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processes

Research Article

Treatment of seawater for rotifer culture uses applying adsorption and advanced oxidation processes

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ABSTRACT. The use of live feed is common in aquaculture processes due to its nutritional advantages, and rotifers comprise one of the most used feeds in finfish aquaculture. However, bacteria present in water used for rotifer culture are a significant source of infection and consequently diseases could cause mortality in fishes. Advanced oxidation process (AOPs) are methods that may be useful to eliminate bacterial infections present in aquaculture wastewater and may allow the reuse of this water. Therefore, the prospect of using different advanced oxidation processes to inactivate *Vibrio* spp. and total bacteria cultures (TCB) present in this kind of water was evaluated. In particular, $UVc/O_3/H_2O_2$, UVc/H_2O_2 and O_3/H_2O_2 and the adsorption process were applied to seawater coming from a fish farming tank. The results obtained were highly promising because it was possible to inactivate 100% of bacteria present in the seawater when UVc/ozone/hydrogen peroxide and UVc/hydrogen peroxide were applied, needing around 20 and 80 min for *Vibrio* spp. and total culturable bacteria inactivation, respectively. These results are justified due to the strong action between H_2O_2 in the presence of UVc, which may produce a large amount of hydroxyl radicals. Nevertheless, when ozone/hydrogen peroxide was applied, total inactivation of bacteria did not occur. Also, an interesting relationship between bacteria inactivation and oxide reduction potential of seawater was observed during the process. Rotifers put in water after the treatment did not suffer behavior changes (swimming) and no mortalities were observed.

Keywords: rotifers, bacteria, UV irradiation, ozone, hydrogen peroxide, aquaculture.

Tratamiento de agua de mar para el cultivo de rotíferos mediante procesos de adsorción y oxidación avanzados

RESUMEN. El uso de organismos vivos como alimento es común en la acuicultura, debido a sus ventajas nutricionales. Los rotíferos constituyen uno de los alimentos principales en el cultivo de peces. Sin embargo, las bacterias presentes en aguas utilizadas en el cultivo de rotíferos son una fuente importante de infección y pueden causar enfermedades y mortalidad en peces. Los procesos de oxidación avanzados son métodos útiles para eliminar bacterias presentes en aguas residuales de la acuicultura, permitiendo su reutilización. Se evaluó la inactivación de *Vibrio* spp. y bacterias cultivables totales presentes en aguas residuales de la acuicultura a través procesos de oxidación avanzados. Particularmente, se estudió la aplicación de $UVc/O_3/H_2O_2$, UVc/H_2O_2 y O_3/H_2O_2 junto a procesos de adsorción aplicados a agua proveniente de un estanque de cultivo de peces. Los resultados obtenidos son muy prometedores debido a que fue posible inactivar el 100% de bacterias presentes cuando se utilizó $UVc/O_3/H_2O_2$ y UVc/H_2O_2, requiriendo 20 y 80 min para la inactivación de *Vibrio* spp. y bacte-

rias cultivables totales, respectivamente. Estos resultados se explican debido a la acción entre H_2O_2 y UVc, que produce una gran cantidad de radicales hidroxilo, que son la base de estos procesos. Sin embargo, cuando se aplicó O_3/H_2O_2 no se observó una inactivación elevada de bacterias. También se apreció una relación entre la inactivación bacteriana y el potencial óxido reductor en el agua luego de los procesos de oxidación. Los rotíferos sometidos al agua tratada no sufrieron cambios de comportamiento ni se observaron mortalidades.

Palabras clave: rotífero, bacteria, UV, irradiation, ozono, peróxido de hidrógeno, acuacultura.

INTRODUCTION

In intensive aquaculture, conditions such as over-feeding, fast growth, high temperature, infrequent water renewal rate, improper solids removal, and insufficient removal of dead and moribund fishes from the farming area produce favourable conditions for the emergence of bacterial diseases (Almeida *et al.*, 2009).

The use of live feeds is common in marine fish hatcheries all over the world because of nutritional advantages compared to dry feed. For instance, rotifers (*Brachionus plicatilis* Mueller, 1786) are essential live prey in larval rearing of marine fish species. However, they are also considered to be a possible vector for the introduction of pathogens into the rearing system. Mortality of fish larvae fed with rotifers has occurred in the past and has been related to infectious diseases. Live prey such as rotifers are important carriers of contamination of the larval digestive tract, which greatly determines the microbiota of the larvae, and in many cases, is responsible for significant mortality rates (Makridis *et al.*, 2000). Transfer of pathogens from live prey to target organisms can be performed directly, by ingestion of the prey, and/or indirectly, by release of pathogens from prey to seawater (Prol-García *et al.*, 2010).

Bacterial infections, including antibiotic-resistant strains, have been recognized as an important limitation to the development of aquaculture production (FAO, 2009; Oliveira *et al.*, 2012). Vibriosis, caused by bacteria from the family Vibrionaceae, is currently responsible for most outbreaks in aquaculture.

Several *Vibrio* species have been reported to cause high mortality episodes in the culture of some fish species. Fish pathogens *Listonella anguillarum* (Bergeman, 1909; MacDonell & Colwell, 1986) and *Vibrio splendidus* (Beijerinck, 1900) have been found to be associated with live prey (Thomson *et al.*, 2005), causing mortality to fish larvae (Thomson *et al.*, 2005; Reid *et al.*, 2009). The strains *L. anguillarum* and *V. splendidus* are pathogenic to turbot larvae but the infection patterns for both species seem to be different. *V. splendidus* was detected and isolated from the gut of diseased turbot larvae (Thomson *et al.*, 2005; Reid *et al.*, 2009) whereas *L. anguillarum* was found in the epidermis of experimentally infected turbot larvae (Planas *et al.*,

2005). Considering this, bacterial infection present in aquaculture systems is an important problem that must be controlled and strict biosecurity measures must be implemented to avoid introduction of contaminated food to fish.
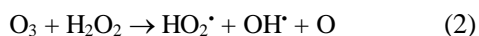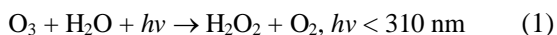
Currently the use of antibiotics is very common, especially when the application of disinfection procedures is ineffective or not sufficient. This strategy could be useful in order to reduce bacterial load (Battaglene *et al.*, 2006); however, it has some drawbacks. For example, the regular use of artificial food supplemented with antibiotics in aquaculture systems to prevent the spread of diseases and their massive use to control infections, has resulted in the development of resistant strains, which have contributed to the inefficacy of antibiotic treatments (Panpan *et al.,* 2012). To reduce the risk of the development and spreading of microbial resistance and to control fish diseases in aquaculture, alternative strategies must be taken into consideration (Defoirdt *et al.*, 2011).

