Integration of Membrane Distillation with solar photo-Fenton for purification of water contaminated with *Bacillus* sp and *Clostridium* sp spores

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Abstract

Although Membrane Distillation (MD) has been extensively studied for desalination, it has other applications like removing all kinds of solutes from water and concentrating non-volatile substances. MD offers the possibility of producing a clean stream while concentrating valuable compounds from waste streams towards their recovery, or emerging contaminants and pathogens present in wastewater in order to facilitate their chemical elimination. This paper analyses the elimination of bacterial spores from contaminated water with MD and the role of MD in the subsequent treatment of the concentrate with photo-Fenton process. The experiments were performed at Plataforma Solar de Almería (PSA) using a plate and frame bench module with a Permeate Gap Membrane Distillation (PGMD) configuration. Tests were done for two different kinds of spores in two different water matrixes: distilled water with 3.5 wt% of sea salts contaminated with spores of *Bacillus subtilis* (*B. subtilis*) and wastewater after a secondary treatment and still contaminated with *Clostridium* sp spores. An analysis of the permeate was performed in all cases to determine its purity, as well as the concentrated stream and its further treatment in order to assess the benefits of using MD. Results showed a permeate free of spores in all the cases, demonstrating the viability of MD to treat biological contaminated wastewater for further use in agriculture. Moreover, the results obtained after treating the concentrate with photo-Fenton showed a shorter treatment time for the reduction of the spore concentration in the water than that when only photo-Fenton was used.

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1. Introduction

Water scarcity is one of the main challenges of the XXI century that many societies around the world are already facing. Throughout the last century, use and consumption of water grew at twice the rate of population growth and, although there is not water scarcity globally, the number of regions with chronic levels of water shortages is increasing. Water scarcity is not only a natural phenomenon, but is also caused by human action. Nowadays, there is enough freshwater to supply people who inhabit the planet, however, it is distributed unevenly, wasted, polluted and unsustainably managed [1]. Due to the development of industry and agriculture together with the rise of population and living standard, water consumption is increasing [2]. Agriculture consumes at least 70 % of water in the world. The use of wastewater in agriculture is being increasingly proposed, since it could have agronomic and economic benefits [3]. On one hand, irrigation with wastewater may increase the available water supply and so allow preserving better quality supplies for other uses [4]. On the other hand, the fertilizer content of many kinds of wastewater can be an advantage for agricultural use [5]. According to FAO, wastewater effluent from domestic sources may contain all the nitrogen as well as most of the phosphorus and potassium usually required for agricultural crop production [6]. However, the use of wastewater has several risks for public health [7, 8]. Wastewater, especially domestic wastewater contains pathogens that can cause different diseases, as they contain fecal pathogens. The most common enteric microorganisms found in such wastewaters are bacteria, viruses, protozoa and helminthes [9]. Therefore, the reuse of wastewater for purposes such as agriculture makes necessary the treatment of the water before their use [10]. Some conventional treatments of disinfection including chlorination and ozonation, generate toxic disinfection by-products that make necessary searching for innovative technologies for pathogens removal [11]. An alternative to conventional disinfection systems is the use of advanced oxidation technologies, based on the generation of oxidant radicals which attack indistinctly organic matter and water microorganisms [12]. Advances oxidation processes (AOPs) have been shown successful for the removal of organic contaminants [12, 13]. AOPs are based on the generation of hydroxyl radicals, which are highly oxidant (E^0=2.8 V). They are highly reactive without chemical selectivity. Among AOPs, photo-Fenton is considered as one of the most successful processes due to its high generation rate of radicals in ideal conditions (optimized concentrations of Fe and H_2O_2, and pH close to 3) and effectiveness in organic pollutants degradation [14, 15]. It has also been proven recently as a good option for wastewater disinfection [16-21]. Photo-Fenton process comprehends a complex series of chemical reactions that all together represent a catalytic process that converts Fe(III) to Fe(II) and vice versa and generates hydroxyl radicals, while H_2O_2 and UV-vis light must be present [22]. The process can be summarized as follows.

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$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO + HO \quad (1)$$

$$Fe^{3+} + H_2O + h\upsilon \rightarrow Fe^{3+} + HO + H^+ \quad (2)$$

Membrane Distillation (MD) is an emerging technology that consists in a thermal process in which only vapour molecules pass through a porous hydrophobic membrane. The feed is in direct contact with the surface of the membrane but it does not penetrate thanks to the hydrophobic nature of the membrane. The driving force of the process is the vapour pressure difference through the membrane. This can be established by increasing the temperature of the feed at one side of the membrane, which increases its vapour pressure and therefore establishes a gradient across the membrane [23-25]. MD presents a number of advantages that make it competitive in more applications. The most important advantages of MD are: I) normally, a pretreatment of feed is not necessary to enlarge the membrane life; II) the good quality of the permeate is independent from the concentration of the feed; III) possibility of treating corrosive and acid effluents [26]. These positive characteristics make MD suitable for production of ultra-pure water, desalination of brines, removal of dyes and treatment of textile wastewater, concentration of acids and corrosive substances, juice and whey concentration in food industry, and finally treating water contaminated with pathogens [27]. A previous article [28] showed a first demonstration of the removal of two types of microorganisms, i.e. *Fusarium solani* spores and *Escherichia coli* (*E. coli*), in water using MD to obtain an effluent free of pathogens. The main conclusion was that thermal increase by MD provoked a complete abatement of *Fusarium* sp and *E. coli* within the module, as they are quite sensitive to T > 50ºC [29, 30] and MD temperatures reach up to 80 ºC. Although *E. coli* is frequently used as indicator of fecal contamination, in this case new thermo-tolerant microorganisms are required as indicator for a deep assessment of MD for wastewater disinfection. As this work aims at the disinfection of wastewater using MD followed by solar photo-Fenton, the selected microorganism should be not only thermo-tolerant but also highly resistant to disinfection processes commonly used, i.e. chlorine, ozone or UVC. This is the case of some spore forming bacteria; *Bacillus* and *Clostridium* species are among the most resistant microorganisms known [31]. Bacterial spores are very resistant to a variety of environmental conditions, including heat, radiation and many toxic chemicals [32-36]. Wet heat is the most commonly used method to inactivate spores. *Bacillus subtillis* (*B. subtilis*) is a non-pathogenic bacterium, easy to culturing and molecular genetic manipulation of some strains. This has made *B. subtilis* a good model microorganism for both lab-scale and full-scale tests. *Clostridium* sp is a pathogen that causes infection diseases, constituting a health risk for humans and animals. It lives in different habitats such as soils and water. *Clostridium* sp spores and in general spores of sulphite-reducing Clostridia

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(SRC), are suggested as indicator of oocysts of *Cryptosporidium* sp and other highly resistant pathogens [37].

