work, it needs an UV light, in general meaning an added cost of electrical energy.

Table 2 - Treatment processes used in the wine industry (K.P.M. Mosse 2011).

References	Treatment process	Type of treatment	Advantages	Disadvantages	Typical COD reduction	Applicability
Maynard et al. (1999); Montalvo et al. (2010)	Aerated lagoons	Aerobic	Easy management. Widespread	Energy intensive. Do not always cope well with high carbon loads and volumes during vintage.	91%	All wineries.
Fumi et al. (1995)	Activated sludge	Aerobic	Easy management. Widespread.	Energy intensive. Requires nutrient (N, P) addition.	98%	Medium-large wineries.
Torrijos and Moletta (1997)	Sequencing batch reactor (SBR)	Aerobic	Widespread. Low capital costs. Relatively simple automation.	Requires storage tanks to permit batch feeding.	>90%	Medium-large wineries.
Artiga et al.(2005)	Membrane bioreactor (MBR)	Aerobic	Small footprint. Lower sludge production. Improved treated water quality.	High establishment costs. Membrane fouling.	>97%	Large wineries. Only if high value reuse intended.
Petruccioli et al. (2000)	Jet-loop activated sludge	Aerobic	High efficiency. Lower energy requirements.	Limited number of applications to date.	94-98%	Potentially all wineries.
Petruccioli et al. (2000); Oliveira et al. (2009)	Air microbubble bioreactor (AMBB)	Aerobic	High biological conversion.	Limited number of applications to date.	>90%	Potentially all wineries.
Ruiz et al. (2002)	Anaerobic Sequencing Batch Reactor (ASBR)	Anaerobic	Potential for biogas capture. Low sludge production.	Batch feeding required.	>98%	Medium-large wineries.
Keyser et al. (2003)	Upflow Anaerobic Sludge Blanket (UASB)	Anaerobic	High sludge activity. Low sludge production.	Relatively high installation costs.	80-98%	Medium-large wineries.
Keyser et al. (2003); Moletta (2005)	Anaerobic digestion (Covered anaerobic lagoon)	Anaerobic	Low cost. Potential for biogas capture.	Longer start-up times.	65-95%	All wineries.

Table 3 - Comparison between Fenton and Photo-Fenton processes for winery effluent treatment (based on Mojtaba Hadavifar, 2009).

Treatment	Operating conditions	Efficiency	Considerations
Fenton	$FeSO_4 7H_2O$ (15%), H_2O_2 (30%), H_2SO_4 and NaOH 1 M to adjust pH. Room temperature. 200 ml beaker consisting of 100 ml vinasse with COD's ranging from 3000 to 39000 mg/l and pH from 2.5 to 8.5. Samples were stirred for 5 min at 30 rpm in order to completely dissolve ferrous sulphate. H_2O_2 was added to the mixtures and stirred for 15 min at 30 rpm. The mixtures were carried into a 100 ml graduated cylinder and solids allowed to settle.	47%	Fenton process is easy to apply for both small or big production.
Photo-Fenton	Treated samples in the first experiment were exposed to UV radiation for 80 min with additional 2 ml/l of H_2O_2. The irradiation was carried out using two parallel adjustable F8T5 UV-B, 302 nm fluorescent tube and radiation values in $\mu w/cm^{-2}$ were obtained by the use of a UVX radiometer. The distance between the UV lamp and reaction vessel surface was fixed at 50 mm.	73%	Photo-Fenton process is easy to apply in small production. The cost increases with the amount of water to be treated because of the costs of the lamp and the electrical energy associated with it.

2.1.2 Parabens

The nature of the contaminant. Parabens (Figure 2) are the most commonly used antioxidants in the cosmetic and pharmaceutical industries, also working as antimicrobial and antifungicidal agents. Parabens are often used in combination in most commercially available personal care products, and therefore a wide spectrum of these compounds may be expected to be released continuously into the aquatic environment through domestic waste.

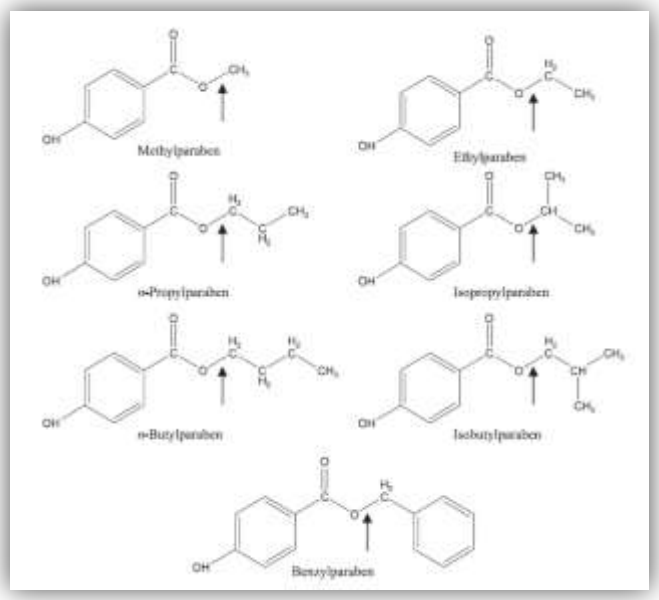

Figure 2 - The chemical structures of seven alkyl esters of p-hydroxylbenzoic acid (parabens) that are commonly used in consumer products. Hydrolysis of the ester linkage (arrow) gives the common paraben metabolite p-hydroxybenzoic acid (Darbre PD 2008).

Parabens have recently been demonstrated to have estrogenic and antiandrogenic properties. There seems to be a potential relationship between breast cancer and prolonged dermal exposure to paraben-containing products based on the discovery of these compounds in breast tumors (Philippa D. Darbre 2008).

Treatment practices and challenges. It is assumed that parabens can be removed by conventional water and sewage treatments, but the prevalence of these compounds in the effluent from WWT plants as well as contaminating natural waters undermines this perception. These findings have also led to growing public concern about the potential impacts of parabens on human health.

Not much research has been carried out on the removal of parabens from aqueous solution.

Ozonation, the technique that was employed in the context of this thesis, has recently appeared as an important technology for the removal of a wide range of organic compounds from water. Table 4 presents data on the use of ozonation to remove parabens from water, showing that this process allows to take away a high percentage of these compounds as well as providing great COD and TOC reductions.

Table 4 - Parabens' remotval by ozonation.

Reference	Parabens'concentration	Experimental conditions	Conclusions
N. A. Kheng Soo Tay (2009)	[MeP] = 0.304 g/l [EtP] = 0.332 g/l [PrP] = 0.180 g/l [BuP] = 0.097 g/l [BzP] = 0.113 g /l Paraben solutions for ozontion were prepared by mixing parabens at equal molar ratio.	Ozone was produced from purified oxygen 99.8% by a OZX03K model ozone generator. Ozone was bubbled into a solution containing parabens in a 1000-ml cylindrical jacked beaker through a gas dispersion tube. Temperature reaction ranged from 20 to 25°C and was maintained at the desired value (± 0.1°C) using a circulating water-bath. Adequate pH was obtained by the addition of sodium hydroxide and phoshoric acid.	For a 500 µM solution, 99% of parabens' removal was achieved within 12 min. Over 3 h of ozonation, removal of COD and TOC was 61% and 32%, respectively.
N. A. Kheng Soo Tay (2011)	[MeP] = 1.9 g/l [EtP] = 2.1 g/l [PrP] = 2.25 g/l [BuP] = 2.4 g/l [BzP] = 2.85 g/l The ozone stock solution was added to a series of solutions (final concentration of 25 µM), containing 250 µM of the selected compound and 20 mM of t-BuOH (radical scavenger).	Ozone was produced from purified oxygen (99.8%) by a OZX-05K model ozone generator. The aqueous ozone stock solution (~1.3 mM) was prepared by sparging ozone at the rate of 0.70 g $L^{-1}h$ into water placed inside a water-jacketed beaker at 2 °C. A 20-mL amber headspace vial was used as the reactor. The experiment was buffered at pH 7 using 20 mM phosphate buffer.	Ozonation of selected compounds in secondary wastewater and surface waters revealed that ozone dose of 1 and 3 mg/L yielded greater than 99% depletion of parabens.

2.2 Chemical treatments used in the proposed integrated remediation approaches

2.2.1 Fenton's reaction

Fenton's reaction is classified as an advanced oxidation treatment process. This process is based on the oxidation power of hydrogen peroxide catalysed by Fe^{2+}. The generated hydroxyl radicals (OH•) are able to unselectively react with a wide range of organic compounds.

Fenton's peroxidation is attractive at the industrial level because it can be operated at ambient conditions of pressure and temperature and it does not require specific equipment. Moreover, it involves low cost and environmentally benign reactants. All these reduce the operating expenses associated with the treatment.

At the industrial scale, the Fenton's process requires a preliminary pH correction step since it is more efficient when pH ranges from 3 to 3.5. In the second stage, ferrous sulphate is added as the iron source and hydrogen peroxide is fed (Bautista et al., 2008). At the end of the reaction, the mixture must be neutralized, which will promote iron separation from the liquid as insoluble $Fe(OH)_3$ is produced. Therefore, the final step of the process involved a settling tank to remove the iron sludge from the treated wastewater. Sludge production is one of the main disadvantages of this treatment process.

Fenton's reaction is able to destroy organic compounds, reduce toxicity levels, remove COD, reduce odour and/or change colour.

The mechanism involved in this process is complex, but it can be described in terms of the following steps. First H_2O_2 is decomposed. This decomposition is catalysed by ions Fe^{2+} in acid ambient:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH\cdot + OH^-$$

The radicals OH· react with organic compounds to give an alchyl radical, which then reacts with dissolved O_2 in water to give an alchyl radical peroxide:

$$OH\cdot + RH \rightarrow R\cdot + H_2O$$

$$R\cdot + O_2 \rightarrow ROO\cdot$$

$$ROO\cdot + RH \rightarrow ROOH + R\cdot$$

Hydroperoxide ROOH can react with Fe^{2+} and Fe^{3+} to give more radicals, increasing the rate of reaction:

$$ROOH + Fe^{2+} \rightarrow RO\cdot + Fe^{3+} + OH^-$$

$$ROOH + Fe^{3+} \rightarrow ROO\cdot + Fe^{2+} + H^+$$

The further oxidation of intermediates of the reaction leads to the formation of carbon dioxide and water.

The OH· radicals can get involved in four kinds of possible reactions:

– addition reaction, where the radical OH· is added to aliphatic or aromatic unsaturated compounds to give a radical ($OH\cdot + C_6H_6 \rightarrow (OH)C_6H_6\cdot$);

– hydrogen reaction removal, in which an organic radical is formed

($OH\cdot + CH_3OH \rightarrow \cdot CH_2OH + H_2O$);

– electron transfers, where ions are formed, having a higher number of oxidation

($OH\cdot + [Fe(CN)_6]^{4-} \rightarrow [Fe(CN)_6]^{3-} + OH^-$);

– interaction radical reactions, when two OH · radicals combine with each other

($OH\cdot + OH\cdot \rightarrow H_2O_2$).

The first two types of reaction must predominate over the others to achieve the intended treatment purpose.

Fenton's efficiency depends on several operating parameters.

Without iron the reaction does not start; increasing iron concentration makes the reaction proceed more rapidly until a moment in which further addition of the ion does not change the speed of the reaction.

The rate of the reaction increases with temperature, but it must not exceed 40-50 ° C as above this temperature the decomposition of hydrogen peroxide in oxygen and water is enhanced. The optimal temperature for the process is between 20 and 40 ° C.

Another important parameter to be taken into consideration in this type of reaction is pH, which should be between 3 and 5. If pH is too high, the iron precipitates as iron hydroxide (III), reducing the catalyst available to promote hydrogen peroxide decomposition into hydroxyl radicals. Moreover, at very low pH the reduction of Fe^{3+} into Fe^{2+} is inhibited lowering the process efficiency since the catalyst recycling step is compromised. It should be noted that there may be a decrease of the pH while adding the Fenton's reactants since the catalyst $FeSO_4$, may contain residual H_2SO_4 and the H_2O_2 is responsible for the fragmentation of organic material into organic acids. Moreover, the reaction between hydrogen peroxide and iron is very exothermic. So, in order to control pH and the temperature

during the reaction it is better to complete the process step by step under continuous adjustment.

The establishment of the H_2O_2 concentration is also a crucial point on Fenton's process. On one hand, the increase on H_2O_2 load improves the treatment efficiency since more oxidant is available. On the other hand, above a certain limit, the excess of this reactant starts to act as radical scavenger reducing the amount of hydroxyl radicals that are able to react with organic matter.

As reviewed in section 2.1.1, Fenton's reaction has been used for winery wastewater treatment. However, application of this process is not limited to this context. Table 5 shows some of Fenton's reaction applications in various fields.

Table 5 - Application of Fenton's reaction.