It is known that some bacteria are important in aquaculture systems, like autotrophic nitrifiers that are able to oxidize ammonia to nitrate, heterotrophs that degrade organic matter (Hagopian & Riley, 1998), or probiotic bacteria that improve the health of their host (Gatesoupe, 1999). The disinfection process may reduce microbial maturation and destabilize microbial communities in aquaculture systems (Attramadal *et al.*, 2012a). Disinfection of intake water is a necessary precaution against pathogenic intruders, but at the same time the procedure temporarily diminishes competition between different types of bacteria (Attramadal *et al.*, 2012b), thus it is imperative to evaluate some technological strategies to reduce the level of bacteria present in aquaculture systems. There are some interesting technological alternatives that may reduce or eliminate bacteria and viruses from intensive aquaculture systems, reducing environmental risks and operational costs. Among them, the use of advanced oxidation processes (AOPs) may be a useful method that could be highly convenient to obtain seawater free of infection.

The success of the basics AOP lies in their ability to produce hydroxyl radicals ($HO^\bullet$), through the use of H_2O_2, Fe^{+2}, UV, O_3, or O_3/UV, and heterogeneous photocatalysis by TiO_2 (Primo *et al.*, 2008; Deng & Englehardt, 2007). The utilization of $H_2O_2/Fe^{+2}/UV$ or

H_2O_2/Fe^{+2} is considered highly important in homogeneous catalysis in AOPs that are used in wastewater treatment. $HO^{\bullet}$ is a stronger oxidant, with the highest oxidation potential after fluorine, and has the advantage of being non-selective, able to oxidize almost all pollutant organic compounds. Also, $HO^{\bullet}$ can oxidize internal cell structures and eventually produce death (Kruszewski, 2003), being very useful to inactivate microorganisms.
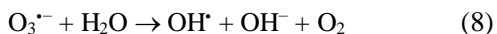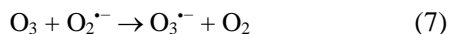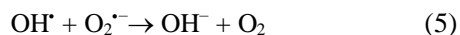
Chemical reactions involved in the action of ozone in water under UV irradiation are represented in equations 1 and 2 (Litter, 2005), including the photodecomposition of ozone in the presence of UVc, a process that generates H_2O_2 and hydroxyl radicals:

$$O_3 + H_2O + h\nu \rightarrow H_2O_2 + O_2, \ h\nu < 310 \ nm \qquad (1)$$

$$O_3 + H_2O_2 \rightarrow HO_2^{\bullet} + OH^{\bullet} + O \qquad (2)$$

Ozone and hydrogen peroxide react with hydroxyl radicals to form hydroperoxyl radical (Eq. 3) and superoxide radicals (Eq. 4).

$$OH^{\bullet} + O_3 \rightarrow O_2 + HO_2 \qquad (3)$$

$$OH^{\bullet} + H_2O_2 \rightarrow O_2^{\bullet -} + H_2O + H^+ \qquad (4)$$
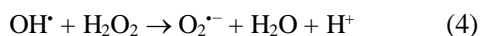
Then, they react with hydroxyl radicals and ozone, producing more hydroxyl radicals (Eq. 5-8).

$$OH^{\bullet} + O_2^{\bullet -} \rightarrow OH^- + O_2 \qquad (5)$$

$$OH^{\bullet} + OH^- \rightarrow H_2O + O_2 \qquad (6)$$

$$O_3 + O_2^{\bullet -} \rightarrow O_3^{\bullet -} + O_2 \qquad (7)$$

$$O_3^{\bullet -} + H_2O \rightarrow OH^{\bullet} + OH^- + O_2 \qquad (8)$$

UVc alone has long been used for the oxidation of several chemicals, but this process is not considered to be an AOP because it is based on the direct photolysis of organic compounds that are able to absorb UVc photons. Nevertheless, when hydrogen peroxide is placed in the solution, the oxidant process is enhanced because the photolytic dissociation of hydrogen peroxide with radiation of 254 nm yields hydroxyl radicals, responsible for an additional oxidation pathway, as can be seen in Eq. 9 (Legrini *et al.*, 1993):

$$H_2O_2 + UVc \rightarrow 2OH \qquad (9)$$

However, it must be taken into consideration that during the ozonation process of sea water, highly toxic and persistent by-products are produced because ozone reacts rapidly with selected halogen ions such as iodide and mainly bromide to different secondary oxidants summed up with the name "ozone-produced oxidants" (Hoigne *et al.*, 1985; Heeb *et al.*, 2014). Bromate is carcinogenic and mutagenic to humans (Ding *et al.*, 2010; Listiarini *et al.*, 2010). Different techniques are currently under investigation for bromate removal: one of these is adsorption by activated carbon (Chitrakar *et*

al., 2011) and the use of UV radiation (Peldszus *et al.*, 2004).

In intensive aquaculture systems, the use of AOPs could potentially improve disinfection efficiency and also oxidize by-products of the process, like toxic compounds generated in the destruction of microorganisms. Considering that there is little research on the mixture of these oxidants to depurate this kind of water matrix, this work is very novel. Therefore, the aim of the present investigation is to study the effect of the use of AOPs, specifically H_2O_2, UVc and O_3, and post-or pre-treatment processes in the depuration of aquaculture wastewater for reuse in rotifer culture.