Inactivation of highly resistant microorganisms in water has a special scientific and technological interest with health and environmental implications. In response to these needs, the objective of this study was to investigate the role of MD in the treatment of two different matrix water contaminated by spores of gram- positive bacteria: salty water inoculated with *B. subtilis* spores obtained after a sporulation process carried out in the laboratory and treated urban wastewater naturally contaminated with *Clostridium* sp spores. Namely, to test if the permeate production is free of microorganisms and to study if MD influences the further treatment of the rejected feed. So, the remaining feed was treated with photo-Fenton at pH 3 due to the high resistance of the spores to conventional disinfection treatment.

2. Materials and Methods

2.1 Water types

The MD experiments were carried out in two types of water, salty water and real urban wastewater effluents (UWW). The first approach aimed at the study of the functioning of MD modules for removal of spores of *B. subtillis* in synthetic salty water. The second approach was done using UWW to demonstrate the capability of MD at pilot scale for purification of a real and complex wastewater to produce a highly pure stream and naturally occurring *Clostridium* sp spores were monitored in this case.

The experiments with saline water were done with distilled water (conductivity: 0.74 µS/cm and organic carbon: 0.5 mg/l) adding 3.5 wt% of sea salts to simulate the standard ocean salinity. Spores of *B. subtilis* were used in this water matrix as they represent a very high resistant biological agent to be inactivated and furthermore they has been isolated from marine invertebrates and detected in seawater of different areas of Pacific Ocean [38]. The salts also served as an indicator of possible wetting on the MD, which would affect the salinity of the permeate. Sea salts used in this study came from raw material from the saltworks of Cabo de Gata, Almería, Spain. In general, the main salt ions that make up 99.9 % are shown in the following table (**Table 1**).

Table 1. Main salt ions of seawater.

Cations	Concentration (mg/l)
Na^{+}	9,600-11,700
Mg^{2+}	1,025-1,400

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Ca^{2+}	375-525
K^{+}	350-500
Sr^{2+}	12-14
Anions	Concentration (mg/l)
Cl^{-}	17,500-21,000
SO_4^{2-}	2,425-3,000
HCO_3^{-}	120-170
Br^{-}	59-120
BO_3^{-}	6-27
F^{-}	1

Freshly UWW from the urban wastewater treatment plant of Almeria was also used to study the evolution of the concentration of *Clostridium* sp spores after MD and photo-Fenton processes. *Clostridium* sp spores are present naturally in the UWW, and thus the sporulation of a dangerous species in the laboratory was avoided. *B. subtilis* was not studied in UWW because this water usually contain a complex mixture of microorganisms and the medium used for the detection of *B. subtilis* is not extremely selective, so it would be very difficult to distinguish it in UWW. The chemical composition and characteristics of UWW are shown in **Table 2**.

Table 2. Ionic composition and physicochemical characteristics (average value during experiments) of UWW.

Cations (mg/l)	Concentration (mg/l)
Na^{+}	302 ± 1
Mg^{2+}	36 ± 0
Ca^{2+}	65 ± 0
K^{+}	27 ± 0
NH_4^{+}	76 ± 1
Anions (mg/l)	Concentration (mg/l)
Cl^{-}	563± 1
SO_4^{2-}	108 ± 0
NO_2^{-}	3 ± 0
NO_3^{-}	4 ± 0
PO_4^{3-}	21 ± 0
pH	8 ± 0
Conductivity (µs/cm)	2277 ± 10
Turbidity (NTU)	12 ± 0
TC (mg/l)	116 ± 0
IC (mg/l)	91 ± 0
E. coli (CFU/100 ml)	10^5 ± 1

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TC (CFU/100 ml)	$5 \times 10^6 \pm 1$
E. faecalis (CFU/100 ml)	$10^3 \pm 1$
Clostridium sp (CFU/100 ml)	$5 \times 10^3 \pm 2$

1

2

3 **2.2. Spores generation**

4 Cultures of *B. subtilis* ATCC 6633 (American Type Culture Collection, Manassas, Virginia,
5 USA), were generated from frozen stock by streaking onto tryptone soya agar (TSA) plate
6 enriched with 0.25 mg/l $MgSO_4$ and were incubated at 37 °C for 24 hours. Afterwards, two
7 colonies from the incubated plate were transferred to tryptone soya broth (TSB) with 0.25 mg/l
8 $MgSO_4$ and incubated at 37 ºC for 24 h shaking at 100 rpm. Then, dilutions of 10^{-1} were
9 prepared using phosphate buffer with 20 mg/l of $MnCl_2$. Afterwards, several times x 1 ml of 10^{-1}
10 dilution were plated in TSA + 0.25 mg/l $MgSO_4$ culture medium and incubated at 37 °C for 15
11 days, for growing and sporulation. The plates were stored at 4 °C until use. To obtain the spore
12 inoculum, the surface of TSA was rinsed with 10 ml of distilled water and scraped with a
13 spreader. This protocol was repeated four times more and the total volume of 50 ml was poured
14 in a 50 ml-tube. Then suspension was centrifuged at 3,000 rpm for 5 min, and the spores were
15 harvested and re-suspended in 10 ml of distilled water. The described procedure resulted in a
16 suspension of $4.7 \cdot 10^{10}$ CFU/100 ml spore concentration. Finally, the spore suspension was
17 placed in a water bath at 80 °C for 15 minutes to eliminate vegetative cells and keep spores
18 only. Spore suspensions were diluted directly in the water sample to achieve an initial spore
19 concentration of 10^7 CFU/100 ml. For experiments of *Clostridium* sp spores, real UWW with
20 naturally occurring *Clostridium* sp spores was used. The concentration of this pathogen in
21 UWW samples was found ca. $5 \cdot 10^3$ CFU/100 ml, which it is enough to permit the monitoring of
22 spore reduction throughout the water processes evaluated in this work, avoiding the need of
23 adding any microbial component to the real UWW sample.