References	Field	Treatment	Operating Conditions	Conclusions
Marco Panizza (2001)	This study was performed to investigate the treatment of industrial wastewater, mainly containing naphthalene- and anthraquinone-sulphonic acids, by electrogenerated Fenton's reagent. The hydrogen peroxide was produced in situ by electrochemical reduction of oxygen on graphite-felt cathodes and the Fe^{2+} ions were also regenerated by cathodic reduction of Fe^{3+}	Electrogenerated Fenton's reagent	The wastewater used for the study was obtained from the regeneration of ion-exchange resin towers by a chemical industry. These towers constitute the final step in the treatment plant. The initial COD was 1361 mg L^{-1}. The experiments were performed using an undivided cell with a volume of 200 ml, supplied with a heat exchanger and a magnetic stirrer.	Maximun % COD reduction (87%) was obtained with Fe^{3+} concentration of 3 mM for the electro-Fenton process.
Huseyin Tekina (2006)	The applicability of Fenton's oxidation to improve the biodegradability of pharmaceutical wastewater to be treated biologically was investigated	Fenton's reaction	The wastewater originated from a factory producing a variety of pharmaceutical chemicals. Treatability studies were conducted under laboratory conditions with all chemicals (having COD varying from 900 to 7000 mg/L) produced in the factory in order to determine the operational conditions to utilize in the full-scale treatment plant. The experiments were conducted in batch reactors and 100–200 mL wastewater samples were used. The pH of the heated sample was adjusted to the required value with sulphuric acid (1 M) and sodium hydroxide (10 M). Required amounts of $FeSO_4 \cdot 7H_2O$ and H_2O_2 were added to the sample. The solution was stirred. Thirty minutes were allowed for the completion of the reaction. Then, another 30 min were allocated for precipitation.	The results in the use of Fenton's reaction indicated that the overall treatment efficiency was best at an initial pH of 3.5 and second stage (coagulation) at a pH of 7.0. For all pharmaceutical products (with a COD range 900–7000 mg/L), average COD removal efficiency was highest when the ratio of H_2O_2/Fe^{2+} was around 150–250. At a constant H_2O_2/Fe^{2+} molar ratio of about 155, 0.3 M H_2O_2 and 0.002 M Fe^{2+} provided 45–65% COD removal. Treatment with Fenton's oxidation improved the biodegradability and reduced the toxicity of the pharmaceutical wastewater. Fenton oxidation was an effective pretreatment method for the non-biodegradable portions of the pharmaceutical wastewater, which renders them more biodegradable for following biological processes.
Miguel Rodriguez (2002)	Photo-Fenton process was explored as photochemical pre-treatment to improve the biodegradability of a wastewater coming from a textile industry	Photo Fenton's reaction	In the coiled photochemical reactor, the pollutant solution circulates through an 8 mm-diameter glass spiral of about 20 m long. A 400 W, 40 cm long, medium-pressure Hg-lamp is positioned in such a way that its center line passes through the axis of the coiled reactor. The pre-dominant radiation is at 366 nm with output equivalent to ~15 W. The runs are carried out at 30, 45 and 60 °C to study the influence of the temperature in the reaction rate. The pH was adjusted around 3 with HCl. The pollutant solution, H_2O_2 and Fe^{3+} solution are added at the beginning into the mixing-vessel. The solution is in batch mode recirculated at 26 L h^{-1} through the illuminated part of the reactor. In order to prepare the photo-treated water for biological treatment, the solution is neutralized and all experiments were carried out	Through this work, the optimal concentrations for the treatment of the textile effluent were set for Fe3+ and H2O2 as 1.43 and 441.2 mmol L−1, respectivel.,. Also, 60 °C was determined as the optimal temperature.

			until H_2O_2 was consumed.	
M.I. Badawy (2006)	As a consequence of population growth, major efforts have been made by the Egyptian government to construct new industrial areas. Tenth of Ramadan City is one of the most important industrial cities in Egypt. The wastewater generated from various industrial activities was highly contaminated with organic matters as indicated by COD (1750–3323 mg/L), TSS (900–3000 mg/L) as well as oil and grease (13.2–95.5 mg/L).	Fenton's peroxidation and coagulation processes	Fenton process was carried out at room temperature by adding various doses of $FeSO_4 \cdot 7H_2O$. The pH was adjusted at 3.0 ± 0.2 and kept at the same value during the reaction. The required amount of H_2O_2 was fed by dosing pump during a period of 15 min, and then the coagulation experiments were conducted with the Jar test method. This method was preceded with rapid mixing of the Fenton treated effluent at 100 rpm for 5 min, slow mixing at 40 rpm for 30 min and then standstill for 30 min. After 30 min settling time, the supernatant was withdrawn, filtered through 0.45 μm, treated with 1N NaOH and heated at 40 °C to remove residual H_2O_2. Physical and chemical characteristics of the treated wastewater were analyzed according to APHA. The pH for oxidation and coagulation experiments was controlled at 3.0 ± 0.2 and 8.5 ± 0.2, respectively, with 0.1N sulfuric acid or sodium hydroxide.	The best treatment results were obtained with the Fenton process, which under the given operating conditions (pH 3, Fe^{2+} dose = 400 mg/L and H_2O = 550 mg/L)provided 100% color removal and more than 90% decrease in COD.. In the case of pretreatment, lower dose of Fenton's reagents can be used.

2.2.2 Ozonation

The ozone molecule consists of three oxygen atoms bound together. It is very unstable and has a short half-life, quickly degenerating as $2O_3 \rightarrow 3O_2$.

Ozone consists of an oxygen molecule that has received an extra oxygen atom. It is formed in the waterfalls, during thunderstorms; as the most commonly known example in the ozone layer, ozone is produced by the ultraviolet radiation (UV), which derives from the sun. Ozone production can also be promoted artificially for several purposes, such as water treatment. Ozone generators can create ozone by means of extremely high voltages or by UV-light. Both methods involve the decomposition of the oxygen molecule, which causes oxygen radical formation. Such oxygen radicals can bind to oxygen molecules, forming ozone. Because of its instability, ozone is always generated on-site. Nowadays, ozone generation by corona-discharge is the most common method, with several advantages as presented in Table 6 by comparison to UV light-based ozone generators.

Table 6 - Advantages and disadvantages of corona-discharge and UV-light generators (Ozone Solutions The element of Success s.d.).

	Corona-discharge	UV-light
Advantages	Can create high quantities of ozone (up to 100-lbs/day). Corona cell life can exceed ten years. Can create a more pure form of ozone without creating other harmful or irritating gases if using dry air or oxygen as a feed gas. Small construction allowing generator to be installed in virtually any area.	Can be feasible where production of small amounts of ozone is desired like in laboratories. Zero nitrogen oxides. Ozone production is not affected or diminished by humidity.
Disadvantages	Ambient air corona discharge systems require periodic cleaning unless pure oxygen or an electronic heated desiccant air drier is used. Production of nitrogen oxides when used without pure oxygen feed gas or an electronic heated desiccant air drier. Humidity affects all ambient air fed corona discharge systems - just as static electricity is reduced or eliminated by humidity.	UV lamps solarize over time, requiring periodic replacement. Lower gas phase concentrations of ozone generated by UV radiation translate into the handling of much higher gas volumes than with corona discharge-generated ozone. Highest concentration of ozone that can be produced by 185-nm UV lamp is 0.2 percent by weight, approximately 10% of the average concentration available by corona discharge. Considerable more electrical energy is required to produce a given quantity of ozone by UV radiation than by corona discharge.

Ozone is a strong oxidant and can be used to “burn” dissolved compounds (oxidation). The extra oxygen radical in an ozone molecule quickly binds to other components that come in contact because of the instability of the molecule and its tendency to return to the original O_2. Organic and inorganic substances can be oxidized by ozone, and hence even

microorganisms such as viruses, bacteria and fungi, meaning that ozone can act as a disinfection agent.

Due to its oxidising power, ozone can be used for a large variety of purposes. It is widely applied in wastewater and drinking water treatment. The application of ozone in the industrial branch is increasing. The food industry uses ozone for disinfection, and the textile industry uses it for colour removal.

Two main mechanisms can be ascribed to ozone reaction with organic compounds. In the direct pathway, molecular ozone reacts with substances encompassing high electronic density sites. However, under some conditions, ozone can decompose into hydroxyl radicals that are able to unselectively react with a wide variety of molecules. The production of hydroxyl radicals through ozone decomposition may be enhanced by alkaline conditions, the introduction of a co-oxidant (such as hydrogen peroxide) or UV light.

2.3 The Asian clam used as biofilter in the proposed integrated remediation approaches

Corbicula fluminea, commonly known as the Asian clam, is a freshwater bivalve mollusk from the family *Corbiculidae* (Figure 3).

Figure 3 - Asian clams.

The Asian clam is native to southeastern China, Korea, southeastern Russia, and the Ussuri Basin (Aguirre 1999), but it is a powerful invasive species, nowadays widely spread across the world. It has been introduced in more than half of the states in the US and its distribution now covers large parts of Europe (ISSG.; DAISIE).

The species has a typical oval-triangular clam shape, with a dorsal "beak" or umbo at the peak of the shell (Figure 4). In the outside, the shell is olive or yellowish to black-brown in colour, with 1-3 brown/purple coloured radial bands (particularly in juveniles) and white erosion rings near the umbo. The shell becomes darker as the clam ages. The inside layer of the shellis typically white-bluish.

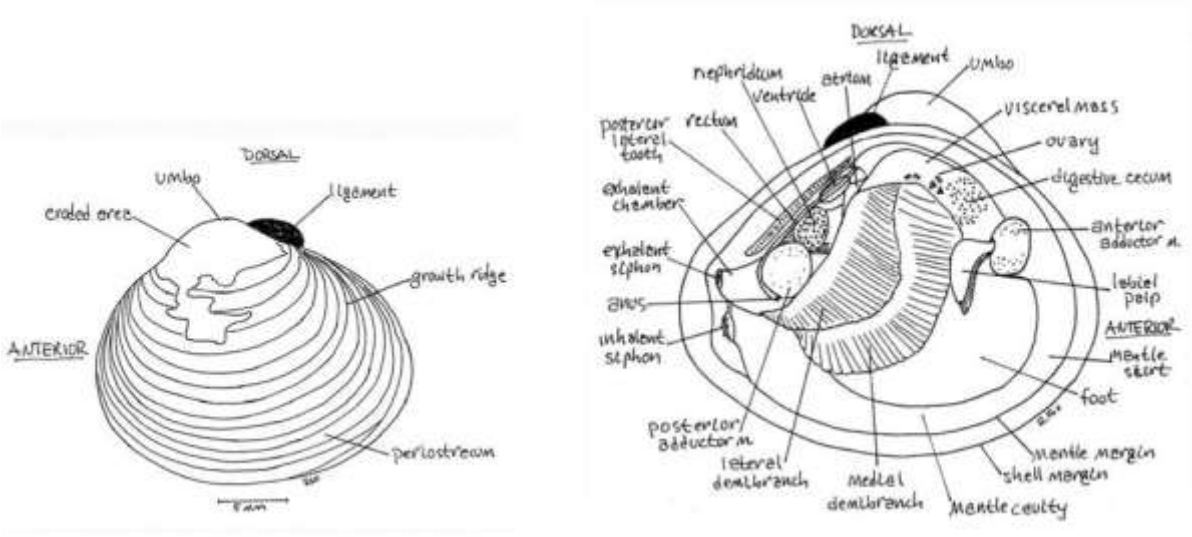

Figure 4 - *Corbicula fluminea* anatomy (Invertebrate Zoology on Line 2007).

An important trait of *C. fluminea* is it powerful filtration capability. The clams use their siphon to filter and feed on suspended particles from the water (particularly Phytoplankton). Laboratory experiments with estuarine bivalves indicate that Asian clam can potentially process large volumes of water, up to 500 ml/h/individual (Rosa *et al.*,). Asian clams also use their fleshy foot appendage to pedal feed on detritus from the sediment (New York Invasive Species Information s.d.).

C. fluminea can be found in lakes and streams with silt, mud, sand and gravel substrate (INHS 1996). It is an infaunal species, meaning that contrary to other invasive biofouling species such as the zebra mussel, the Asian clam does not have the ability to attach to structures. Asian clams will usually be found on the bottom of the structures, but juveniles can be found embedded in moss and mud on the walls of pump wells (Gerald L. Mackie and Renata Claudi 2010).

The table 7 provides data on the tolerance limits of Asian clams for a range of environmental variables. The species can tolerate salinities of up to 13 ppt for short periods (Aguirre and Poss 1999) and temperatures between 2 and 30 degrees Celsius (Balcom 1994).

It prefers fine, clean sand, clay and coarse sand substrates (Aguirre and Poss 1999). It is usually found in flowing water areas because it requires high levels of dissolved oxygen.

Table 7 - Tolerance limits of Asian clams for several abiotic parameters.