MATERIALS AND METHODS

A set of experiments was carried out to determine the effectiveness of bacteria inactivation using various AOPs. To do this, 18 L of seawater were taken from a fish culture tank placed in the aquaculture hatchery of Universidad Católica del Norte (Coquimbo, Chile). This tank has a usable volume of 5 m^3 and the seawater is maintained in aerated conditions. The AOPs evaluated were $UVc/O_3/H_2O_2$, UVc/H_2O_2 and O_3/H_2O_2. Figure 1 shows a schematic configuration of the equipment and the reagent intervening in the AOP pilot plant.

Seawater placed in the tank was pumped using a centrifugal submerged pump (36 W) into the UVc photoreactor and then returned to the tank (Fig. 1), generating a recirculation system with a flow rate of approximately 12 L min^{-1}. The UV lamp was an Atman unit, with 254 nm wavelength and 36 W of power, irradiating 477.7 W m^{-2} of seawater treated with a specific power of 0.028 W mL^{-1}.

Ozone gas was provided by an ozone generator (Pacific Ozone, 180 W of power), which receives molecular oxygen (>90% O_2) from an oxygen generator (Air Product), adding 30 L min^{-1} of ozone to the seawater tank. H_2O_2 was added to the solution avoiding the excess of this reagent, because it is well known that excess hydrogen peroxide reacts with $OH^{\bullet}$ yield during the process, acting as a free-radical scavenger. This decreases the hydroxyl radical concentration and generates $HO_2^{\bullet}$ radicals, which are much less reactive, and inhibit or reduce the $OH^{\bullet}$ effect (Tuhkanen, 2004; Primo *et al.*, 2008). Also, the excess of H_2O_2 could be dangerous for the organisms that are going to use the treated water. Therefore 5 mL of H_2O_2 (30% purity, Sigma Aldrich) was added at the beginning of the procedure, obtaining a concentration of 3.767 mM or 0.13 ppm inside the tank.

The turbulence produced by the pump flow inside the tank and bubbling of ozone input generate adequate
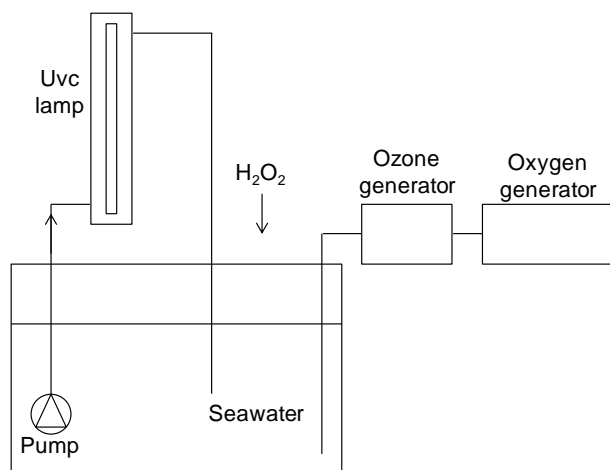
Figure 1. Schematic diagram of the advanced oxidation process pilot plant.

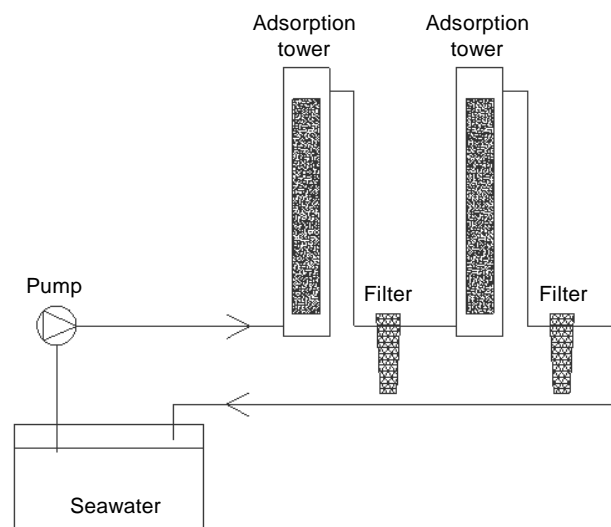**Figure 2.** Schematic diagram of adsorption pilot plant.

mixing of the reagent in the seawater tank, which may help to improve contaminant removal (Feng *et al.*, 2013).

To remove possible undesirable byproducts and residuals generated during AOPs, an adsorption process was carried out using activated carbon (AC), produced with coffee waste, activated with H_3PO_4 and calcined at 350°C for 3 h, with an impregnation ratio of 1:1, obtaining AC with a particle size of 250 mm and pore size of 10 mm (unpublished data). A schematic diagram of the adsorption pilot plant is presented in Fig. 2.

This adsorption pilot plant contains a centrifugal pump with 370 W of power that propels the seawater into two towers containing 20 g of AC. After each adsorption tower there is a cartridge filter unit with a 5 mm pore size that blocks the escape of AC from the adsorption system.

Measured of Total Culturable Bacteria (TCB) and *Vibrio* spp. were carried out using the spread plate method with Marine agar 2216 (Difco Labs, Becton Dickinson and Company, Sparks, MD 21152, USA), and thiosulfate-citrate-bili salts-sucrose agar (TCBS, Difco labs, Becton Dickinson and Company, Sparks, MD 21152, USA), respectively. Appropriate 10-fold dilutions of water samples were prepared and 0.1 mL aliquots were inoculated in triplicate onto agar plates. All marine agars were incubated at 22°C for 5 days and TCBS agars at 22°C for 2 days. Colony forming units (CFU) were counted and the bacterial numbers per millilitre of samples were calculated, based on two or three dilution steps. The detection limit of the culture technique is 2 CFU mL^{-1}.

The parameters measured through the different treatment processes applied were oxide reduction potential (OPR), ozone and pH. ORP was measured with

a Hanna instrument model HI 9828. Ozone was measured using DPD method. H_2O_2 was analyzed using Ti(IV) oxysulfate (Fluka) (DIN 38 402, H15 method) at 410 nm wavelength and using Peroxid-Test (Merckoquant). pH was measured using a WTW 3150i unit.