24 **2.3. Spores enumeration**

25 For *B. subtilis* spores, samples were taken and serially diluted (10 fold series) in distilled water
26 and then spread on TSA+ 0.25 mg/l $MgSO_4$ media petri dishes. For last stage samples, 500 µl of
27 undiluted sample was dropped onto the agar plate for the enumeration of the colonies to reduce
28 the detection limit of this enumeration method. The colonies were quantified after incubation at
29 37 °C for 24 h. The detection limit (DL) of this method was 200 CFU/100 ml.

30 For detection of *Clostridium* sp, water samples were cultured on sulphite polymyxin
31 sulphadiazine (SPS, Cultimed, Panreac) agar medium at 44 ºC under anaerobic conditions for
32 24 h. Dilutions of samples were carried out to reach a DL of 2 CFU/100 ml [37].

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2.4. Membrane Distillation (MD) Module

A bench-scale module with a Permeate Gap Membrane Distillation (PGMD) configuration was used for studying the behaviour of MD processes. In PGMD configuration, the permeate and the cold liquid are separated by a condensing foil creating a channel which is filled with stagnant permeate. The plate and frame module consisted of two transparent half-shells made of polycarbonate with a thickness of 20 mm and dimensions of 290 x 140 mm. The effective membrane area was 131 cm^2. The active layer of the membrane was made of PTFE while the backing material was of PP. The nominal pore size and the porosity were 0.2 μm and 85 % respectively and the membrane thickness was 0.16 mm. The separate channel was established using a 1 mm gap spacer between the membrane and the condenser channel.

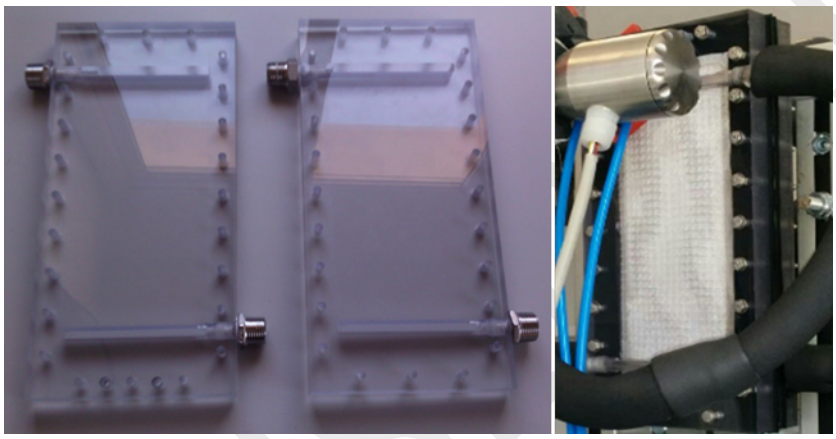

Figure 1. Evaporator and condenser shells (left) and test cell (right).

The test module was integrated into a test facility consisting of two separate hydraulic loops, one for the hot feed and one for the cooling (Figure 2). In the hot loop, the feed water was recirculated from an 80 l storage tank with an electrical heater that can provide a maximum of 3 kW_{th}. In the cooling loop, the cold water was recirculated from another 80 l storage tank connected to a compressor chiller. Finally, the permeate was collected in another tank placed on a precision balance to register continuously the mass of permeate. Four encapsulated Pt100 temperature sensors were positioned at the evaporator inlet, evaporator outlet, condenser inlet and condenser outlet of the test module. The volume flow rates were measured by different flow meters. The feed flow rate was measured by a magnetic inductive flow meter while the cooling flow rate was measured by a variable area flowmeter.

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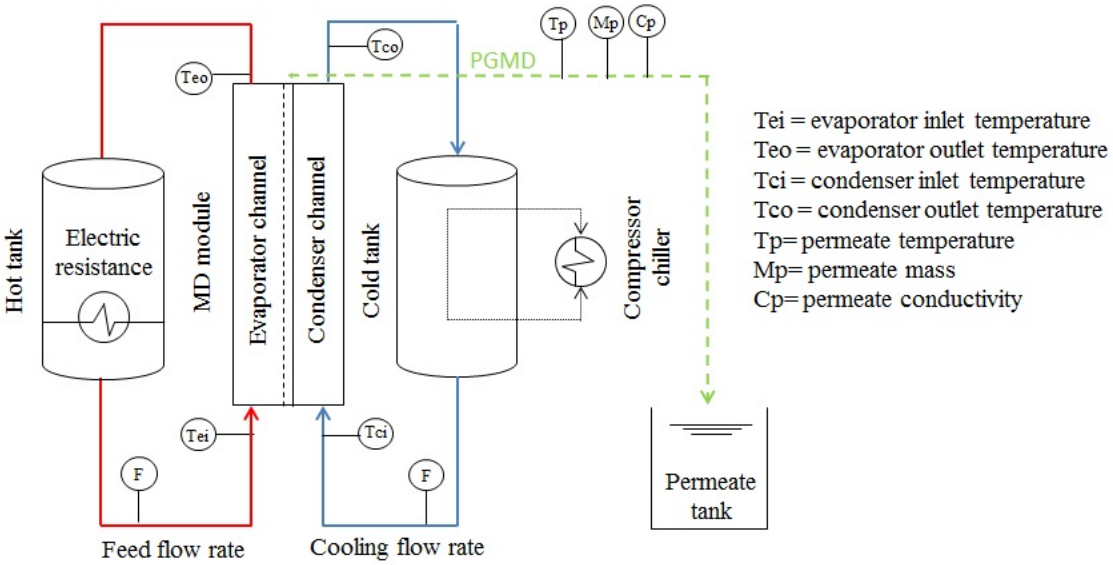

Figure 2. Hydraulic layout of the module test facility.