References	Variables	Tolerances
(Frances E. Lucy 2012)	Upper salinity limit (psu)*	14 - 17
(Janech MG 1995)	Lower temperature limit (°C)	0 - 2
(Rajagopal S 2000)	Minimal temperature for reproduction (°C)	15
(Dreier H 1981)	Upper temperature limit (°C)	36 - 37
(P 1982)	Lower pH limit	5.6
(Karatayev AY 2005)	Lower calcium limit (mg l^{-1})	3
(SE 1991)	Lower oxygen limit at 25-30°C (mg l^{-1})	1 - 3

* *psu, Practical Salinity Units, correspond to the ratio between the conductivity of a sample of sea water and that of a standard solution of KCl formed by 32.4356 grams of salt dissolved in 1 kg of solution at 15 ° C. The ratios are dimensionless and 35 psu is equivalent to 35 grams of salt per liter of solution.*

Asian clams are hermaphrodites, being capable of both cross and self-fertilization. Adults can live 3-4 years, and typically reproduce two times a year. The species is characterised by very high productivity, it takes only 1 individual to start a population. A single adult can produce 1000-100,000 juveniles per year. Juveniles are tiny (0.25 mm) and capable of long distance dispersal via stream transport, water currents, or hitchhiking on animals, floating objects, or vegetation. Juvenile clams can reach maturity in 3-6 months. *C. fluminea* can rapidly grow into dense populations (>2000 individals/m^{-2}). Substantial die-offs even been shown to affect established populations, probably due to sudden changes in temperature and dissolved oxygen decrease. However, the species' life history (i.e. quick maturity, high fecundity) enable rapid recolonization and population rec overy, even after near extirpation. Overall, these traits turn the Asian clam into a very successful and damaging invasive species able to spread out of the native range and colonize new habitats, where it becomes a serious pest. Asian clams cause important changes in the infested ecosystems at the individual, population, community and ecosystem levels (Ronaldo

Sousa et al., 2013). For instance, by being prodigious filter feeders and establishing dense populations, they tend to promote a pronounced decline in the level of phytoplankton and an increase in the transparency of the infested water bodies, the effects of which extend to the whole aquatic food web. In addition to their ecological impacts, as a biofouling bivalve, *C. fluminea* also cause major problems in the industrial context and other socio-economic scenarios (G.L. Mackie and Robert C. Bailey 2007). By colonising civil, recreational and commercial structures, the clams affect their functioning and can render them useless. Drinking water treatment facilities and industrial cooling systems are also especially vulnerable to the effects of the bivalve's biofouling action. Some of the problems experienced by freshwater-dependent industries as a result of invasive bivalve infestations include pipe and equipment blockage, reduced efficiency of water cooling systems, increased corrosion, safety hazards when systems such as fire protection units are affected, and plant operation disturbance associated with the need for biofouling removal.

Once invasive Asian clam populations are established in an ecosystem, eradication becomes extremely difficult and in most situations the invasion process cannot be reverted. Under these circumstances, there is interest in seeking ways to take advantage from this species and thus, to some extent, offset the pest's damaging effects. A possible way of assigning a value to Asian clams is to capitalise on the species' filtering capabilities for water remediation purposes. Other bivalve species, such as the blue mussel and the zebra mussel, have been used in eutrophication scenarios for the improvement of water quality. Another application of particular interest would be incorporating these biofilters into wastewater treatment, using them to reduce effluents' toxicity. Such a concept, explored in this thesis, has not been thoroughly investigated yet. Amongst the few works addressing this idea are the testing of the capacity of dreissenid mussels to remove particulate-phase contaminants from an effluent (T P Diggins et al., 2002), the assessment of the potential use of zebra mussels to improve the quality of diluted activated sewage sludge (G L MAckie et al., 1994) and the study of Asian clams as part of metal-rich wastewater treatment strategies (Rosa et al.,).

3. Combining Fenton's reaction and biofiltration by Asian Clams to treat winery effluent - Pilot scale study

3.1 Introduction

Several factors contribute to the challenging character of winery effluents. They have high contents in phenolic compounds, which are very toxic to aquatic biota and not easily biodegradable. Their production, both in terms of quantity and quality, is strongly seasonal. For these reasons, wastewater from wineries cannot be directly treated at urban sewage treatment plants because it inhibits microbial growth. While some wine producers attempt to implement their own treatment processes, the discharge of untreated or poorly decontaminated winery effluents remains a serious environmental problem in Southern Europe.

To be viable and widely accepted, any wastewater treatment approach must be affordable for the typically small-to-medium size wineries. A common approach that has been increasingly accepted involves the use of Fenton's process followed by SBR processing. The major issue with such an approach is that it needs ten days or more to remove 84 - 90 % of COD and sometimes it require the addition of extra nutrients, to balance the C/N/P ratio.

In this chapter, an alternative treatment approach for winery effluent, based on the integration of the well-established Fenton's process with biofiltration by Asian clams, is explored. The study builds on previous proof-of-principle laboratory studies by Rita Ferreira (2015), which has shown that it is possible to remove 98% of COD in 7 days of biofiltration after Fenton's reaction.

In the present study, the results by Rita Ferreira (2015) were taken further to evaluate the potential of the proposed combined treatment approach by conducting tests at the pilot scale. A 80-L scale biofiltration unit was designed and built, and real effluent provided by a winery was treated by Fenton's reaction and then processed by the clams.

3.2 Materials and Methods

3.2.1 Chemicals

The origin of the most relevant chemicals and materials are presented in Table 8. All solutions were prepared with distilled water from the equipment Autostill 4000X.

Table 8 - ***List of chemical chemicals.***

Name (chemical formula)	Purity	CAS-number	Supplier
Iron Sulfate $FeSO_47H_2O$	≥99%	7720-78-7	Riedel-De-Haën
Hydrogen Peroxide H_2O_2	33% w/v	7722-84-1	Panreac
Sulfuric Acid H_2SO_4	96%	7664-93-9	Panreac
Silver Sulfate Ag_2SO_4	≥99.5%	10294-26-5	Sigma-Aldrich
Potassium Dichromate $K_2Cr_2O_7$	≥99.8%	7778-50-9	Sigma-Aldrich
Mercury Oxyde HgO	99%	21908-53-2	ACROS ORGANICS
Potassium Sulfate K_2SO_4	99%		Panreac
Methyl Red			Panreac
Methyl Blue			Panreac
Ethyl Alcohol	96°	64-17-5	Carlo Erba Reagents
Sodium phosphate monobasic monohydrate (NaH2PO4)	≥98%	10049-21-5	Sigma-Aldrich
Sodium phosphate dibasic (Na2HPO4)	Basic		
Sodium Hydroxide NaOH	Microbeads	1310-73-2	Cmd Chemicals

3.2.2 Effluent treatment by Fenton's reaction

Four winery effluent samples, with different characteristics (Table 9), were analysed.

Table 9 - Composition of the original winery effluent samples.

Effluent sample	Initial COD [mg L^{-1}]	pH	N-NH_4 [mg L^{-1}]
A	1267.15	5.7	0
B	770.41	5.1	0
C	1366.49	5.0	0
D	340.68	5.3	0

The original effluent samples were treated separately by Fenton's reaction in a batch reactor of 20 L at the following conditions (Rita Ferreira, 2015): (i) 4.31 mL of H_2O_2 per 300 mL of effluent; (ii) 1.38 g of $FeSO_4$ per 300 mL of effluent; (iii)-pH 3. The pH of the effluent being treated was corrected using a 0.4 M H_2SO_4 solution to have the best conditions for the reaction. After pH correction, $FeSO_4$ was added and the effluent was continuously stirred at 170 rpm (mixer HEIDOLPH RZR1 50 Hz) for 6 h. Then H_2O_2 was added and stirring proceeded for 10 h more, giving an overall reaction time of 16 h (Figure 5a). After that time the reaction was stopped by increasing the pH to 7 using a 5 M NaOH solution. The effluent was left to settle for 24 h, and then the supernatant was separated from the sludge using a pump (ISMATEC BVP 60/50 Hz) (Figures 5b, 5c). At the end of the process, clear effluent

samples, thereafter designated as Fenton-treated, were available. The original and Fenton-treated effluent samples were characterized COD, pH, N-NH_4 presence by implementing the methods described in section 3.2.4.

Figure 5 - Treating the winery effluent by Fenton's reaction. (a) Fenton reaction involving 16 hours of continous stirring. (b) Sedimentaion after stopping Fenton's reaction. (c) Fenton-treated effluent separation from Fe-rich sediment. (d) Effluent (A) before and (B) after Fenton's reaction (original and Fenton-treated samples, respectively).

3.2.3 Effluent treatment by biofiltration by Asian clams

The Asian clams used in the biofiltration experiment were obtained from a sandy–muddy shallow creek located in Casal de São Tomé, Mira, Portugal (40°25'06.90'' N, 8°44'13.18'' W), where an invasive population is well-established (clam density in the range 2000 to 4000 individuals m^{-2}). Individuals of all size classes were collected by sieving sediment though a 1-mm mesh bag and immediately transported to the laboratory in field water. The animals were maintained at least one week under continuous aeration at constant temperature (20 ± 2°C) and photoperiod (16 h light and 8 h dark) prior use. The laboratory culture water was fully renewed once a week.

The biofiltration experiments were conducted in two 80-L tanks (Figure 6b), one containing dechlorinated municipal water, working as a blank control, and the other containing Fenton-treated effluent (Figure 4c). Air was bubbled into the tanks throughout the experiments to ensure adequate dissolved oxygen levels for the clams.

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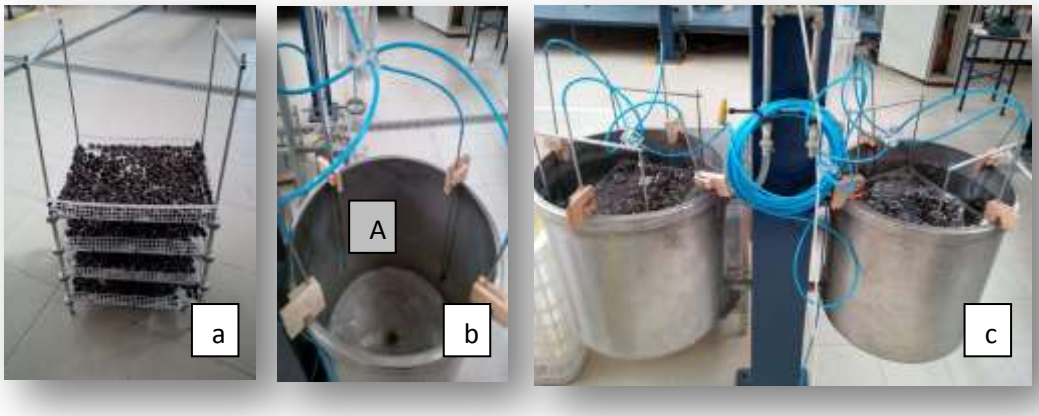


Figure 6 - Biofiltration of Fenton-treated winery effluent by Asian clams. (a) Details of a biofiltration tank, equipped with an air bubbling system with stone diffusors (A). (b) Clam holding structure with 4 levels. (c) Control and treatment tanks used in each replicate biofiltration experiment.

The biofilter structure used to hold the clams in the tanks (Figure 6a) was a key component in the pilot testing unit, and had to be purposely designed and constructed (in-house) before initiating the experiments. The structure was designed to hold a clam ratio (R) of 10/0.5 clams L^{-1}, defined based on the previous testing experience with clams at the laboratory scale. Considering the tank volume (V, 80 L) and the need not to fully fill it to avoid overflow upon aeration of the tank contents, the total number of clams to use in each tank (N) was estimated based on a volume V' of 75 L:

$$N = V' \times R = 1500 \text{ clams} \quad (4.1)$$

Assuming a fixed area (A) of 0.105 m2 for the rectangle of each level of the structure, which would fit the cross section of the tank, and schematizing a clam like a square of side 0.018 m, the density of clams in the tank in number of animals per m^2 (M') was calculated as

$$M' = 3086 \text{ [clams/m}^2\text{]} \quad (4.2)$$

At M' was added 2% of M' to take account of the dead spaces, so the real M' was:

$$M = M' + 0.2 \times M' = 3073 \text{ [clams/m}^2\text{]} \quad (4.3)$$

then, the area S to contain clams and then the number of trays was calculated:

$$S = \frac{N}{M} = 0.408 \text{ [m}^2\text{]} \quad (4.4)$$

The calculation of number of trays was done taking into account that A was the area of each tray:

$$P = \frac{S}{A} = 4 \text{ [number of trays]} \quad (4.5)$$

The available height to arrange the trays was 40 cm, taking a margin of safety from the bottom and from the free surface of the water, the distances between the last tray and the bottom of the reactor and between trays were set as 5 cm and 7 cm, respectively. The dimensions of each tray were defined as 37x28.5x3 cm.

Each biofiltration experiment was initiated by randomly selecting a set of 3000 clams from the laboratory culture. The length of 300 clams from the testing set was measured to assess the size structure of the population at the beginning of the experiment. The test animals were distributed by the trays of the holding structure in both the control and the treatment tanks. The two tanks contained dechlorinated municipal water, and the clams were let to acclimate for 48 h. After the acclimation period, the contents of the tanks were replaced by new dechlorinated water (control tank)) and Fenton-treated effluent (treatment tank), the latter obtained from original wastewater described in the previous section. Effluent sample A (Table 9) was used in the biofiltration experiments because it was the one available in the amount necessary for use in the pilot unit. Moreover, this sample (before and after treatment by Fenton's reaction) had similar characteristics to the effluent used in the previous laboratory proof-of-principle studies (Rita Ferreira, 2015). Biofiltration lasted for 8 days, which would allow for comparison with the alternative treatment SBR applied in the current practice of wineries after Fenton treatment. Mortality within the tanks was evaluated daily by assessing samples of 10 clams, which were discarded after the mortality check. The animals were considered alive if they showed evident siphoning activity or offered resistance to valve opening as carefully forced with a blunt dissection needle. At the end of the test, the size

structure of the filtering populations was checked again by measuring the length of 300 clams taken from each tank. Water samples (of 180 mL) were drawn from both tanks and used to determine COD, pH, dissolved oxygen daily and N-NH4 at days 0, 5 and 8. The analytical procedures were implemented as described below. To ensure reproducibility, the biofiltration experiments were conducted in duplicate (two independent replicates, each consisting of a control plus a treatment tank).