Samples were withdrawn each 20 min to measure bacteria concentrations, pH, ozone, bromine, H_2O_2, dissolved oxygen percentage saturation (DO%), oxide reduction potential (ORP), temperature and conductivity. ORP measures the potential of water to oxidize and reduce, and is thus an indication of process to disinfect an aquaculture system. ORP can be used to control ozone addition and thus ensure the desired treatment objective (Tango & Gagnon, 2003).

All the analyses were conducted in duplicate, from which the average was calculated. The physical and chemical characteristics of the seawater before treatments are summarized in Table 1.

RESULTS

Bacteria inactivation using complete AOPs

Bacterial inactivation in seawater obtained using complete AOP systems were evaluated in this investigation (UV/H_2O_2/O_3) (Fig. 3). The initial concentration of TCB was 2.93×10^6 CFU mL^{-1} and total *Vibrio* spp. equalled 2.27×10^3 CFU mL^{-1}. After 80 min of treatment time TCB was totally inactivated, without apparent recovery (Fig. 3a). Total *Vibrio* spp. was completely inactivated (or below the detection limit of the method) in the first 20 min of the process. For the two types of bacteria removal evaluated, TCB and total *Vibrio* spp. the inactivation was 100% (or below the detection limit of the method).

Table 1. Value range of parameters of seawater before treatments.

Parameter	Value range
pH	7.38-7.41
Oxide reduction potential (mV)	-10.73-11.00
Conductivity (mS cm^{-1})	32.75-33.20
Temperature (°C)	22.8-23.0
Dissolved oxygen (%)	70.20-92.32
Bromide (mg L^{-1})	145-151
Chemical oxygen demand (mg L^{-1})	6972-7400
Ammonia (mg L^{-1})	0.22-0.31

The high level of removal of TCB and total *Vibrio* spp. may be due to the strong action of hydroxyl radicals produced during the process, and will be discussed below. In the UV/H$_2$O$_2$/O$_3$ experiment, the initial seawater pH was 7.38 before the process and after it increased slightly to 8.03. The initial temperature of the water was 23°C and remained relatively stable, finishing the process at 23.4°C. The variation of DO and ORP during the AOPs (UV/H$_2$O$_2$/O$_3$) was studied and both, DO% and ORP (Fig. 4), increased greatly during the process, beginning at 70.2% and -10.73 mV, and finishing at 358% and 79.6 mV, respectively.

Bromine concentration was relatively stable throughout the process at 147 mg L^{-1} (Fig. 4). The concentrations of ozone and COD, however, showed changes. The initial concentration of COD was 6,972 mg L^{-1}, increasing to 7,927 mg L^{-1} after 60 min of experimentation, staying relatively stable until min 100, and after that the concentration diminished to 7,400 mg L^{-1} at the end of the AOPs. The increase in the COD level could be due to the fact that AOPs form complex by-products in the presence of H$_2$O$_2$, causing increased COD concentration. An expected increase of ozone concentration was observed from 0 mg L^{-1} in the beginning to 19 mg L^{-1} at the end of experimentation.

The presence of bromine in the solution can produce bromate when ozonation is applied due to processes from the oxidation of bromide through a combination of ozone and OH$^\bullet$ reactions (Von Gunten & Hoigne, 1994). Bromate was not measured in this investigation; however, we can infer that the concentration of bromine in this experiment is enough to produce this undesirable by-product due to the action of ozone. Therefore, after the AOPs, an adsorption process was applied using AC following the protocol detailed in the Materials and Methods section.

During the adsorption process COD was reduced 32%, going from 7,400 to 5,000 mg L^{-1}, after 2 h of treatment (see Fig. 5). Hydrogen peroxide concentration was reduced 84.6% from 0.13 to 0.02 mg L^{-1}. The bromine concentration was reduced 71.4%, going from 147 to 42 mg L^{-1}. This reduction is not sufficient to adequately prevent the formation of bromate.

Adsorption and complete AOP

Because of a relatively high concentration of bromine in the solution (42 mg L^{-1}) after the adsorption process, which was applied following the AOPs, an experiment with a different configuration of water treatment was carried out. In this experiment, the aim was to evaluate the effect of the adsorption process on AC before applying the AOPs, so as to reduce the concentration of bromine using the same adsorption pilot plant described in the Materials and Methods section. Therefore, 18 L of seawater from the same fish culture tank described in the Materials and Methods section were placed in a new tank. This water was pumped across the adsorption towers and samples were taken every 20 min to measure bacteria concentration, COD, H$_2$O$_2$ and bromine 5 mL of H$_2$O$_2$ (30% purity, Sigma Aldrich) was added at the beginning of the complete AOPs (UVc, H$_2$O$_2$, O$_3$), obtaining an initial concentration of 3.7 mM or 0.13 ppm inside the tank.

Respect to the evolution of the concentration of bacteria present in seawater during the adsorption process and the complete AOPs treatment, in the first 60 and 20 min of the adsorption process, bacteria, TCB and *Vibrio* spp., were eliminated (Fig. 6).

These results are unexpected, because AOPs were used in order to inactivate bacteria and other contaminants present in seawater, but the above process (adsorption) removed the bacteria in question. It was evaluated the evolution of ORP and DO% present in seawater during the adsorption process and AOPs. DO% and ORP increased greatly during the process, beginning at 85% and -10 mV, and finishing at 390% and 92 mV, respectively.