2.5. Solar CPC photo-reactor

The photo-Fenton process was carried out in a compound parabolic collector (CPC) reactor (Ecosystem S.A., Environmental Services, Spain) (**Figure 3**) consisting of two CPC-mirror modules with a total of 20 tubes manufactured in borosilicate glass. This has been described elsewhere [28]. The CPC mirror geometry has a concentration factor of 1, it is made of MiroSun highly reflective anodized aluminium (Alanod, Germany). The total volume of the system was 60 l, 45 l of which were illuminated, corresponding to a ratio of illuminated volume to total volume of 0.75. The flow rate used was 30 l/min. Temperature (Checktemp instruments, Spain) and pH (Crison) values were measured directly in the CPC reactor during the experiments.

Figure 3. Solar CPC reactor used in this study.

2.6. Solar UVA radiation

Solar UVA radiation was measured by a global UV radiometer (Model CUV4, Kipp&Zonen, the Netherlands) with a typical sensitivity of 264 μV/W/m^2, and a central wavelength of 200-400 nm which provides data in terms of incident W_{UV}/m^2, placed on a platform tilted 37 °. Q_{UV} (eq. 3) estimates accumulated UV energy in the solar reactor per unit of treated water volume for given periods of time. It allows evaluating and comparing experimental results carried out in different days with different meteorological conditions.

$$Q_{UV} = \sum_n \overline{UV}_{n-1} \cdot \frac{A_r}{V_t} \cdot \left(t_n - t_{n-1}\right) \quad (3)$$

Where t_n is the experimental time for n-sample, $\overline{UV}_{n-1}$ is the average solar ultraviolet radiation measured during the period (t_n-t_{n-1}), A_r is the illuminated of reactor surface, and V_t is the total water volume. In this study, besides Q_{UV}, experimental time was used as a result too, because it is important to give an idea of the effectiveness of the process and of the treatment time.

2.7. Reagents

Ferrous sulphate heptahydrate ($FeSO_4 \cdot 7H_2O$, PANREAC, Spain) was used as source of Fe^{2+} to obtain an initial concentration of iron of 10 mg/ l^{-1}. This concentration was selected based on our previous studies that evidenced the important role of light penetration in the photo-reaction and the detrimental effect of suspended precipitated iron when the added iron was higher than 10 mg/L [19, 30]. The concentration of Fe^{2+} was measured with a spectrometric method, ISO 6332. With this method is possible analyze concentrations between 0.01 and 10 mg/l. The samples were filtered with NY 0.2 μm CHROMAFIL® Xtra PET-20/25 (PANREAC, Spain) to remove precipitated iron. Then, 1 ml of 1,10-phenanthroline and 1 ml of buffer solution was added to each sample to generate an acid pH and to favour the formation of a colored complex between Fe^{2+} and 1,10-phenanthroline. Ascorbic acid is added to reduce Fe^{3+} in Fe^{2+}. The coloured complex was measured with a spectrophotometer (PG Instruments Ltd T-60-U) at 510 mm in glass cuvettes (1 cm path length). H_2O_2 (30 wt%, Riedel- de- Haën, Germany) was used as received to obtain a concentration of 20 mg/l in the CPC reactor. Its consumption was measured with the spectrophotometer too but with a wavelenght of 410 nm following DIN 38409 H15. H_2O_2 forms a yellow complex with titanium (IV) oxysulfate. Absorbance was read after 5 min incubation time against a H_2O_2 standard curve linear in the 0.1-10 mg/l concentration range. Sulphuric acid (H_2SO_4, Merk, Germany, analytical grade) was used to obtain the required acidic conditions for photo-Fenton experiments.

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2.8 Lab-scale experiments

A series of control tests were conducted at lab-scale to find out the effect of individual parameters including the effect of salts, temperature and acid pH on the viability of spores of *B. subtilis* and *Clostridium* sp. These tests were performed using 200-ml borosilicate glass bottles filled with synthetic salty water spiked with *B. subtilis* (10^7 CFU/100 ml initial concentration) or with real UWW containing naturally occurring *Clostridium* sp spores (ca. $5 \cdot 10^3$ CFU/100 ml initial concentration). Each parameter was experimentally evaluated as follows:

i) *Salt effect*: Bottles with 200 ml of marine salts (3.5 wt%) sterile solution and spores of *B. subtilis* were kept under constant stirring in the dark for 5 hours.
ii) *Temperature effect:* Bottles with 200 ml of sterile distilled water and spores of *B. subtilis* or real UWW containing *Clostridium* sp spores were heated up to 80 °C placing the bottles in a thermal bath in the dark for 10 hours.
iii) *Acid pH effect:* pH 2.8 was set using sulphuric acid in a bottle with 200 ml of marine salts (3.5 wt%) sterile solution spiked with spores of *B. subtilis*; it was kept under constant stirring in the dark for 5 hours.

In all cases, the samples were taken every hour and analyzed according to enumeration technique used for *B. subtilis* or *Clostridium* spores, respectively.

Finally, to test the potential detrimental effect of mechanical stress and membrane retention in MD, an experiment was carried out in the MD test module without heating for 5 hours. In addition, the feed consisted of 80 l of marine salts (3.5 wt%) solution with an initial concentration of 10^7 CFU/100 ml. Flow rates used in both sides of the membrane were the same and equal to 400 l/h. Water temperature varied with ambient temperature between 20 and 30 °C. Samples were taken and analyzed every hour over 5 hours.