3.2.4 Analytical Methods

Chemical Oxygen Demand (COD). COD was determined by the potassium dichromate method. In this procedure, potassium dichromate is used as an oxidizer of the organic matter under acidic conditions (promoted by sulphuric acid) with a catalyst (silver sulphate). Hence to perform the analysis a digestion solution (potassium dichromate) and an acidic solution were necessary. For use in the COD range 0-2000 mg L^{-1}, the digestion solution contained 10.210 g L^{-1} of potassium dichromate, 33 g L^{-1} of mercury (II) sulphate and 167 g L^{-1} of sulphuric acid. The acidic solution consisted of a 9.6 g L^{-1} silver sulphate solution of 10 g L^{-1}sulphuric acid. Each effluent was analysed in duplicate for its COD value - two vials, each containing 1 mL of effluent sample, 1.2 mL of digestion solution and 2.8 mL of acidic solution, were prepared. Along with these two effluent vials, two blank control vials containing 1 mL of distilled water instead of the effluent were prepared according to the same procedure. The vials were slightly shaken and placed in a thermo-reactor (Eco 25 from VELP Scientifica) at 150ºC for 120 min. After this period the absorbance at 605 nm of the vials' contents was measured using a Photolab S6 photometer from WTW. For each pair of vials the values were compared and if they were uneven the measurements were repeated. Potassium dichromate is reduced forming Cr^{3+} and the amount of Cr^{3+} that is measured after digestion is an indirect indicator of the organic contents of the sample. A calibration curve relating absorbance to COD was prepared in advance by measuring the absorbance of samples with known COD (refer to Appendix – COD – Calibration curve).

It is important to mention that the COD measurement is affected by the presence of hydrogen peroxide in the samples. Therefore, for the effluent samples that had been treated with hydrogen peroxide, some drops of a 200 mg L^{-1}solution of catalase from bovine liver (Sigma Life Science, Sigma-Aldrich, C9322-1G) were added before proceeding to the COD measurement. Furthermore, a cuvette containing 1 ml of effluent sample and 1 ml of ammonium metavanadate was placed in a T60 UV/VIS spectrophotometer to check the presence of H_2O_2.

pH. The equipment Crison micro pH 2002 in conjunction with a glass electrode was used for pH measurements. The calibration equipment was frequently done using the buffer solutions from Scharlau SO2070 for pH = 7 and SO2040 for pH = 4.

Dissolved O_2. The equipment WTW Terminal INOLab740 was used for O_2 dissolved measurements and for temperature measurements.

N-NH_4 presence. To determinate N-NH_4 presence in water VELP SCIENTIFICA DKL Fully Automatic Digestion Units in conjunction with VELP UDK 129 Kjeldahl Distillation Unit were used.

For the measurement of organic Nitrogen was used Kjeldahl's method, which consist of three stages: digestion, distillation and titration.

Kjeldahl digestion converts nitrogen compounds (proteins, amines, organic compounds) into ammonia compounds.

The goal of the digestion is to break down the bonds that hold the polypeptides together and convert them into simpler molecules (such as water, carbon dioxide and ammonium sulphate).

These reactions can be speeded up by the temperature used during *Kjeldahl digestion* (the higher the temperature used, the faster the digestion can be obtained) and by the presence of acid, salt and catalysts (selenium, copper, titanium). Vapors that escape from the tubes are aspirated through the suction cap by a JP recirculating water vacuum pump and eliminated in an SMS scrubber.

Every digestion tube was filled by 50 mL of sample, 7 g of potassium sulphate anhydrous K_2SO_4, 350 mg mercuric oxide, red HgO, 10 ml sulphuric acid concentrated H_2SO_4.

Digestion occurred for 60 minutes at 200 °C (initial heating is aimed to evaporate water) and then for 120 minutes at 370 °C, after this the digestion tubes had to cool to 50-60 °C.

The ammonium sulphate present in the digested sample are converted into ammonia gas, heated and distilled. The ammonia gas is led into an acid trapping solution where it dissolves and becomes a trapped ammonium ion once again.

Distilled or deionized water were added to the tests tube containing the digested sample to dilute it. In this way it is easier to detect all the ammonia.

Nitrogen was separated from the digested mixture by steam distilling (10-100%), in order to extract ammonia from the alkaline solution.

pH was raised of the digested mixture using sodium hydroxide (35%) (automatically on UDK Kjeldahl Distillation Units) to convert NH^{4+} (in solid format) into NH_3 (gaseous), that will be detected with titration.

Distilled vapors were trapped in a dedicated solution of 25-30 ml of boric acid, to trap all the nitrogen, eliminating the risk of loss.

The test tube with the digested sample was drained and the final titration of the ammonia distilled from the sample was performed, considering that if the nitrogen content of the sample is high, a high-concentrated acid for the titration is needed. Another solution is reducing the quantity of the sample used for the analysis, but in some cases it may cause errors giving wrong results.

For the titration Tashiro's indicator was used and it was made in this way: 0.6 g of methyl red are dissolved by 50 ml of 95% ethyl alcohol and then added to a methylene blue solution (0.1 g in 50 ml of distilled water). This solution presents green colouri in alkaline range and gray to pink (pH 4.9) in acid medium to red with an excess of acid.

Using this procedure the Sample range is 2 -150 mg of organic Nitrogen and 1 ml = 0.028 mg $N\text{-}NH_4$.

3.3 Result and Discussion

3.3.1 Effluent treatment by Fenton's reaction

Figure 3 shows the results of the Fenton treatment for the four working effluent samples. For those with higher original pollutant charge (samples A and C), COD removal rate ranged from above 80% for sample A and none in sample C (Figure 7a). As the initial COD decreased (samples B and D), the Fenton's reaction seemed to remain an efficient treatment approach although COD removal rates lower than those for sample A could be achieved (Figure 3a). For example, sample B, having an initial COD of around 770 mg L^{-1}, ended the treatment with a COD of almost 322 mg L^{-1}, corresponding to a removal rate of just below 59%. As far as the pH values are concerned, they were not very different amongst the original samples nor were they for the Fenton-treated effluents, as one could expect by the fact that pH adjustment to 7 was used to stop the reaction (Figure 7b).

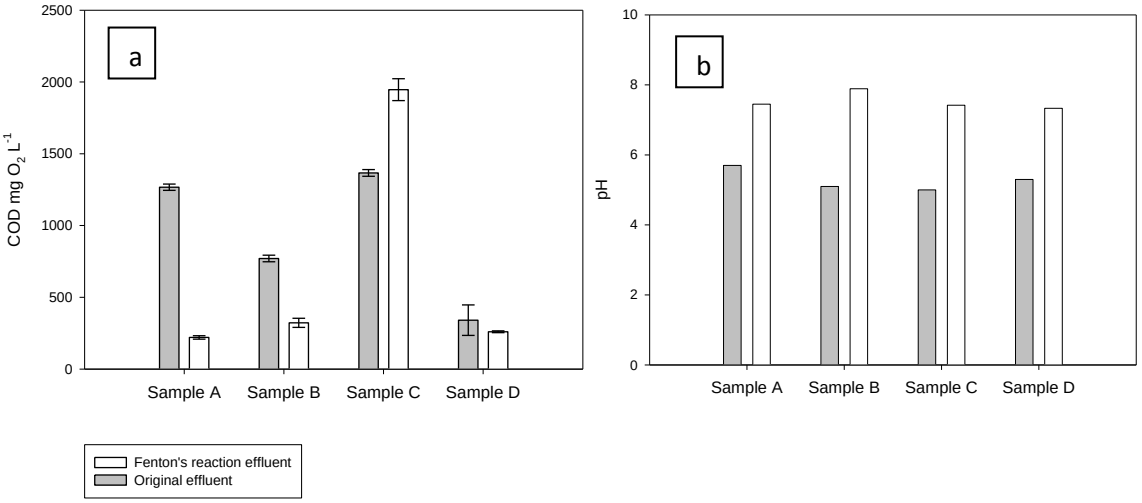


Figure 7 - Characteristics of the four effluent working samples before and after treatment by Fenton's reaction. (a) COD. (b) pH.

3.3.2 Effluent treatment by biofiltration by Asian clams

Figure 8 gives information on the survival of the Asian clams throughout the biofiltration experiments. Mortalities in the control tanks were lower than 15 % (Figure 8a). In general, the animals seemed to fairly tolerate the effluent being treated, which still contained considerable organic charge – mortalities no higher than 70 % were obtained throughout the tests (Figure 8a). Ammonium contents, dissolved oxygen and pH are key parameters for clam survival. Given the static configuration of the pilot unit, these parameters were monitored throughout the biofiltration experiments. As expected, N-NH_4 increased towards the end of the 8-day period, reaching higher values in the treatment tanks compared to the blank controls (Figure 8b). The ammonium variation should be linked to the accumulation of excretion products as well as the degradation of dead animals, more frequent in the treatment tanks than in the controls. At the 5^{th} day of biofiltration, when the effluent was already fairly clean and suitable for discharge as far as COD values were concerned (see below), the N-NH_4 levels in the treatment tanks were around 5 and 7 mg L^{-1} in replicate 1 and 2 respectively. These values are lower than the legal limit for discharge – 10 mg L^{-1} (MINISTÉRIO DO AMBIENTE, Decreto-Lei n.º 236/98). The dissolved oxygen levels in the treatment tanks (Figure 6c) stayed in the range 6.45 - 6.61 mg L^{-1} and 4.58 - 6.33 mg L^{-1} in replicates 1 and 2, respectively, always well above the survival limit for clams (1-3 mg L^{-1}; SE 1991). The pH of the effluent being treated slightly increased over the experiments, never exceeding 8.5 and not significantly affecting the animals' survival (Figure 8d). Overall, the

quality of the media should not have significantly compromised clam mortality. The mortalities observed amongst clams exposed to the Fenton-treated effluent were probably due to some residual toxicity. Even so, at the middle of the treatment period, when appropriate COD values had already been reached (see below), more than 50% of the mussels were still alive.

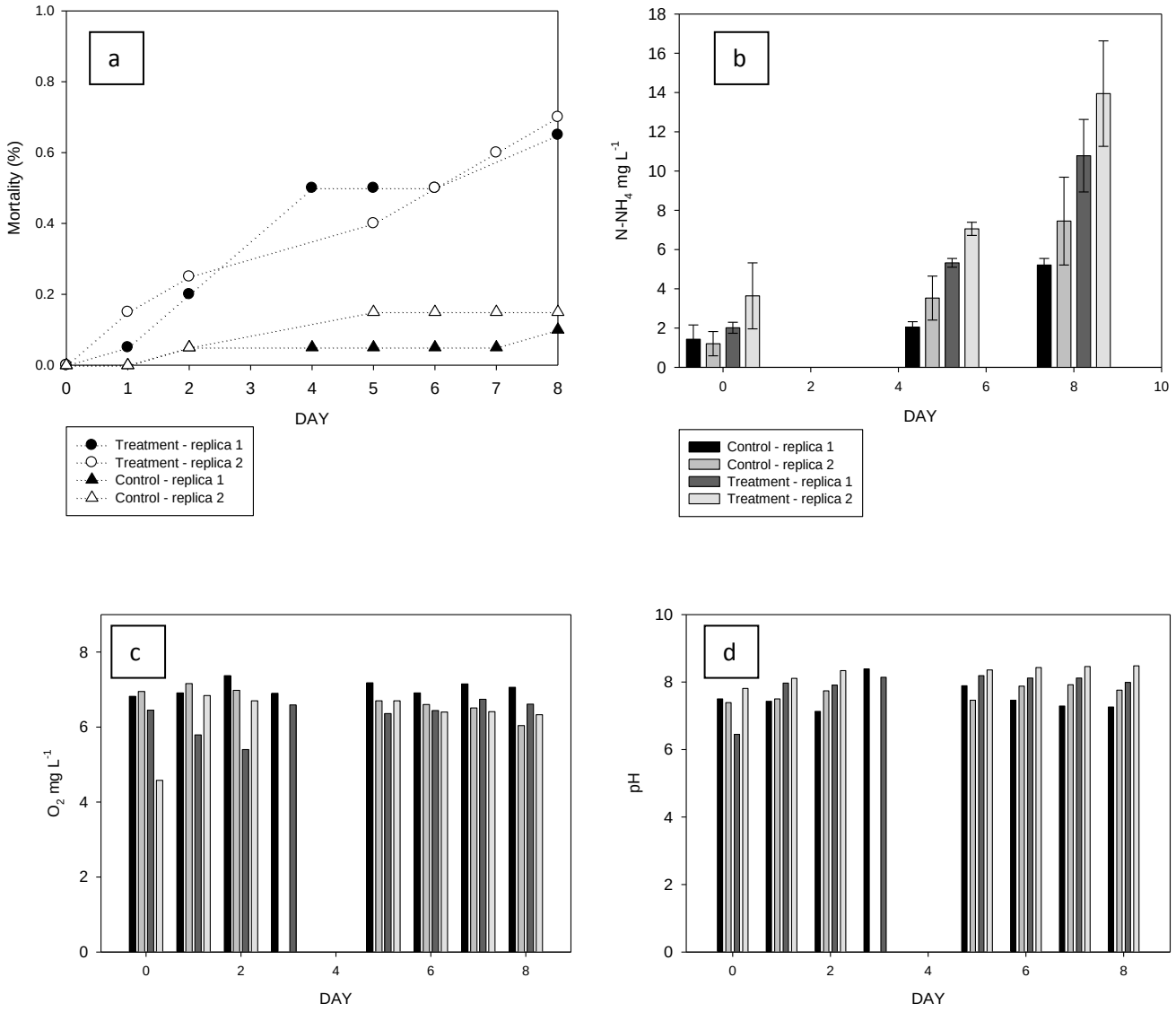


Figure 8 - Survival of the Asian clams working as biofilters in the control and treatment tanks in the two test replicates. (a) Mortality rates. (b) N-NH_4 conditions. (c) Dissolved oxygen levels. (d) pH values.