Chemical oxygen demand is reduced during the adsorption process, going from 7,050 to 4,500 mg L^{-1} after 1 h treatment, reaching 36.1% reduction (Fig. 7). After the adsorption process, the first 20 min of AOPs produced an increase in the concentration of COD, probably due to the formation of more complex by-products and the presence of H$_2$O$_2$. COD then decreased even more, reaching a final concentration of 7,200 mg L^{-1}. The concentration of H$_2$O$_2$ was 0.13 ppm at the beginning of the AOPs, and its level was reduced throughout the process, reaching a final concentration of 0.02 mg L^{-1}. This reduction may be due to the consumption of hydrogen peroxide demonstrated in Eq. 2, 4 and 9. Bromine reduction was very high (99.8%), going from 160 to 0.1 mg L^{-1} after 60 min of the adsorption process.
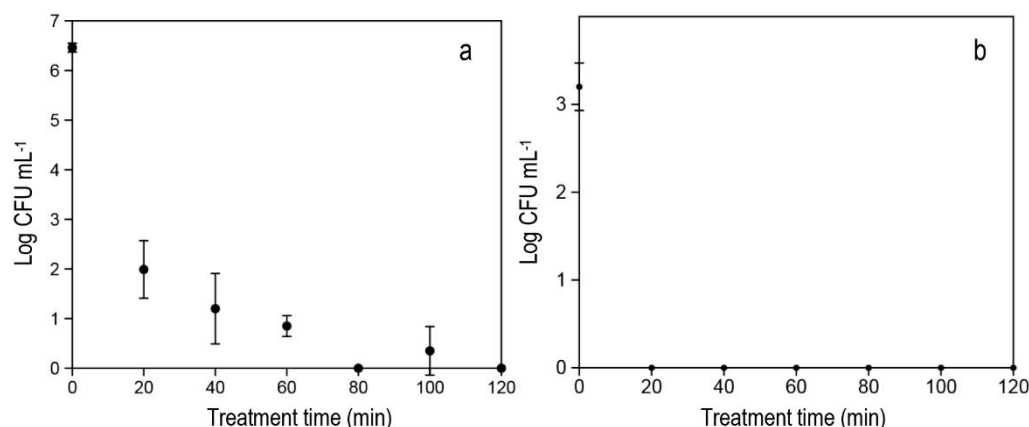
Figure 3. Bacterial concentration during the complete advanced oxidation process for a) TCB and b) *Vibrio* spp. Error bars represent the standard deviation of the results (n = 3).
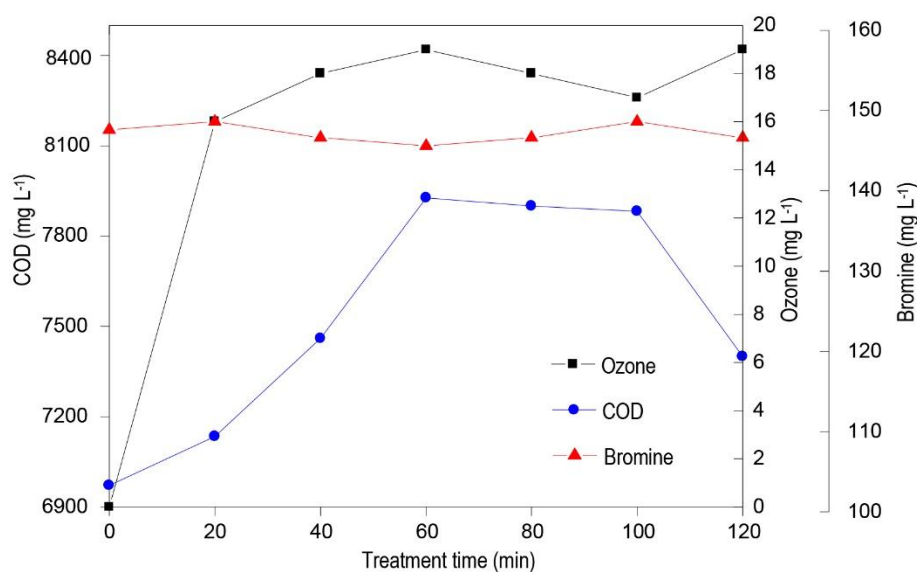
Figure 4. Evolution of chemical oxygen demand (COD), ozone and bromine during complete advanced oxidation process.

Bacteria inactivation using H_2O_2/UV

As can be observed, after 80 min of treatment all TCB were inactivated, both kinds of bacteria removal evaluated reached 100% (or below the detection limit of the method). Seawater pH was 7.38 before the process and decreased ending at 6.7. The initial temperature of the water was 22.8°C and finished at 24.3°C (Fig. 8).

Both parameters, DO% and ORP, increase during the H_2O_2/UV process, but less so than in the $H_2O_2/UV/O_3$ process for ORP and slightly more than in the $H_2O_2/UV/O_3$ process for DO%, beginning at 92.32% and -10 mV and finishing at 419.5% and 73.2 mV, respectively.

There was an increase in the concentration of COD at the beginning of AOPs, due to the presence of H_2O_2 and the appearance of oxidation byproducts, which could be more complex (see Fig. 9). However, after 60 min of treatment, COD suffered a reduction in its concentration, probably due to the formation of more simple compounds in the solution (Poblete *et al.*, 2012), going from 7,100 to 6,500 mg L^{-1}. The concentration of H_2O_2 decreased, going from 0.13 to 0.02 mg L^{-1}. The reduction of the concentration of H_2O_2 could be due to its consumption in the oxidation reduction, a reaction with UV (Eq. 2 and 9) and with $OH^\bullet$ (Eq. 4).