2.9 MD & solar photo-Fenton experiments

For MD experiments, the hot tank was filled with 80 l of water (salty water or UWW), and then heated up to 80 °C. The cold tank was filled with distilled water and kept at 20 °C. Once the desired temperatures were reached, *B. subtilis* spores were added with an initial concentration of 10^7 CFU/100 ml in the hot tank and the suspension was homogenized by pumping in a closed loop. When real UWW was used, there was no addition of *Clostridium* sp spores to the water, being the initial concentration the natural presence of this bacterium in the effluent ($5 \cdot 10^4$ CFU/100 ml). Then, the first sample (t = 0 min) was taken and the water started to pass through the evaporator channel of the MD test cell with a flow rate of 400 l/h. In the other side, cooling water with a flow rate of 400 l/h started to flow too. Both fluids were recirculated continuously to their respective tanks (figure 2). These temperature and flow rate values were chosen to

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obtain a high permeate production in MD. Experiments were performed during 10 hours, taking samples of the feed and permeate streams every hour. After MD process, the remaining feed was stored in a sterilized tank for further treatment (solar photo-Fenton) the following day. For photo-Fenton process, solar CPC- reactor was filled with 60 l of MD feed recirculating at 30 l/min. The pH was adjusted closed to 3 using sulphuric acid and then iron source was added. After that, the hydrogen peroxide was added and recirculated in the dark for homogenization. Finally, the reactor was uncovered and an initial sample was taken, and every 30 min afterwards. All experiments started between 10:30 and 11:00 am local time and lasted 5 h exposed to sunlight; temperature and pH in the reactor were measured. When only photo-Fenton was applied, the CPC- reactor was filled with 60 l of salt water inoculated with *B. subtilis* spores or real UWW with natural presence of *Clostridium* sp spores. The procedure followed was the same as described above.

3. Results and Discussion

3.1. *B. Subtilis* spores removal by MD and Photo-Fenton

Prior to carrying out the experiments in MD, several tests at lab scale were performed to check whether the effect of salinity, heat and mechanical stress suffered by the circulation inside the MD module could mask the real effect of the MD process on the *B. subtilis* spores. Figure 4 shows the results obtained for the lab-scale experiments to test the individual effect of salts, heat pH and mechanical stress on *B. subtilis* spores viability. Results showed that the concentration of spores was constant during all time in the three bottles, evincing that they can resist a concentration of 3.5 wt% of marine salt, a pH of 2.8 and a temperature of 80 °C for 5 and 10 hours respectively. Moreover, spores did not suffer mechanical stress or adsorption on the membrane after 5 hours.

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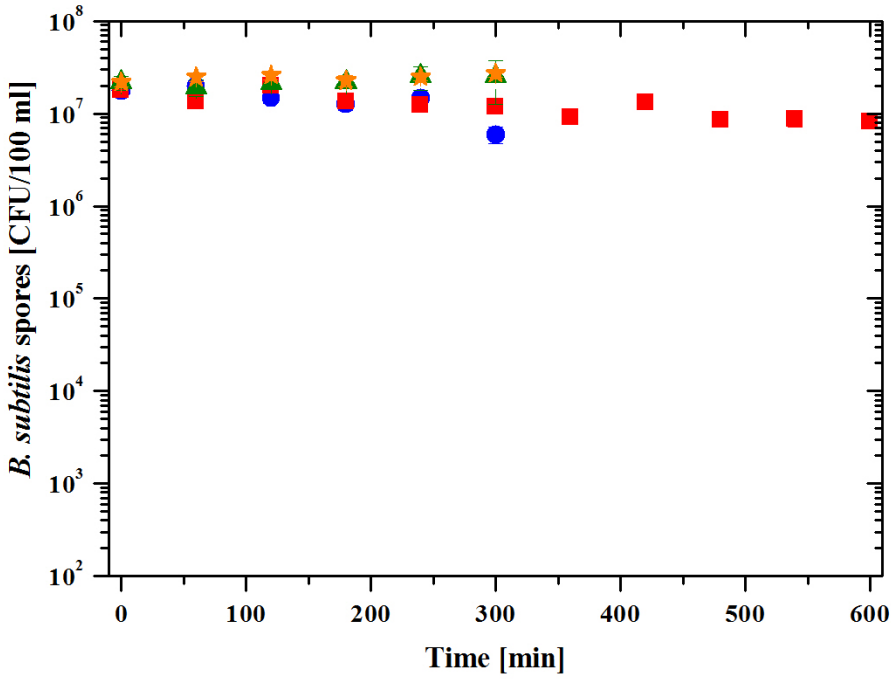

Figure 4. Effects of salts, heat, pH and mechanical stress in *B. subtilis* spores. 3.5 wt% salts ( ), T = 80 ºC ( ), pH ( ) and mechanical stress ( )

In MD treatment of saltwater with *B. subtilis* spores, a total volume of 5 l of permeate free of *B. subtilis* spores was obtained (concentration below DL) with a permeate flux of 38 l/h.m^2. The concentration of spores in the feed tank decreased only 1 log after 10 hours of MD functioning (**Figure 5**). This small decrease in spore concentration can be attributed to the combined effect of the three stress factors occurring simultaneously (i.e. T, salt and mechanical stress) inside the MD module. The following day, 60 l of the remaining feed were treated in a CPC- reactor with photo-Fenton at pH 2.8 (i.e. ideal pH). Initial iron concentration added was 10 mg/l. H_2O_2 was measured and added in doses of 20 mg/l when the concentration was below 2 mg/l along the process as this reagent is consumed during the photo-Fenton reaction (1). A total of 80 mg/l of H_2O_2 was used in 5 hours of experiment.