The core of the biofiltration experiments focussed on assessing whether the clams could work as a polishing treatment tool for Fenton-treated winery effluent. Figure 9 provides some hints towards an answer to the question. Figure 9a shows the COD removal, defined as the ratio 100 - COD/CODinitial, in the treatment pots throughout the tests. As the clams got in contact with the effluent, the COD started to steadily decrease. By the middle of the exposure

period (around day 4-5), the clams seem to have been able to fully clean the effluent's organic charge, with COD removal rates of around 100% being observed. Towards the end of the test, the COD removal rate was found to decrease, which translated an increase of COD after a minimum had been reached. Such an increase coincided with increasing morality rates (Figure 8a), and hence decomposition of dead clams resulting in an increase of the organic matter in the water column. Figure 9b further supports the potential of the *C. fluminea* biofilter. As shown in the figure, at the middle of the test period when the COD removal peaked, the actual COD values in the effluents being treated were 0 and 7.97 mg O_2 L^{-1} in replicates 1 and 2, respectively. Such values are well below the legal emission limit for wastewater discharge that is 150 mg O_2 L^{-1} (MINISTÉRIO DO AMBIENTE, Decreto-Lei n.º 236/98). Furthermore, the SBR generally used to reduce COD of this effluent needs two weeks to reduce 84% of COD.

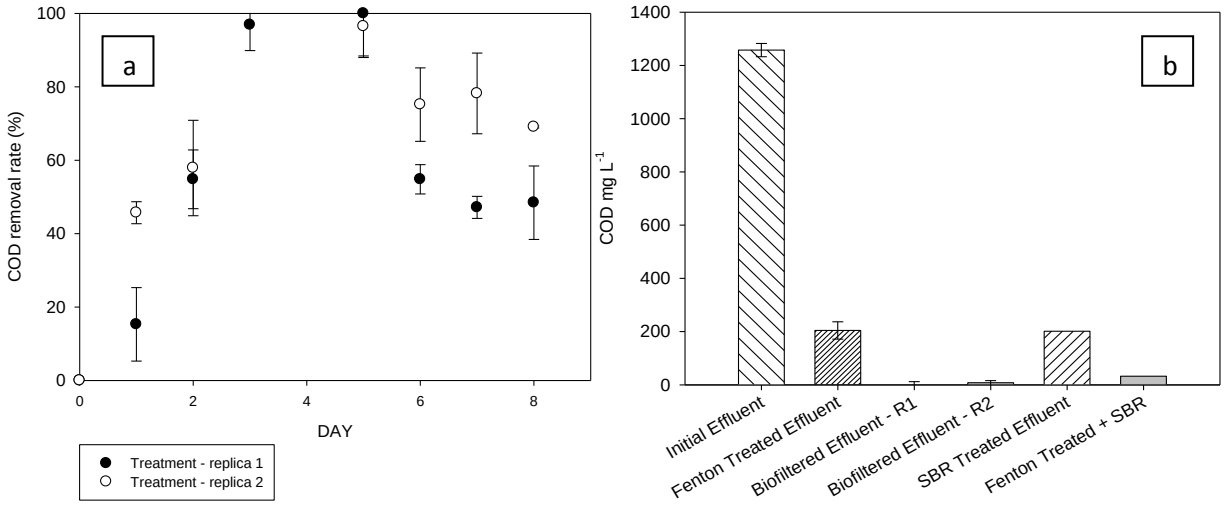


Figure 9 - Performance of the biofilter as a polishing tool for Fenton-treated winery effluent. (a) COD removal rates. (b) Sequential removal of COD through Fenton's reaction followed by biofiltration by Asian clams; comparison with legal limit for discharge and the performance of current treatment solution.

One last comment on clams' mortality is worth being made here. Although some mortality has been observed (Figure 8a), the biofilter still managed to perform its intended function, i.e. lower the effluent's COD to legally acceptable values. Moreover, as discussed above, it seems possible to define the biofiltration period to reach optimal COD values before significant clam mortality occurs. The size structure of the biofilter population at the beginning and at the end of the test shows that clam mortality did not depend on their size/age i.e. mortality was transversal to all size classes (Figure 10).

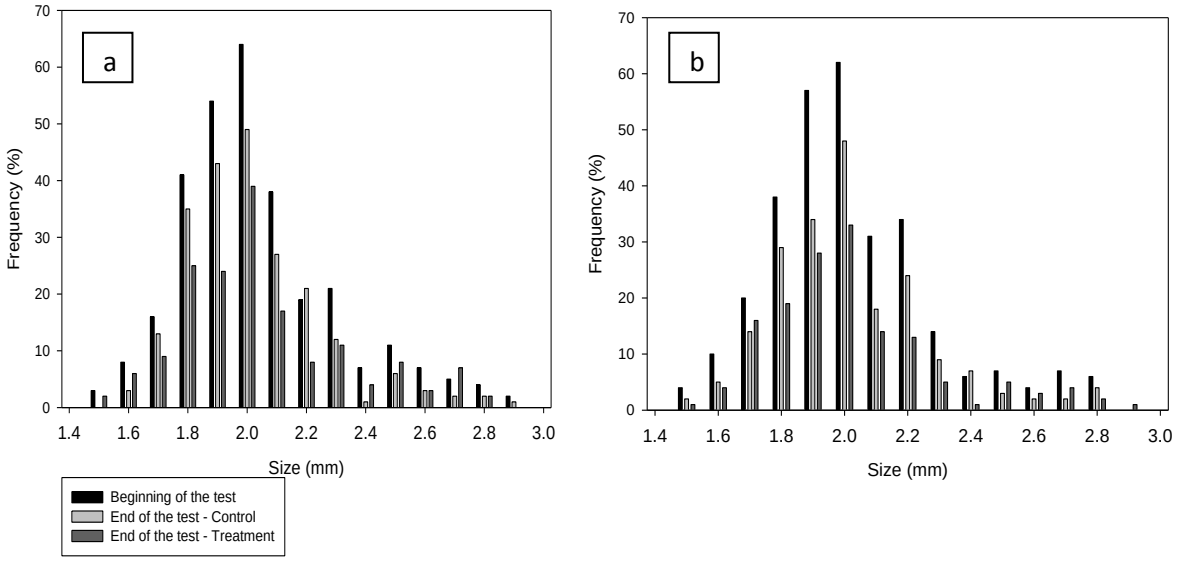


Figure 10 - Size structure of the biofilter and control populations at the commencement and at end of the biofiltration experiments. (a) Replicate 1. (b) Replicate 2.

3.4 Concluding remarks

The results obtained showed that if mild Fenton treatment is applied to partially remove the effluent's organic charge, the clams are able to bring it to legally acceptable quality in a period as short as 5 days. Such a biofiltration period is much shorter than the duration of the post-Fenton treatment approach currently employed – activated sludge SBR. Further advantages of C. fluminea filters as compared to SBR technology, include the fact that they allow assigning a value to an invasive pest and do not require storage tanks to permit batch feeding.

While the pilot study reported here presents a significant advancement in assessing the potential of Asian clams as biofilters for wastewater treatment, some key issues remain to be addressed for a full viability study. Amongst these issues is the economic and environmental cost-balance linked to the use of a damaging invasive species vs the need to dispose of the dead clams after they perform their function.

4. Combining ozonation and biofiltration by Asian Clams to treat parabens - developing the ozonation component

4.1. Introduction

Parabens are a group of compounds widely used as antimicrobial preservatives in food, pharmaceutical and cosmetics products, including underarm deodorants since the 1950s.

Parabens have recently been demonstrated to have estrogenic and antiandrogenic properties and also there seems to be a potential relationship between breast cancer and prolonged dermal exposure to paraben-containing products. The efficiency of conventional biological processes used in waste water treatment plants in processing and degrading parabens is very low and the work presented in this chapter start to find a solution to remove parabens from municipal waste water.

Combined ozonation-biofiltration by *C. fluminea* is proposed as a treatment method for parabens. A Parabens solution, artificially prepared to simulate an effluent rich in these compounds, was treated by Ozonation at optimal work conditions founded during this study. Then toxicity was evaluated by mortality test with *Asian Clams* and by luminescent test with *Vibrio Fischeri*. Investigation of such a decontamination approach is still at an incipient stage, being initiated with this thesis. The first step towards the combined remediation tool is the development of the chemical treatment component (ozonation), which is addressed in this chapter. Laboratory-scale studies using a model, synthetic paraben-rich effluent were carried out to examine the effect of ozonation on parabens and eventually gather data to optimize the chemical treatment conditions. Preliminary toxicity tests to assess the tolerance of *C. fluminea* to the contaminants were also conducted, but biofiltration of paraben-containing effluent after ozonation could not be done yet due to time constraints.

4.2 Materials and methods

4.2.1 Chemicals

The most relevant chemicals that were used in the experiments are presented in Table 10. All solutions were prepared with distilled water from the equipment Autostill 4000X.

Table 10 - *List of chemicals*.

Name (chemical formula)	Purity	CAS-number	Supplier
Hydrogen Peroxide H_2O_2	33% w/v	7722-84-1	Panreac
Sulfuric Acid H_2SO_4	96%	7664-93-9	Panreac
Silver Sulfate Ag_2SO_4	≥99.5%	10294-26-5	Sigma-Aldrich
Potassium Dichromate $K_2Cr_2O_7$	≥99.8%	7778-50-9	Sigma-Aldrich
Sodium phosphate monobasic monohydrate (NaH2PO4)	≥98%	10049-21-5	Sigma-Aldrich
Sodium phosphate dibasic (Na2HPO4)	Basic		
Sodium Hydroxide NaOH	Microbeads	1310-73-2	Cmd Chemicals

4.2.2 Searching for optimal ozonation conditions

A semi-batch reactor of 500 mL was used to perform the ozonation of a model paraben-containing effluent (Figure 10). The ozone was produced in situ by an ozone generator (802N, BMT) that was fed by pure oxygen (99,9%) supplied by Praxair. There was a gas ozone meter (BMT 963 vent, BMT), which determined the ozone concentration produced by the generator. This concentration was the same entering the reactor, which is designated by CO3,in. It was also possible to measure the ozone exiting the reactor (CO3,out) by changing the positions in a valve system. To ensure chemical regime into the reactor, it was agitated at the approximate speed of 300 rpm with by means of a magnetic stirrer (Agimatic-N, P Selecta).

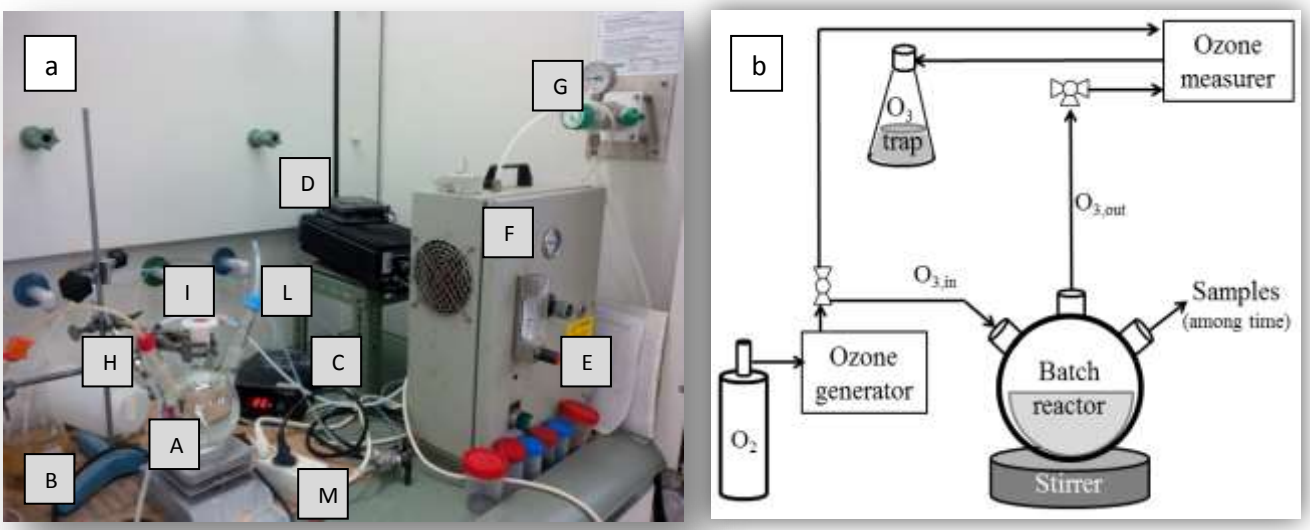


Figure 11 - Ozonation set-up. (a) Layout of the equipment in the laboratory (A - semi-batch reactor; B - ozone trap KI Potassium Iodide; C - ozone measurer; D - Ozone generator; E - flowmeter for gas supply; F - manometer for gas supply; G - oxygen supply; H - ozone inlet with air diffusor stone; I - orifice for sample colletion; L - ozone outlet; M - stirrer). (b) Schematic representation. of ozone equipment.