Bacterial inactivation using H_2O_2/O_3

Respect to the evolution in the concentration of bacteria present (both TCB and *Vibrio* spp.) in seawater during the H_2O_2/O_3 treatment, significant removal of microorganisms did not occur, obtaining only 40% removal of TCB in 120 min of treatment time (Fig. 10).
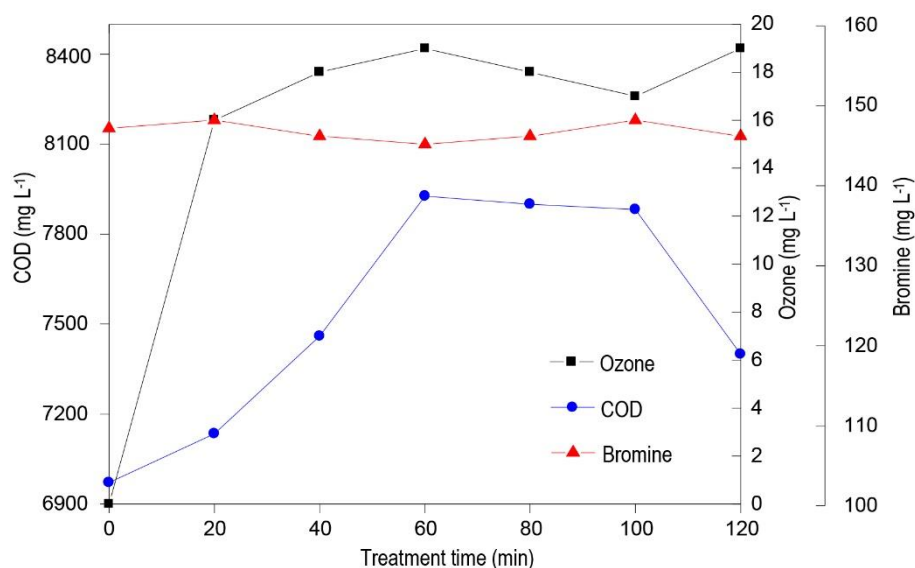
Figure 5. Evolution of chemical oxygen demand (COD), ozone and bromine during the adsorption process.
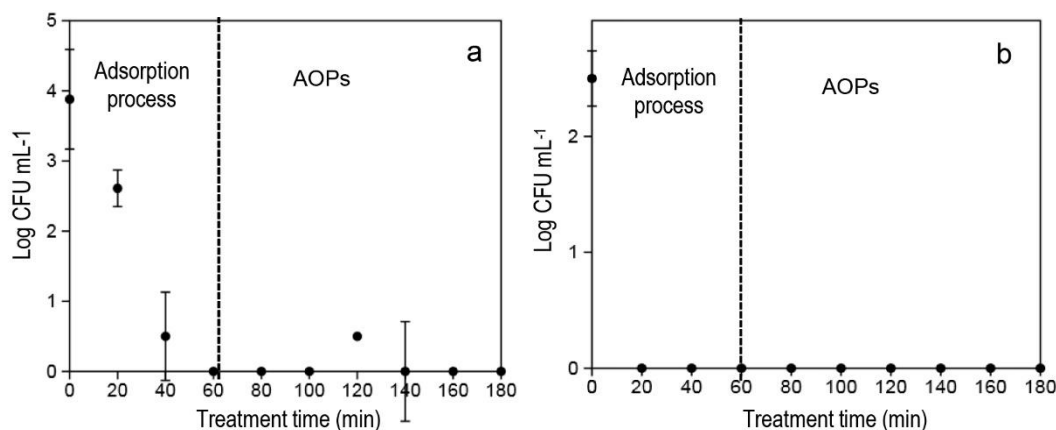
Figure 6. Bacterial concentration during the adsorption process and complete advanced oxidation process for a) TCB and b) *Vibrio* spp. Error bars represent the standard deviation of the results (n = 3). Vertical dashed line represents the end of a process and the start of the next one.

Considering the evolution of DO% and ORP during the H_2O_2/O_3 process both parameters increased during the process, but less than in the $H_2O_2/UV/O_3$ process with ORP going from 70.2% and -11 mV, to 360% and 10.2 mV, for DO% and ORP, respectively.

Observing the low level of bacteria inactivation using H_2O_2/O_3 AOPs, further analysis regarding elimination of bromine or its applications in the culture process was discarded.

The results of this study show an interesting relationship between ORP and the level of bacteria removal. As was demonstrated in this study, $UV/H_2O_2/O_3$ and UV/H_2O_2 processes achieved complete bacteria inactivation, coinciding with a higher increase of ORP, (90.33 and 83.3, respectively). However, in the $H_2O_2/$ O_3 process, the bacteria removal obtained was small and the ORP increase was smaller as well.