Dissolved iron concentration was measured, and the initial concentration was much lower (2.6 mg/l) than the initially added (10 mg/l). Moreover, a variation from 2.6 mg/l to 0.3 mg/l was observed along the 5 h of the experiments, while pH remained stable at values 2.8-3.0. This is a normal behavior for Fe-complexes in water when the salinity is very high, as this case. For example, Rubio et al. [39] reported dissolved iron very close to zero during ~~on~~ photo-Fenton water disinfection at near neutral pH in artificial seawater, the authors attributed this to the pH and the presence of high concentrations of salts (chloride, sulphate, hydrogen carbonate).

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Some contributions attribute the loss of dissolved iron to the formation of iron complexes at low pH (<5) in the presence of high concentrations of chloride ions (>0.2 M). Ideally, in the optimal conditions of photo-Fenton, at pH 2.8, predominant iron complex is $Fe(OH)^{2+}$, the most photoactive. When pH increases, photoactivity of this complex decreases [22, 40]. Nevertheless, other factors also influence this, as for example the presence of natural organic matter in natural waters or wastewaters, and also the presence of certain concentrations of ions. This is the case of seawater and brackish. Some articles have studied the influence of the high salinity in the Photo-Fenton process at several pH values, and specifically the influence of high concentrations of chloride. These articles report on the inhibition of the oxidation rate of 4-chlorophenol and aniline by photo-Fenton with concentrations up to 50g/L of NaCl and 0.2 M of Cl^-, respectively [41]. At high concentrations of chloride ions, the complexation of Fe–Cl occurred (reactions 4-9). This effect will decrease the iron solubility under these conditions, and it will alter the generation of radicals, and therefore inhibiting the oxidation process even at low pH values. Moreover, scavenging of hydroxyl radicals (reaction 10), will generate chloride radicals $Cl^{\bullet}$, which are less reactive than hydroxyl radicals.

$$Fe^{2+} + Cl^- \rightleftharpoons FeCl^+ \quad (4)$$

$$FeCl^+ + Cl^- \rightleftharpoons FeCl_2^0 \quad (5)$$

$$Fe^{3+} + Cl^- \rightleftharpoons FeCl^{2+} \quad (6)$$

$$Fe^{3+} + 2Cl^- \rightleftharpoons FeCl_2^+ \quad (7)$$

$$*Fe^{3+}(aq) + Cl^- \rightarrow Fe^{2+}(aq) + Cl^{\bullet} \quad (8)$$

$$*FeCl_3 + RH \rightarrow FeCl_2 + HCl + R^{\bullet} \quad (9)$$

Where * symbol means that the specie is photoexcited.

$$Cl^- + HO^{\bullet} \rightarrow [ClOH]^{\bullet -} \quad (10)$$

Moreover, a lower inactivation in photo-Fenton in saltwater may be also due to the decrease in the transmittance of light in water with high salt concentration. Cl^- and SO_4^{2-} in high concentrations can absorb light and prevent microorganisms to be inactivated by direct or indirect action of photons [39].

The water temperature varied between 24 and 32 °C in the CPC reactor. This little thermal increase is the commonly observed in this type of non-concentrating solar collectors [42] which

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does not affect significantly the viability of spores (figure 4) but has an Arrhenius relationship which explains some grade of accelerating effect on the disinfection rate of the photo-Fenton [43]. The average solar UVA irradiance along the experiment was 33.9 ($\pm$1.2) W/m^2, with minimum and maximum values of 23.8 ($\pm$1.2) W/m^2 and 38.2 ($\pm$1.2) W/m^2, respectively. This corresponds to a variation of Q_{UV} from 3.5 to 45.8 kJ/l. After photo-Fenton process, the spore concentration decreased from 10^6 to $5 \cdot 10^3$ CFU/100 ml, therefore photo-Fenton accounted for a reduction of ca. 2.5 logs.

It is important to check that the reduction of the concentration of spores of *B. subtilis* was not due to the acid pH of the experiment (**Figure 6**). Results showed that *B. subtilis* spores resist perfectly the acid pH. Therefore, acid pH did not affect the concentration of spores.

To find out the effect of photo-Fenton without prior MD treatment, another experiment was carried out. In this case, the contaminated solution had initial concentration of 10^6 CFU/100 ml to reproduce the concentration as when photo-Fenton was used after MD treatment. The experiment required a total adding of 10 mg/l of Fe^{2+} and 100 mg/l of H_2O_2 in doses of 20 mg/l, in spite of that, no reduction of spores at all during the 5h of experiment was observed. Water temperature varied between 27 and 35 °C. The average solar UVA irradiance during all the experiment was 35.1 ($\pm$1.2) W/m^2, with minimum and maximum values of 20.1 ($\pm$1.2) W/m^2 and 43.1 ($\pm$1.2) W/m^2, respectively. This corresponds to a variation of Q_{UV} from 3.7 to 47.3 kJ/l. Results showed the concentration of *B. subtilis* spores was kept totally constant and equal to 10^6 CFU/100 ml, which clearly indicates that *B. subtilis* is completely resistant to photo-Fenton in acidic conditions. Bandala et al [44] achieved the inactivation of *B. subtilis* spores using photo-Fenton process. However, the amount of iron and H_2O_2 used for only 25 ml of solution was very high (initial concentration of iron and H_2O_2 was 2.5 mM and 100 Mm respectively) compared to this study (only 0.036 mM and 0.588 mM of iron and H_2O_2 were added to 60 l of contaminated solution).

Thus, it can be concluded that the treatment of contaminated water with *B. subtilis* spores with MD resulted very satisfactory for two reasons. On one hand, a permeate totally free of spores was produced, and on the other hand, MD favoured the 2.5-log reduction of *B. subtilis* spore concentration when photo-Fenton was used, while photo-Fenton without a previous MD process did not achieve any significant reduction of spores in the same time of operation with the same amount of solar radiation and using a similar amount of reagents. This difference can be attributed to the heating effect suffered by the spores during the MD process, which although is not enough to significantly kill all the spores, it may generate a sub-lethal effect that produce a high susceptibility to additional stress factors, as the great inactivation by solar photo-Fenton.