A parabens solution containing = 10 mg L^{-1} of each MeP (Metilparaben), EtP (Etilparaben), PrP (Propilparaben), BuP (Butylparaben) and BzP (Benzylparaben) in distilled water was prepared and used as model effluent for the ozonation studies. To maintain pH at 7, Na_2HPO_4 and KH_2PO_4 were added respectively at 1.4 g L^{-1} and 0.24 g L^{-1}.

Several ozonation trials were performed in order to assess the effect of ozone and hydrogen peroxide concentrations on the treatment performance, and hence produce a dataset that can be used to choose the most favourable conditions for ozonation in the integrated ozonation-biofiltration decontamination method. The tests were conducted at room temperature, using an ozone pressure of 1 bar and an ozone flow rate of 0.2 L min^{-1}. The test conditions were set following a full factorial with the three levels presented in the Table 11. Table 12 labels and links the 10 trials conducted to the ozone and hydrogen peroxide conditions used. All trials lasted for 180 minutes. 20-mL samples were collected during minute 0, 2, 5, 10, 15, 30, 45, 60 120 and 180.

Table 11 - Hydrogen peroxide and ozone concentrations used in the ozonation trials.

	-	0	+
$[H_2O_2]$ mg L^{-1}	0	2.5	5
$[O_3]$ g Nm^{-3}	20	32.5	45

Table 12 - Combination of hydrogen peroxide and ozone concentrations in the ozonation trials.

Experiment number	$[H_2O_2]$ mg L^{-1}	$[O_3]$ g Nm^{-3}
1	-	+
2	+	+
3	0	+
4	-	-
5	0	-
6	+	-
7	-	0
8	+	0
9	0	0
10	0	0

Each sample drawn from the ozonation trials was assessed for its COD. The analysis was performed as described in the previous chapter (section 3.2.4). A calibration curve specific for the ozonation samples was prepared with COD values in the range 0-100 mg L^{-1}(Appendix I – COD – Calibration curve). The digestion solution used contained 4 x 10^{-3} M of potassium dichromate, 25 g L^{-1} of mercury (II) sulphate (HgSO4) and 125 g L^{-1} of

sulphuric acid (H2SO4). The acidic solution was the same as previously described. The vials for COD analysis contained 2.5 mL of ozonation sample, 1.5 mL of digestion solution and 3.5 mL of acidic solution.

As an illustration, Figure 12a shows samples taken during the ozonation trial # 3 (Table 15) in the thermo-reactor for COD determination and Figure 12b shows them after processing in the thermo-recator. The difference of yellow intensity shows Cr^{+3} accumulation, which means a low COD value.

Figure **12 -** Illustration of samples in COD determination. (a) Samples in the thermo-reactor. (b) Samples before absorbance reading.

4.2.3 Testing the toxicity of untreated and ozonation-treated effluent

From the results of the search for optimal ozonation conditions described above, a specific set of conditions (see Results section) was selected and used to treat 4.5 L of model parabens-containing effluent. Untreated and ozonation-treated effluenst were then tested for their toxicity against two aquatic species: *Vibrio fischeri* and *C. fluminea*. The main aim of these bioassays was to ascertain whether ozonation could not only reduce the effluent's COD but also its toxic potential. Without supporting data, a COD reduction should not be blindly accepted as a reduction of toxicity (e.g. particularly toxic compounds may remain in a lower COD effluent while other less harmful but with higher contribution to the COD original may have been removed). The bioassays with *C. fluminea* served an additional purpose – as the ultimate goal is to integrate ozonation with biofiltration by this species to remove parabens from water, the mortality tests conducted at this stage with Asian clams also intended to provide a basis to examine their tolerance to parabens and define the conditions for future biofiltration studies.

The bioassays were conducted as described below.

Bioassays with luminescent V. fischeri. *V. fischeri* is a very sensible marine luminescent bacteria, used as a standard organism in ecotoxicological analysis. When exposed to harmful contaminants, the capacity of this species to emit light is diminished, which

provides a way of measuring the toxicity of the sample under analysis (usually reported as percentage of luminescent inhibition).

Bioassays using untreated and ozonation-treated model effluent were carried out by following the described in ISO 11348 – Part 2, with lyophilized bacteria being used. Comercial bacteria samples were defrost and introduced in a commercial glycose solution. 0.5 mL of untreated and treated water samples were then added to 0.5 mL of bacteria in the culture media. The luminescence obtained under exposure to the test solutions after 15 and 30 min was measured and compared to that of bacteria in clean culture media to which no effluent had been added. The samples were run at least in duplicate. A LUMISTherm and a LUMIStox with the software LUMIStox 300 (version 4.00) from Hach-LANGE were used to perform the tests. The 2% NaCl solution used to dilute the effluents for the test solutions was prepared from a standard 7.5% solution (LUMIStox). The luminous bacteria kit used was LCK 480.

Bioassays with *C. fluminea*. The adult clams used as test organisms were collected and maintained in the laboratory as described in the previous chapter (section 3.2.3). Mortality tests were conducted under static conditions (Figure 13). Blank control treatments (dechlorinated municipal water) were applied along with a dilution series of untreated and ozonation-treated model effluent. Exposure to all treatments lasted for 72 h. Three replicates, consisting of 10 clams in 500 mL of test media each, were used per treatment. Prior to dosing, the clams were kept overnight in the test vessels, in dechlorinated municipal water and under continuous aeration, for acclimation purposes. After the acclimation period, adequate volumes of the effluents were dosed (and an equivalent volume of water was removed) to achieve the intended concentrations in the test posts (20, 40, 60, 80 and 100 %v/v effluent). The pots were fully aerated throughout the exposure period. Clam mortality was assessed daily based on siphoning activity and resistance to valve opening as described in chapter 3 (section 3.2.3). Dead clams were discarded at each mortality assessment.

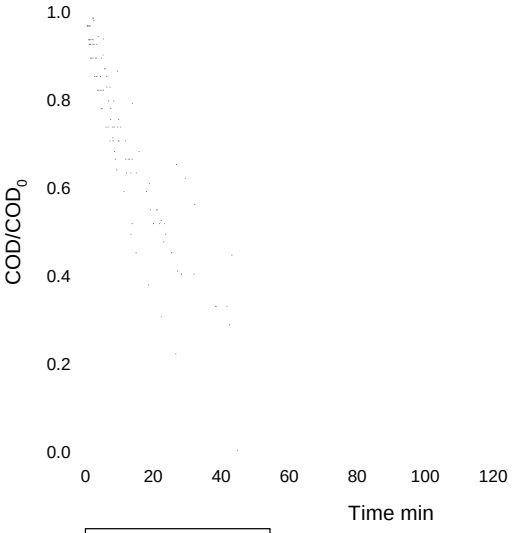
Figure 13 - Set-up for the mortality tests with Asian clams.

4.3 Results and discussion.

4.3.1 Searching for optimal ozonation conditions

In genetal, the experimental conditions that promoted the highest COD reduction in the shortest time were those of experiment #3 (Tables 11 and 12) –after 45 minutes of treatment, 100% of COD was removed (Figure 14).

If only the ozonation process is considered, optimal conditions will be those that reduce COD to a larger extent and more rapidly. However, as the ultimate goal is to integrate ozonation with biofiltration, overall optimal conditions will have to be selected in view of such an integration. This means that not only maximum COD removal is the target for ozonation – in the context of the combined treatment approach, ozona
COD to a value that represents an effluent that is well tolerated and
the clams. The optimal conditions for ozonation as such will the
removal that has to be achieved by ozonation prior to biofiltration. T
yet because the capacity of the clams to process the effluent and red
examined so far and no information on their survival in water conta
available at this stage. Therefore, the data generated in this study
analysis and should, in the future, be used iteratively and combined w
gathered on the biofiltration of parabens by the clams.



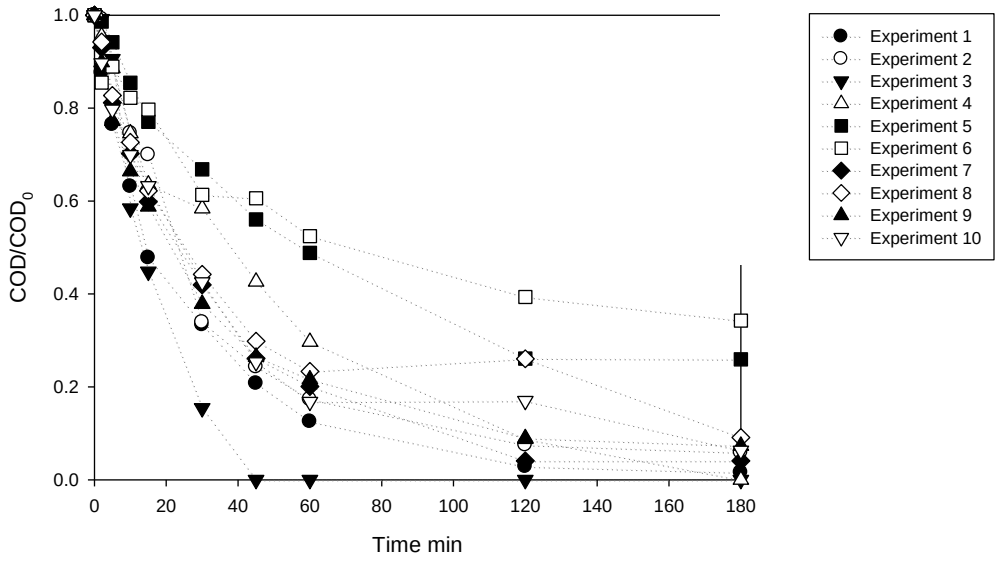


Figure 14 - COD **ıent being treated at the beginning and at**

Experiment 1
Experiment 2
Experiment 3
Experiment 4
Experiment 5
Experiment 6
Experiment 7
Experiment 8
Experiment 9
Experiment 10

Analysing Figure 14, some considerations can be made on the performance of ozonation as a decontamination tool for parabens. For the ozone and hydrogen peroxide concentrations used, achieving around 50% COD removal represented a sort of breaking point performance. If higher COD removal rates are to be achieved, then the conditions in experiment #3 have been shown to always provide better (i.e. faster) results. At 15-minutes of ozonation both trials 1 and 3 resulted in the breaking point performance. For that treatment duration, experiment 3 corresponded to a COD removal of 55.19% and experiment 1 to a COD removal of 52%, the latter achieved at the same ozone concentration as in trial 3, but without hydrogen peroxide (Tables 14 and 15). This means that optimal ozonation conditions in the breaking point performance correspond to those in trial 1 as only one reactant was used. For COD removals lower than the 50% breaking point performance, Figure 14 shows that the experimental points are very close to each other, meaning that no major differences could be seen between the different trials.

The option for the toxicity tests was to assess ozonation conditions providing the breaking point performance, not only because the "hinge" character of such performance as discussed above, but also because a 50% COD removal should result in a fairly tolerable effluent for the clams to clean further. That breaking point performance could be rapidly achieved at 15 minutes through conditions in trial 1 and 3. Because only trial 3 used hydrogen peroxide, those conditions were used (i.e. 15 minutes ozonation 2.5 mg $L^{-1}$of H_2O_2 and 45 g Nm^3 of O_3) so that the effect of possible intermediates resulting from the presence of hydrogen peroxide could also be addressed at this preliminary toxicity tests.

4.3.2 Testing the toxicity of untreated and ozonation-treated effluent

Both the tests with *V. fischeri* and *C. fluminea* showed that after ozonation the effluent became considerably less toxic (Table 13). The luminescence of *V. fischeri* was much less inhibited by ozonation-treated effluent than by the untreated one. The LC_{20} and LC_{50} values for Asian clams increased by factor of 2 and 1.5, respectively, as the animals were exposed to ozonation-treated effluent instead on untreated effluent. Figure 15 showing complete concentration-response data provides a fuller picture of the mortality tests with *C. fluminea* and further corroborates the ability of ozonation to reduce the toxicity of parabens-containing waters. The figure shows that the after being processed by ozonation the model effluent killed considerably less clams.

Table 13 - Summary of the toxicity tests with *V. fischeri* and C. fluminea.