During the present research, it was demonstrated that it was possible to completely inactivate (or below the detection limit of the method) measured bacteria present in the seawater and also highly reduce the concentration of bromide and H_2O_2 that could negatively affect the rotifers that could use this water after the studied treatment. However, in order to be sure about this and confirm that this treated water does not have undesired byproducts or residuals from the treatment, it was placed approximately 100,000 adult rotifers (*B. plicatilis)* in 10 L of the water submitted to adsorption and complete AOPs and in the H_2O_2 and UVc processes with the aim of observing their behaviour
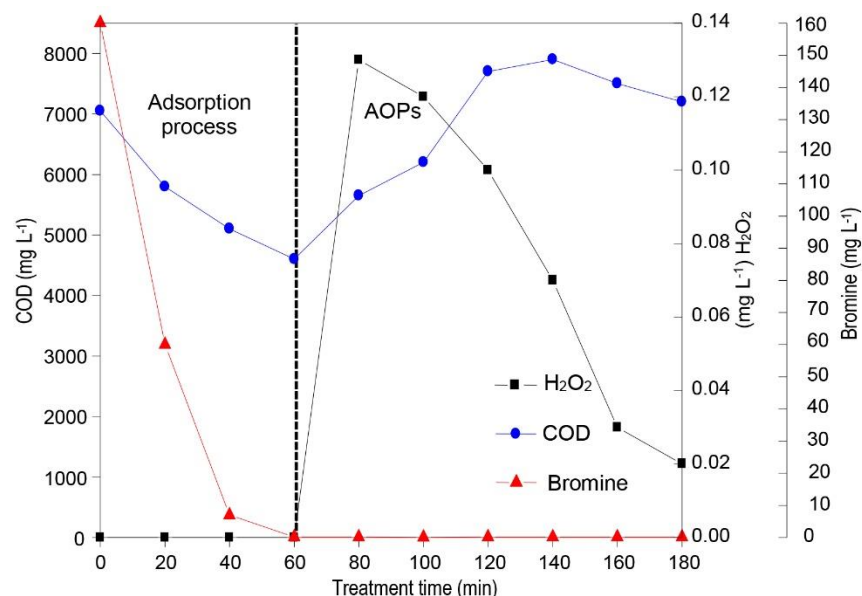
Figure 7. Evolution of chemical oxygen demand (COD), H_2O_2 and bromine during adsorption and advanced oxidation process. Vertical dashed line represents the end of a process and the start of the next one.
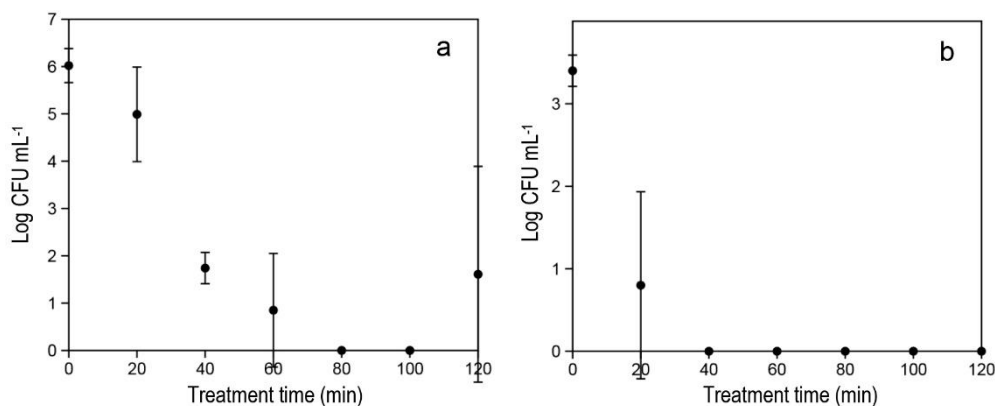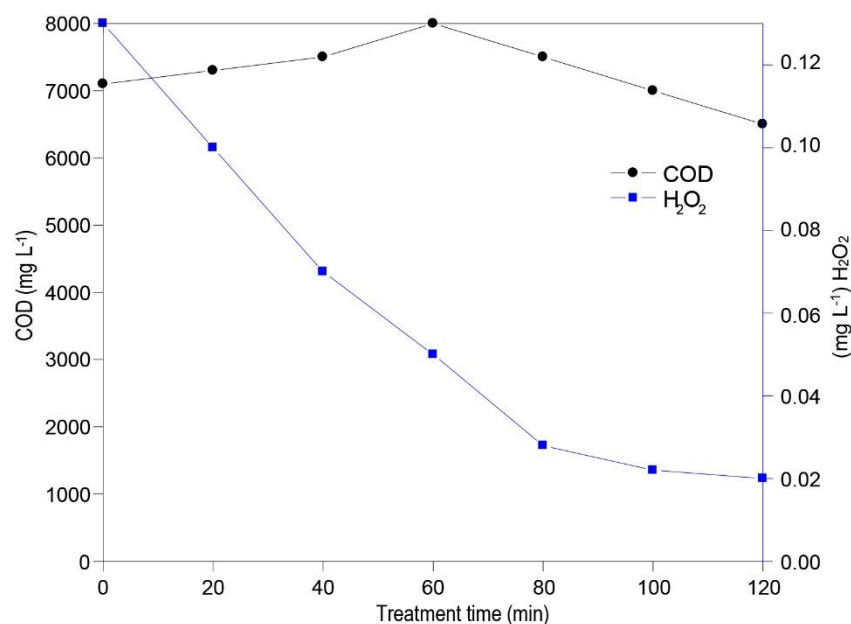

Figure 8. Bacterial concentration during H_2O_2/UV advanced oxidation process for a) TCB and b) *Vibrio* spp. Error bars represent the standard deviation of the results (n = 3).

and confirming their survival (swimming and motility). The treated water in contact with the rotifers was maintained aerated in a batch process and every 1 h, 3 mL of samples were taken to observe the response of the organisms. After 8 h of exposure the survival of rotifers was confirmed and they demons-trated normal swimming behaviour. The analysis of the reproductive behaviour of the rotifers maintained in this treated water will be carried out in a future study.

DISCUSSION

It was observed that DO% and ORP increased during the UV/H_2O_2/O_3 process, this behaviour was also observed by Pedersen & Pedersen (2012), who reported increased values of these parameters when H_2O_2 was

added. The reduction of COD during the adsorption process may be caused by AC's decomposition of H_2O_2, producing an exchange of a surface hydroxyl group with a hydrogen peroxide anion (Bansal *et al.*, 1988). The bromine concentration was reduced due to the adsorption processes after AOPs (Asami *et al.*, 1999; Bao *et al.*, 1999; Kiristis *et al.*, 2000) however it the relatively low level of elimination of bromine could be caused by the presence of a high DO in the solution because of the ozonation process, which restricts the adsorption of bromate in AC (Kiristis *et al.*, 2001). The higher elimination of bromine obtained using adsorption process before AOPs may be due a lower concentration of DO in the solution submitted to the adsorption process (Kiristis *et al.*, 2001). This final con-centration of bromine does not represent a risk of gene-

Figure 9. Evolution of chemical oxygen demand (COD) and H_2O_2 in seawater during the H_2O_2/UV advanced oxidation process.
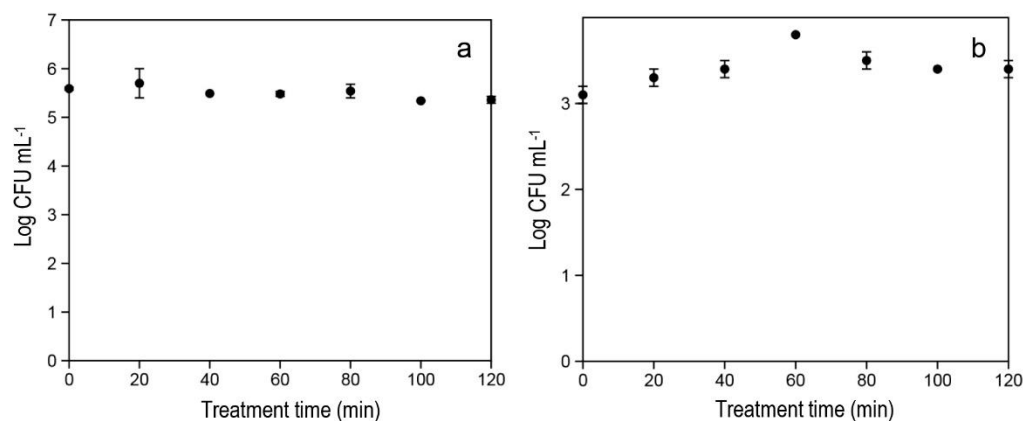
Figure 10. Bacterial concentration during H_2O_2/O_3 advanced oxidation process for a) TCB and b) *Vibrio* spp. Error bars represent the standard deviation of the results (n = 3).

rating enough bromate that might negatively affect the organisms that use this water (Schroeder *et al.*, 2010).