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Therefore, the combination of these technologies represents clear advantages for inactivation of highly resistant waterborne pathogens.

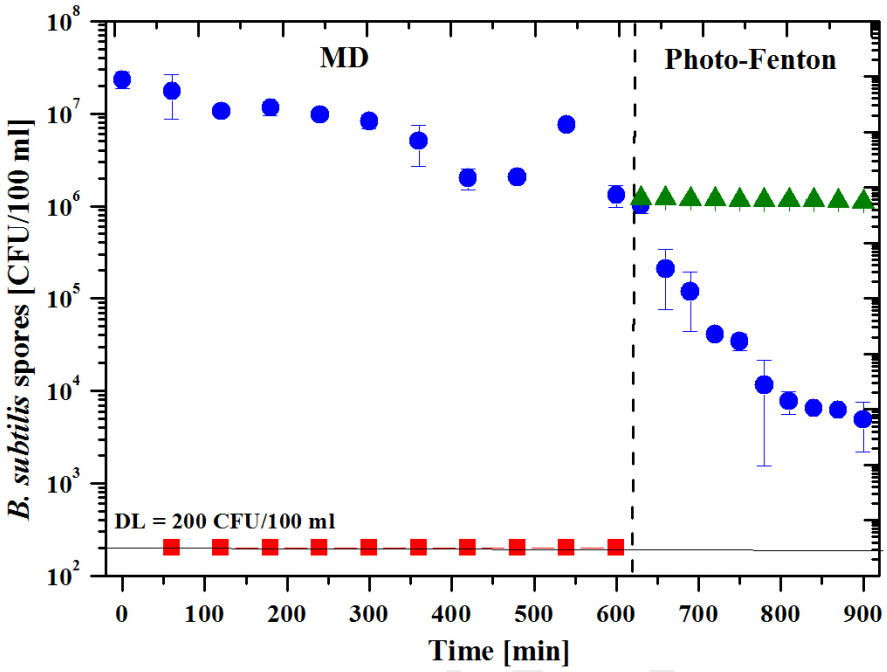

Figure 5. Evolution of *B. subtilis* spores concentration after MD and photo-Fenton treatments. Feed (MD + photo-Fenton) (●), red square: permeate from MD (■), Feed (only photo-Fenton) (▲).

3.2. *Clostridium* sp spores elimination by MD and photo-Fenton processes.

UWW containing *Clostridium* sp spores ($5 \cdot 10^3$ CFU/100 ml) was treated by MD over 10 hours. A total volume of 4.4 l of permeate free of *Clostridium* sp spores was obtained. This corresponds to a permeate flux of 34 l/h.m^2. Spore concentration in the feed tank decreased to below 10^2 CFU/100 ml (**Figure 6**). To check whether this reduction was due to the effect of the thermal stress, a lab-scale experiment was performed. **Figure 7** shows that *Clostridium* sp spores can resist 80 °C for 4 hours but from this time the concentration decreased 1.5 log. This decrease was observed after 4 h, similar to the time (after 5 h) that spores concentration was reduced in the MD module. Therefore, this indicates that the principle responsible for the reduction of the concentration of the spores was the heat supplied in the MD process. The following day, 60 l of the MD feed were treated by photo-Fenton at pH = 2.8 (Fe at 10 mg/l, H_2O_2 at 20 mg/l) in solar CPC reactor. Since H_2O_2 was consumed along the experiment, it was added four times to maintain 20 mg/l of the oxidant, making a total of 100 mg/l of H_2O_2 added for 5 hours of operation. A variation of the initial dissolved iron concentration from 4.65 to 3.65 mg/l was observed along the 5 h of experiment. The possible causes of this reduction have been

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widely discussed in the previous section. The temperature varied between 35 and 45 °C in the solar reactor. The average solar UVA irradiance during all the experiment was 33.0 (±1.2) W/m^2, with minimum and maximum values of 23.7 (±1.2) W/m^2 and 36.9 (±1.2) W/m^2, respectively. This corresponds to a variation of Q_{UV} from 3.4 to 44.6 kJ/l. After 2.5 h of photo-Fenton process, the spore concentration depleted completely, below the DL of 2 CFU/100 ml (**Figure 6**). Therefore the combination of MD and photo-Fenton processes achieved the total elimination of *Clostridium* sp spores in UWW. Another control photo-Fenton test at pH = 2.8 without prior MD was carried out with *Clostridium* sp spores (**Figure 7**, green triangle symbols). The proportion of the iron: H_2O_2 was the same as previous experiments, adding doses of H_2O_2 when the concentration was below 2 mg/l. The temperature varied from 30 to 37.5 °C. The average solar UVA irradiance during all the experiment was 35.5 (±1.2) W/m^2, with minimum and maximum values of 28.7 (±1.2) W/m^2 and 39.2 (±1.2) W/m^2, respectively. This corresponds to a variation of Q_{UV} from 4.1 to 38.4 kJ/l. A reduction of something more than 1 log (from $2x10^3$ to 10^2 CFU/100 ml) after 4 h was observed. The reduction rate when only photo-Fenton was used was lower than that when MD was used as a pretreatment. It seems like the thermal stress suffered at the MD treatment favoured the reduction of spores in the photo-Fenton process afterwards. For this reason, it was concluded that the combination of MD and photo-Fenton process resulted beneficial for the removal of *Clostridium* sp spores, achieving full elimination in only 2.5 hours of solar photo-Fenton.