	Untreated effluent	Ozonation-treated effluent
LC_{20} (95% CI in brackets) (% v/v)	14.05 *(4.72 - 38.18)*	28.68 *(15.78 - 50.29)*
LC_{50} (95% CI in brackets) (% v/v)	81.9 *(75.17 - 89.93)*	126.43 *(110.39 - 161.16)*
Luminescence inhibition after exposure to effluent for 15 min (%)	95.6	57.1
Luminescence inhibition after exposure to effluent for 30 min (%)	95.1	61.5

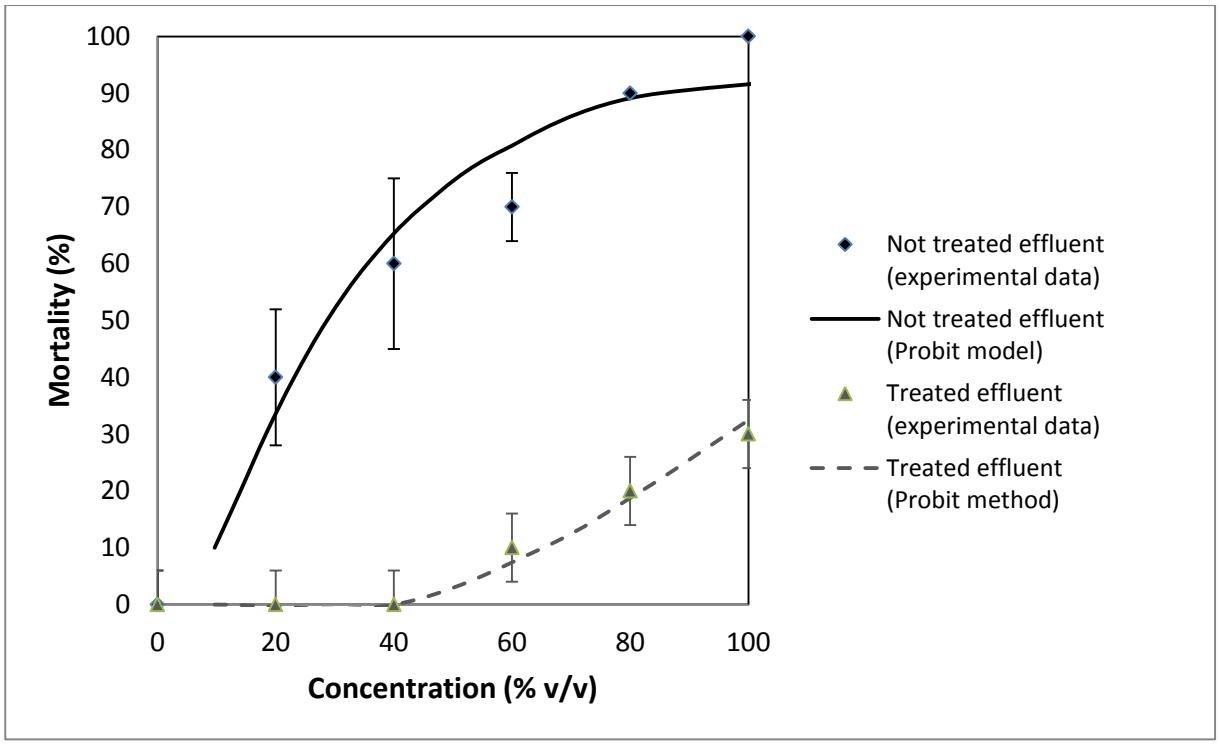


Figure 15 - Concentration-response data for C. fluminea exposed to ozonation-treated and untreated parabens-containing water for 72 h.

4.4 Concluding remarks

The results reported in this chapter showed that it is possible to remove parabens from water by ozonation, resulting in significant COD reductions. It has also been shown that it is possible to define optimal working conditions for ozonation, which will depend on the COD removal required for such a step when it precedes biofiltration as part of a combined treatment tool. Finally, this study showed that ozonation not only reduces the organic load of paranbens-containing waters, but also their toxicity towards aquatic biota, Asian clams in particular. Therefore, integration with biofiltration by the clams as a subsequent treatment level seems promising at this stage and should be investigated in the future.

5. Conclusions and future work

This thesis addressed the treatment of winery wastewater and paraben-rich effluents as two challenging contaminants for which improved remediation tools are yet to be found. While these are two distinct contaminants, a common integrated approach involving chemical treatment followed by biofiltration by the Asian clam has been pursued here. The Asian clam is one of the world's worst pests, having great damaging impacts in nature as well as in industrial and other man-made structures due to the species' biofouling activity. Eradication of well-established populations from infested ecosystems is extremely difficult, in most situations impossible. Therefore, finding applications for the pest is an attractive research line as it would allow, to some extent, offsetting its damaging effects. By capitalising on the Asian clams' great filtration capacity, high productivity and reasonable tolerance to contaminants, it may be possible to take profit from them by using them as biofilters for wastewater.

The main conclusions and directions for future work for each of the two projects reported here are described below.

Combining Fenton's reaction and biofiltration by Asian Clams to treat winery effluent - pilot scale study. Commercial production of wine results in the generation of large volumes of wastewater, which typically contains large amounts of organic material and salts as a result of product loss and cleaning processes. The treatment and management of these wastewaters are of significant concern, especially considering increasing environmental restrictions. There are numerous treatment options available to process winery effluent, which vary with respect to efficacy, cost and reliability. As Fenton's process is a fairly well-established method for this effluent, it was selected as the chemical component of the combined remediation approach being sought.

A pilot scale study involving the treatment of real winery effluent by Fenton's reaction followed by biofiltration was conducted. The results of the study show that this seems to be a suitable decontamination alternative, ensuring high COD removal. The COD removal observed experimentally is comparable to that achieved by the treatment processes currently used in practice. Typical activated sludge treatment after Fenton's reaction removes around 98 % of COD with a residence time of 10 days in the biological treatment; COD removals from 96.5% to 100% were achieved through the pilot integrated treatment with 5 days of biofiltration. Compared to Fenton-activated sludge treatment, the combined decontamination method is advantageous in the sense that it performed more rapidly and it does not require the addition of extra nutrients, to balance the C/N/P ratio.

With the pilot scale study reported here, the development of integrated Fenton's reaction- biofiltration for winery effluent treatment is getting to a fairly advanced stage. The

pilot unit operation now needs an optimisation - it is important to ascertain at what COD contents can the Fenton's reaction be stopped, i.e. at what COD contents can Asian clams start to process the Fenton-treated effluent. By doing such a study it will be possible to define the conditions that ensure maximum savings in terms of chemicals and reagents in Fenton's reaction.

Another aspect that will be interesting to work out is the supporting structure holding the clams in the biofiltration tanks. Eventually the structure configuration may be defined to maximise clams survival and filtration.

In the future, it will also be important to study the balance between the number of biofilters vs their size and the need to replace them periodically to limit the death of clams and control $N\text{-}NH_4$ production.

A key issue that will have to be addressed as the proposed combined treatment reaches a point of practical application is deciding what to do with the dead clams taken from the biofilters. Discharge in landfill is a possibility that has to be balanced against cost and environmental impacts. Some invasive bivalves such as the zebra mussel have been proposed as a supplement for chicken food. Such an use may be given to the clams discharged from biofilters, but first exhaustive studies have to be done to ensure that the bivalves exposed to the effluent do not have compromising contamination levels. Other ends for the clams used in the biofilters should be sought in the future.

Combining ozonation and biofiltration by Asian Clams to treat parabens - developing the ozonation component. The efficiency of conventional biological methods in processing the increasingly common parabens is very low, and these are becoming concerning pollutants in natural waters.

As improved decontamination methods for parabens must be found, the second study reported here was concerned with combined ozonation- biofiltration for the removal of these compounds from wastewater. The development of such an integrated treatment approach is still at an incipient stage, and this thesis focused on the development of ozonation as the chemical treatment step of the intended remediation tool.

The experimental results showed that ozonation is able to provide significant reduction of COD as well as toxicity of a model effluent containing parabens. The dataset produced also indicates that it is possible to work in optimal ozonation conditions, which are a function of the COD removal requested for the chemical treatment step prior to biofiltration by the clams.

As biofiltration of ozonation-treated effluent has not been studied yet, future further work should start from this point. As an insight into the biofiltration capacity of the calms is

got, COD contents at the feed of the biofilters can be defined and optimal conditions for ozonation can also be iteratively set. It will be important to know what happens when parabens are in a real rather than a model effluent, so it will be useful to run experiments using a real municipal waste water containing parabens.

6. References

Ozone Solutions The element of Success. s.d. http://www.ozonesolutions.com (consultato il giorno April 29, 2015).

(ISSG), National Biological Information Infrastructure (NBII) & IUCN/SSC Invasive Species Specialist Group. *Global Ivasive Species Database.* s.d. http://www.issg.org.

Aguirre, W. and S.G. Poss. «Non-indigenous Species in the Gulf of.» 1999.

Antonio G. Brito, Joao Peixoto, José M. Oliveira, José A. Oliveira, Cristina Costa, Regina Nogueira, and Ana Rodrigues. «Brewery and Winery Wastewater Treatment: Some Foca Points Of Design and Operation.» 2007.

Antonio G. Brito, Joao Peixoto, José M. Oliveira, José A. Oliveira, Cristina Costa, Regina Nogueira, and Ana Rodrigues. «Brewery and Winery Wastewater Treatment: Some Foca Points Of Design and Operation.» s.d.

Artiga, P., Ficara, E., Malpei, F., Garrido, J.M. and Mendez, R. « Treatment of two industrial wastewaters in a submerged membrane bioreactor. Desalination.» 2005: 179, 161–169.

Bader, Hoigné J. and H. «Ozone Initiated Oxidations of Solutes in Wastewater: A Reaction Kinetic Approach.» *Progress Water Technol.*, 1978: 10(516):657.

Bader, Hoigné J. and. «The Role of Hydroxyl Radical Reacting in Ozonation Processes in Aqueous Solutions.» *Water Resources*, 1976: 10:377.

C. MCLaughlan, D.C. Aldrige. «Cultivation of zebra mussels (Dreissena polymorpha) within their invaded range to improve water quality in reservoirs.» *elsevier*, 2013.

Claire McLaughian, Paul Rose, David C.Aldrige. «Making the best of a pest: the Potentioal for using invasive Zebra Mussel (Dreyssena Polymorpha) biomass as a supplement to commercial chicken feed.» *Enviromental Management*, 2004.

DAISE. s.d. http://www.europe-aliens.org/.

Dana M. Franka, J. Evan Warda, Sandra E. Shumway, Bridget A. Holohana and Callum Gray. «Application of particle image velocimetry to the study of suspension feeding in marine invertebrates.» *Marine and Freshwater Behaviour and Physiology*, 2008.

Darbre PD, Harvey PW. «Paraben esters: review of recent studies of endocrine toxicity, absorption, esterase and human exposure, and discussion of potential human health risks.» 2008.

Decreto-Lei n.o 236/98. (MINISTÉRIO DO AMBIENTE,1998).

Dreier H, Tranquilli JH. « Reproduction, growth, distribution, and abundance of Corbicula in an Illinois cooling lake.» *Illinois Natural History Survey Bulletin*, 1981: 32: 378–393.

Frances E. Lucy, Alexander Y. Karatayev, and Lyubov E. Burlakova. «Predictions for the spread, population density, and impacts of Corbicula fluminea in Ireland .» *Aquatic Invasions*, 2012.

Fumi, M.D., Parodi, G., Parodi, E., Silva, A. and Marchetti, R. «Optimisation of long-term activated-sludge treatment of winery wastewater. Bioresource Technology.» 1995: 52, 45–51.

G L Mackie, C A Wright, Water. «Thorp and Covich's Freshwater Invertebrates: Ecology and General Biology.» 1994: 28, 1123–1130.

Gerry L. Mackie, Robert C. Bailey. *Biology of freshwater corbiculid and sphaeriid clams of North America.* 2007.

Glaze, W. H., et al. «The Chemistry of Water Treatment Processes Involving Ozone, Hydrogen Peroxide, and Ultraviolet Radiation.» *Ozone: Sci. Engrg*, 1987: 9(4):335.

Glaze, W.H. «Reaction Products of Ozone: A Review.» *Environ. Health Perspectives,*, 1986.

Glaze, W.H., and J-W Kang. «Advanced Oxidation Processes for Treating Groundwater Contaminated With TCE and PCE: Laboratory Studies.» *J. AWWA.* , 1988: 88(5): 57–63.

Hoigné, J. and H. Bader. «Ozonation of Water: Role of Hydroxyl Radicals as Oxidizing Intermediates.» *Science*, 1975: 190(4216):782.

Huseyin Tekina, Okan Bilkaya, Selale S. Ataberka, Tolga H. Baltaa, I. Haluk Ceribasia, F. Dilek Saninb, Filiz B. Dilekb, Ulku Yetisb. «Use of Fenton oxidation to improve the biodegradability of a pharmaceutical wastewater.» *Journal of Hazardous Materials*, 2006: 258–265.

Ines Correira Rosa, Raquel Costa, Fernando Goncalves, Joana Luisa Pereira. «Bioremediation of Metal-Rich Effluents: Could the invasive bivalve Corbicula Fluminea work as a biofilter?» *Journal of Environmental Quality*, s.d.

Invertebrate Zoology on Line. s.d. http://lanwebs.lander.edu/faculty/rsfox/invertebrates/.

Janech MG, Hunter RD. « Corbicula fluminea in a Michigan River: implication for low temperature tolerance. .» *Malacological Review* , 1995: 28: 119–124.

K.P.M. Mosse, A.F. Patti, E.W. Christen and T.R. Cavagnaro. «Review: Winery wastewater quality and treatment options in Australia.» *Winery wastewater review*, 2011.