The complete elimination of bacteria due the adsorption process was observed for Moreno-Castilla *et al.* (2003) and Hijnen *et al.* (2010), who reported that it is possible to remove microorganisms with this process, because AC offers the advantages of a large adsorptive capacity and an irregular shape, which acts as a shelter for bacteria from high fluid shear forces (Raihan *et al.*, 1997) and due to the strong attractive forces between the microorganisms and the adsorbents (Li *et al.*, 2011).

The percentage of DO and ORP increased greatly during the adsorption process and this behavior may be due to the turbulence generated in circulated water, principally in the moment when it is returned to the tank, increasing the concentration of DO in the system and consequently the increase in ORP (Pankaj *et al.*, 2008; Yu *et al.*, 2014).

The reduction of COD during the adsorption process is due to the adsorption of organic matter from the solution (Almazán-Sánchez *et al.*, 2014; Islam *et al.*, 2015). The very high level of bacteria removal using the H_2O_2/UV treatment has also been documented and demonstrated in other studies (Bhatti *et al.*, 2011; Penru *et al.*, 2012). This method has been shown to improve water quality via disinfection (Klausen &

Grønborg, 2010). The use of a mixture of two or more oxidants produces a synergic oxidation effect, which enhances microbial destruction and improves the disinfection process (Sharrer & Summerfelt, 2007).

For complex water treatment the $UV/H_2O_2/O_3$ and UV/H_2O_2 could be considered a real alternative, due to their high performance and lack of disadvantages present in other AOPs, like the post-separation required with the use of semiconductor TiO_2 (Chon *et al*., 2010) and the need to adjust the pH solution to acid conditions, with an optimum around 3 for the Fenton and photo-Fenton process (Feng *et al*., 2006; Ahmed *et al*., 2011; Zhang *et al*., 2011), which remains an important obstacle to industrial application of these technologies (Chong *et al*., 2010).

Bacteria inhibition or death obtained in $UVc/H_2O_2/O_3$ and UVc/H_2O_2 treatment can be explained by the direct action of photoinactivation and Haber-Weiss reactions leading to internal cell injuries. This process occurs when small and uncharged H_2O_2 molecules diffuse through membranes into the cell, increasing intracellular H_2O_2 levels (Malato *et al*., 2009; Polo-López *et al*., 2011).

As is well known, exposure of biological compounds to $HO^{\bullet}$ can result in reactions such as hydroxylation, oxidation, H-abstraction, addition to double bonds, bond breakage, also affecting its DNA, and inhibiting its replication and consequently the microorganism's reproduction (Ravanat *et al*., 2001). However, these damages can be reversible and one could observe apparent recovery (or resurgence) of cells when UVc is applied alone, due to the self-repairing mechanism of the microorganism. Nevertheless, the UV_C/H_2O_2 process produces a real inactivation effect in the bacteria, due to the production of hydroxyl radicals (Yasar *et al*., 2007, Pablos *et al*., 2013).

Values achieved of ORP in UVc irradiation experiments were higher than in non-irradiated experiments, and the difference may be due to the hydroxyl radicals generated by the photocatalytic reactor, a yield that is enhanced when UVc radiation and H_2O_2 are acting in the same reactor (see Eq. 9), consequently reducing the concentration of bacteria in the system. A similar observation was made in Lin *et al*. (2012) and Ruey-Fang *et al*. (2014), who noticed a direct relationship between UV and ORP. Also, Rico *et al*. (2008) reported a direct relationship between ORP and the bactericidal effect of an oxidant process. According to Lucas *et al*. (2009), the reaction described by Eq. 2 is slow, therefore in the absence of radiation (only in the presence of O_3/H_2O_2), less radical generation kinetics are observed and subsequently, a lower ORP value results. The dissolved oxygen in the tests reaches a similar maximum value because the

solubility of O_2 in the aqueous solution is the same, with a mean of 379 DO%. Similar observations were made by Lin *et al*. (2012) and Yu *et al*. (2014), who noticed a relationship between hydroxyl radical production and ORP, producing a high level of contamination removal.

CONCLUSIONS

The viability of inactivation of TCB and *Vibrio* spp. (microorganisms present in wastewater from fish farming tanks) was evaluated using different mixtures of H_2O_2, UVc and O_3 and adsorption processes, with the objective of studying bacteria removal, bromide and COD. The adsorption process applied after complete AOPs was not efficient enough in the removal of bromine, obtaining a final concentration of 42 mg L^{-1}. However, when the process was applied before complete AOPs the removal of bromide and COD was very high, getting a concentration of 0.1 mg L^{-1}. The results revealed that the mix of $UVc/O_3/H_2O_2$ (complete AOPs) and UVc/H_2O_2 quickly and completely removed bacteria, especially for *Vibrio* spp., which was inactivated faster than TCB and different level of COD removal. Also, the adsorption process carried out before complete AOPs achieved complete removal of the bacteria studied and as well as high COD removal. However, when the O_3/H_2O_2 treatment was applied it did not achieve a high level of bacteria removal (only 40% removal of TCB). Considering the similar final results of bacteria removal and that it is not necessary to use the adsorption process to reduce the concentration of bromide in the second one (UVc/H_2O_2) because it does not produce bromate, this method seems more suitable, because of reduced energy consumption and less investment due that because the ozone generator is not necessary. An interesting relationship was observed between bacterial inactivation and ORP of seawater during the process that may be explained by the yield of hydroxyl radicals. Regarding the effect on bacteria, it was observed that bacterial death was associated with a higher presence of radicals in the aqueous solution than in the O_3 and H_2O_2 concentrations. Results obtained in this investigation demonstrate the viability of inactivation of TCB and total *Vibrio* spp. present in aquaculture system using AOPs, specifically $H_2O_2/UVc/O_3$ and H_2O_2/UVc, suggesting that this treatment method may be very useful in the aquaculture wastewater treatment and could be considered for future projects that needs water free of bacteria and pathogenic microorganisms.

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