Clostridium sp in real wastewater effluents, as indicator of *Giardia* sp and *Cryptosporidium* sp, is recognized as a very resistant model pathogen that cannot be easily inactivated by standard tertiaty treatments, i.e. UV and UV followed by chlorine [45, 46], by mixed oxidant disinfectants and chlorine [33], or even by solar AOPs. Specifically, Agulló et al [37] studied the inactivation of SRC oocysts by H_2O_2, TiO_2 and photo-Fenton process under natural solar radiation. In all the cases, SRC oocysts showed a slow sensitivity against different treatments studied. In this work, we demonstrated that the combination of two solar driven processes accelerates the disinfection of real wastewater effluents containing *Clostridium* spores. There are no other results of this pathogen in the literature. This has clear implications in the use of solar membrane distillation for water purification when applied to real wastewater, as it produces a high quality clean permeate, and the concentrate can be successfully disinfected by solar photo-Fenton.

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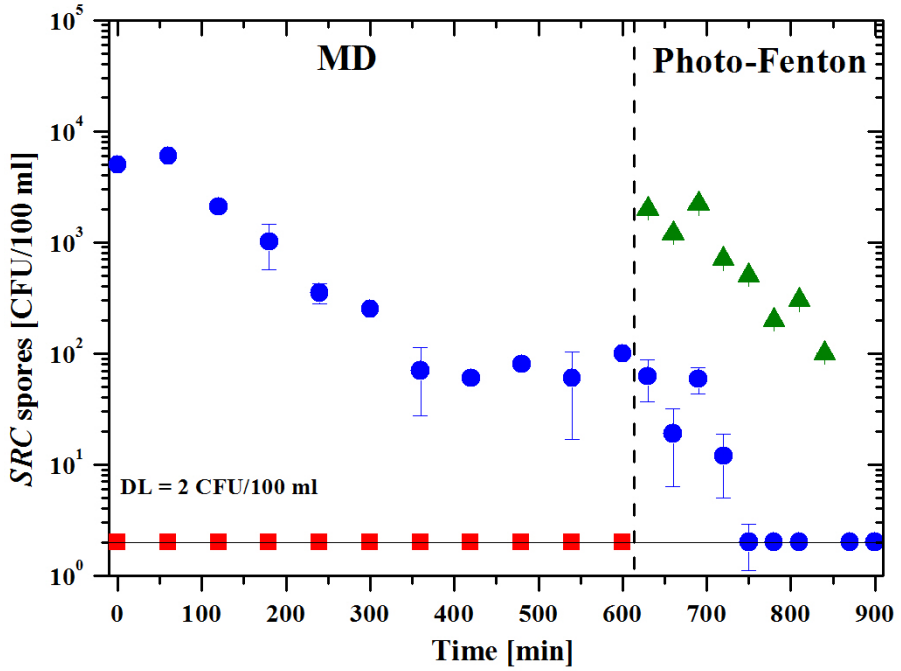

Figure 6. Evolution of *SRC* spores concentration after MD and photo-Fenton treatments. Feed (MD + photo-Fenton) (●), permeate from MD (■), Feed (only photo-Fenton) (▲).

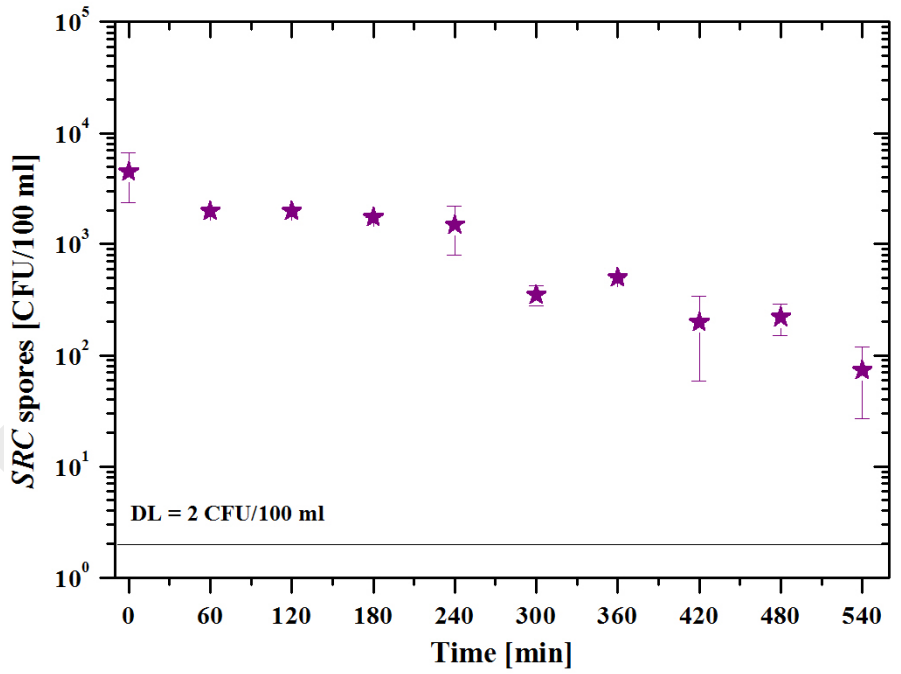

Figure 7. Effects of heat in *SRC* spores. T = 80 ºC.

4. Conclusions

This study contributed to establish an alternative process for the elimination of highly resistant spores, specifically *B. subtilis* and *Clostridium* spores, from wastewater towards its use in

agriculture. The proposed mechanism was MD process. For both cases, MD was satisfactory in obtaining a permeate totally free of spores. Therefore, MD can be used to obtain an effluent free of spores that can be discharged into rivers or reused in the field of agriculture. Moreover, the effect of MD as a pretreatment of a tertiary treatment like photo-Fenton was also proven. For both pathogens *B. subtilis* and *Clostridium* spores, the photo-Fenton process did not affect the concentration of spores while the combination of MD and photo-Fenton process achieved a significant reduction. These are important results because these spores are very resistant to a wide variety of tertiary treatments and the proposed treatment here can be a solution for the removal of highly resistant endospores. Moreover, it is expected that if the combination of MD and photo-Fenton is capable of reducing *B. subtilis* and *Clostridium* spores, it could be able to eliminate other less resistant pathogens under the same conditions.

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