Karatayev AY, Howells RG, Burlakova LE, Sewell BD. «History of spread and current distribution of Corbicula fluminea (Müller) in Texas.» *Journal of Shellfish Research*, 2005: 24: 553–559.

Keyser, M., Witthuhn, R.C., Ronquest, L.C. and Britz, T.J. « Treatment of winery effluent with upflow anaerobic sludge blanket (UASB) – granular sludges enriched with

Enterobacter sakazakii. Biotechnology Letters .» 2003: Keyser, M., Witthuhn, R.C., Ronquest, L.C. and Britz, T.J. (2003) Treatment of winery effluent with upflow25, 1893–1898. .

Kheng Soo Tay, Noorsaadah Abd. Rahman, Mhd. Radzi Bin Abas. «Kinetic studies of the Degradation of Parabens in Aqueous Solution by Ozone oxidation.» Springer, 2009.

Kheng Soo Tay, Noorsaadah Abd. Rahman1, Mhd. Radzi Bin Abas. «Removal of Selected Endocrine Disrupting Chemicals and Personal Care Products in Surface Waters and Secondary Wastewater by Ozonation.» *Water Environment Research*, 2011: 684–691.

M.I. Badawy, M.E.M. Ali. «Fenton's peroxidation and coagulation processes for the treatment of combined industrial and domestic wastewater.» *Journal of Hazardous Materials*, 2006: 961–966.

Marco Panizza, Giacomo Cerisola. «Removal of organic pollutants from industrial wastewater by electrogenerated Fenton's reagent.» *Water Research*, 2001: 3987–3992.

Maynard, H.E., Ouki, S.K. and Williams. « Tertiary lagoons: a review of removal mechanisms and performance. Water Research .» 1999.

Miguel Rodriguezb, c, Victor Sarriaa, Santiago Esplugasc, Cesar Pulgarin. «Photo-Fenton treatment of a biorecalcitrant wastewater generated in textile activities: biodegradability of the photo-treated solution.» *Journal of Photochemistry and Photobiology A: Chemistry*, 2002: 129–135.

Mojtaba Hadavifar, Ali Akbar Zinatizadeh, Habibollah Younesi and Maedeh Galehdar. «Fenton and photo-Fenton treatment of distillery effluent and optimization of treatment conditions with response surface methodology.» *ASIA-PACIFIC JOURNAL OF CHEMICAL ENGINEERING*, 2009.

Montalvo, S., Guerrero, L., Rivera, E., Borja, R., Chica, A. and Martín, A. « Kinetic evaluation and performance of pilot-scale fed-batch aerated lagoons treating winery wastewaters. Bioresource Technology.» 2010: Montalvo, S., Guerrero, L., Rivera, E., Borja, R., Chica, A. and Martín, A. (2010) Kinetic evaluation and performance101, 3452–3456.

New York Invasive Species Information. s.d. http://nyis.info/.

Odd Lindahl, Rob Hart, Bodil Hernoth, Seven Kollberg, Lars Ove Loo, Lars Olrog, Ann Sofi Rehnstam-Holm, Jonny Stevenson, Susanne Svensson and Ulf Syversen. «Improvising Marine Water Quality by Mussel Farming: a Profitable Solution for Swedish Society.» 2005.

Odd Lindhal, Rob Hernroth, Sven Kollberg, Lars-Ove Loo, Lars Olrog, Ann-Sofi Rehnstam-Holm, Jonny Svensson, Susanne Svensson and Ulf Syrversen. «Iproving Marine

Water Quality by Mussel Farming: AProfitable Solution for Swedish Society.» *Royal Swedish Academy of Sciences 2005*, 2005.

Oliveira, M., Queda, C. and Duarte, E. « Aerobic treatment of winery wastewater with the aim of water reuse. Water Science and Technology.» 2009: 60, 1217–1223.

P, Kat. «Shell dissolution as a significant cause of mortality for Corbicula fluminea (Bivalvia: Corbiculidae) inhabiting acidic waters.» *Malacological Review*, 1982: 15: 129–134 .

Petruccioli, M., Duarte, J. and Federici, F. «High-rate aerobic treatment of winery wastewater using bioreactors with free and immobilized activated sludge. Journal of Bioscience and Bioengineering.» 2000: 90, 381–386.

Philippa D. Darbre, Philip W. Harvey. «Paraben esters: review of recent studies of endocrine toxicity, absorption, esterase and human exposure, and discussion of potential human health risks.» *JOURNAL OF APPLIED TOXICOLOGY*, 2008.

Rajagopal S, van der Velde G, bij de Vaate A. «Reproductive biology of the Asiatic clams Corbicula fluminea in the River Rhine.» *Archiv fur Hydrobiologie*, 2000: 149: 403–420.

Rita Ferreira «Integração do Processo de Fenton e Biofiltração com Corbicula Fluminea para o tratamento de efluentes vinicolas.» 2015.

Ronaldo Sousa, Adriana Novais, Raquel Costa, David L. Strayer. «Invasive bivalves in fresh waters: impacts from individuals to ecosystems and possible control strategies.» 2013: 233–251.

Ruiz, C., Torrijos, M., Sousbie, P., Lebrato Martinez, J., Moletta, R. and Delgenes, J.P. « Treatment of winery wastewater by an anaerobic sequencing batch reactor. Water Science and Technology.» 2002: 45, 219–224.

SE, Belanger. «The effect of dissolved oxygen, sediment, and sewage treatment plant discharges upon growth, survival and density of Asiatic clams.» *Hydrobiologia* , 1991: 218: 113–126.

T P Diggins, R E Baier, A E Meyer, R L Forsberg. «Marine Biotechnology in the twenty-first century: Problems, Promise and Prodouct.» 2002: 18, 29–36.

Technology, Wineryanddistillerywastewatertreatmentbyanaerobic digestion. Water Science and. «Wineryanddistillerywastewatertreatmentbyanaerobic digestion. Water Science and Technology .» 2005: 51, 137–144.

Torrijos, M. and Moletta, R. « Winery wastewater depollution by sequencing batch reactor. Water Science and Technology .» 1997: 35, 249–257.

Appendices

Appendix I – Further information on analytical techniques for liquid samples.

COD - Calibration curve.

Calibrations curve, one for winery effluent (range 0 - 2000 mg O_2 L^{-1}) and one for parabens solution (range 0 - 100 mg O_2 L^{-1}), was prepared by the determination of the absorbance corresponding to samples with known COD that were under this procedure (figure A winery effluent and figure B parabens solution).

The mean absorbance value for the blanc was subtracted to the mean absorbance values for vials and that result was considered in the calibration curves to obtain the COD value in milligrams of oxygen per liter of solution.

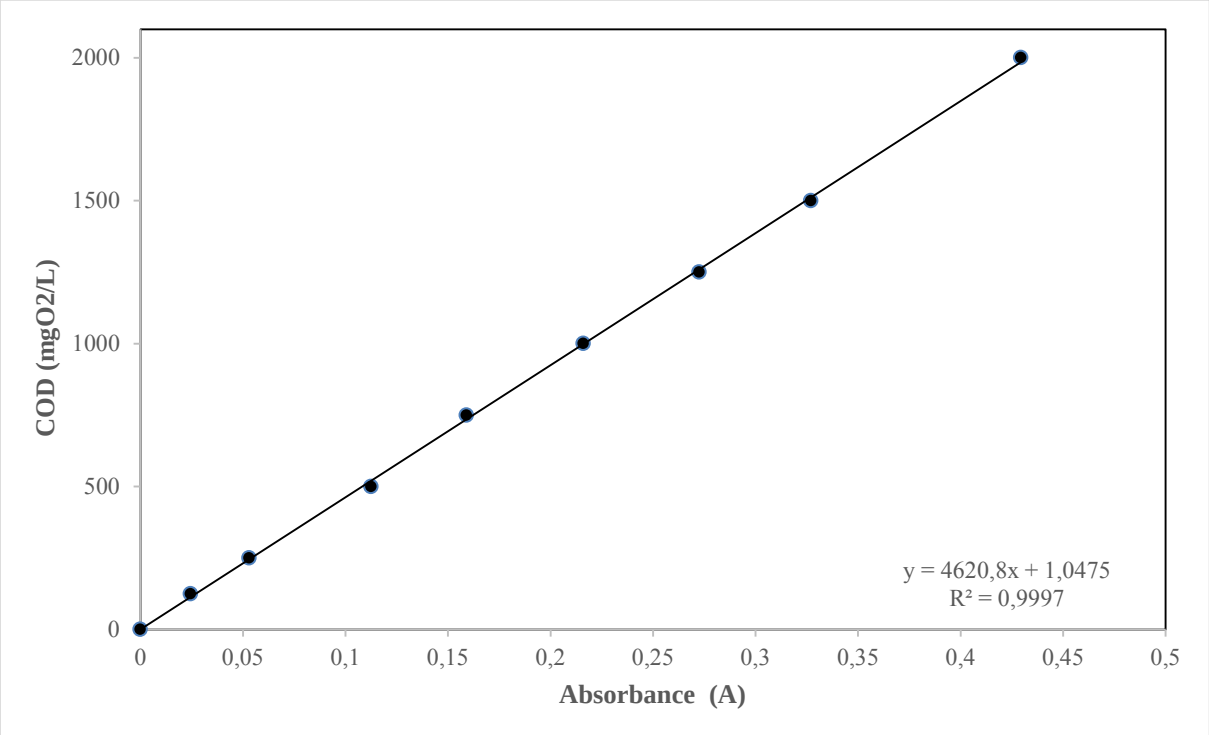


Figure A - Calibration curve for winery effluent.

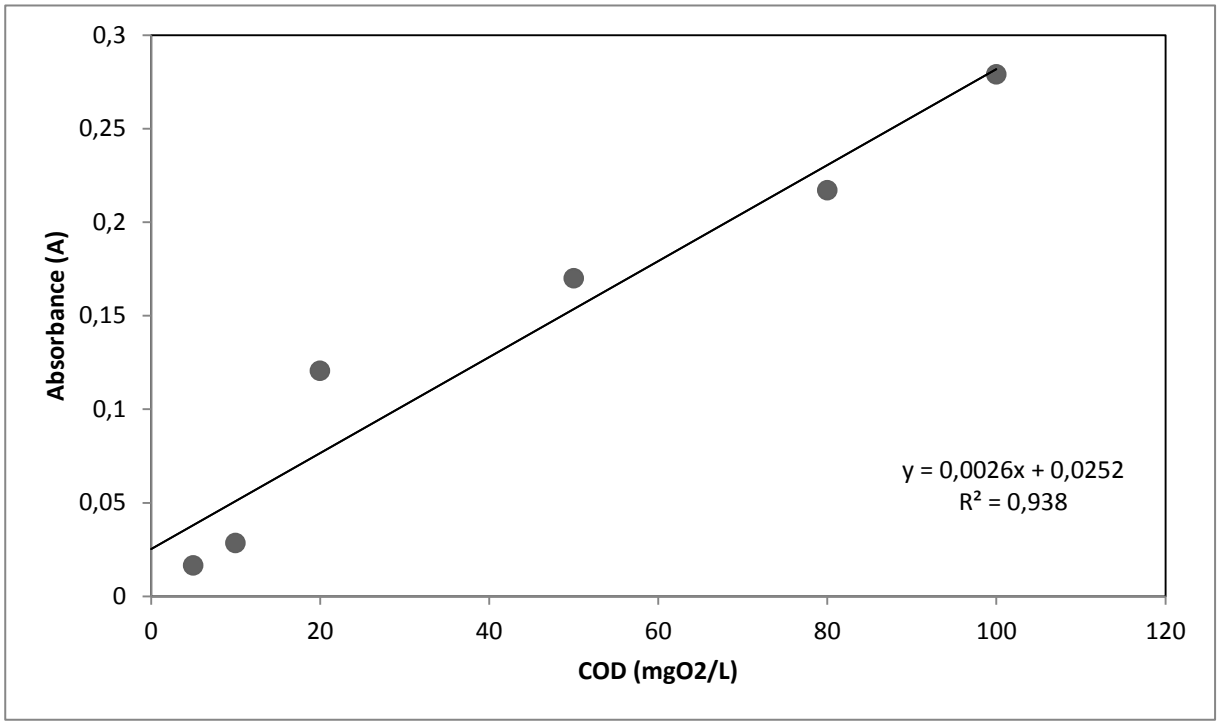


Figure B - Calibration curve for Parabens solution.

Appendix II – Probit Method.

Probit analysis is used to analyze many kinds of dose-response or binomial response experiments in a variety of fields. Probit Analysis is commonly used in toxicology to determine the relative toxicity of chemicals to living organisms. This is done by testing the response of an organism under various concentrations of each of the chemicals in question and then comparing the concentrations at which one encounters a response. The response is always binomial (e.g. death/no death) and the relationship between the response and the various concentrations is always sigmoid. Probit analysis acts as a transformation from sigmoid to linear and then runs a regression on the relationship. Once a regression is run, the researcher can use the output of the probit analysis to compare the amount of chemical required to create the same response in each of the various chemicals. There are many endpoints used to compare the differing toxicities of chemicals, but the LC50 (liquids) or LD50 (solids) are the most widely used outcomes of the modern dose-response experiments. The LC50/LD50 represent the concentration (LC50) or dose (LD50) at which 50% of the population responds.