

Photocatalytic ozonation for a sustainable aquaculture: a long-term test in a seawater aquarium

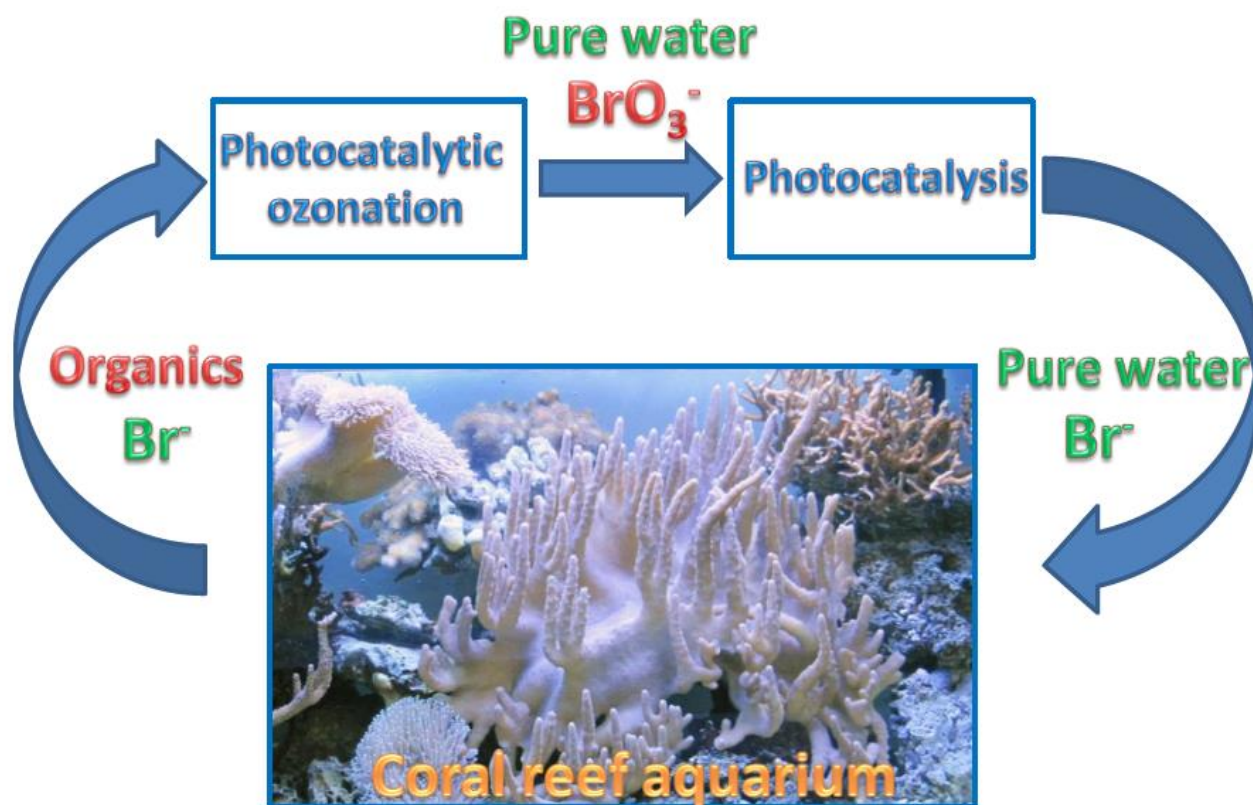
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Graphical Abstract



Abstract

A long-term test at a laboratory scale with a closed system (a coral reef aquarium) has been carried out to study the potentialities of photocatalytic ozonation and photocatalysis for the removal of noxious organic matters compounds in recirculating systems (aquaria and recirculating aquaculture systems) working with a minimum make up of new water. Attention has been focused not only on the depuration capabilities but also on possible negative phenomena, some of which could become apparent only at long times.

After having tuned the system by setting appropriate operating conditions and procedures, positive results were obtained. In particular, it was observed that the alternation of photocatalytic ozonation with photocatalysis with definite cycle allowed to severely limit the presence of the organic compounds, which otherwise could accumulate in the system, and at the same time to control the formation of bromate, the ozonation side-product of main concern. In addition, the values of other parameters, which are important for water quality, remained at satisfactory values. It was also verified

that the prepared photocatalytic films maintained a good photocatalytic activity even after several months of utilization in seawater.

These results demonstrate that photocatalytic ozonation is a good candidate for water purification in recirculating systems in view of sustainable aquaculture.

Keywords: Photocatalysis, Ozonation, Photocatalytic Ozonation, Depuration, Recirculating Aquaculture Systems, Aquarium.

1. Introduction

Aquaculture is the food sector that experienced the fastest growth in the latest years. Both the whole and per capita food fish consumption are increasing, with nowadays almost 50 % of the world's food fish coming from aquaculture whilst this percentage was only 5-6% in the 1970s [1,2]. The production of fish food is expected to continue to increase in the next decades, because fish represents an important part of healthier and more nutritious diets and the potential of the oceans and inland waters can be exploited in a more effective and sustainable way [2].

Aquaculture, unlike fisheries, looks to be the best way to meet this increasing demand for fish [3,4]. In fact, the future of the fisheries industry is constrained by the unsustainable overexploitation of marine resources in nature with about 33.1 % of fish stocks overfished and 59.9 % fully fished [2]. However, the potential of aquaculture strongly depends on the extent to which sustainability requirements can be fulfilled [5,6,7]. In particular, the sustainability of intensive aquaculture, which is the most attractive for economic reasons and for the opportunity of land and resources saving, must be considered. Under this aspect, recirculating aquaculture systems (RAS) could represent the best solution at least for now. In fact, it may provide several advantages in comparison with flowthrough or cage systems [8,9]. Among the several advantages, the limited consumption of the new makeup water [10] and the reduced emissions [6,11] are the most important for sustainability.

On the other hand, RAS need extremely efficient depuration technologies for the purification of the recirculating water in order to grant, with a minimum makeup of new water, a suitable environment for the cultured species and a safe final product even in systems characterized by particularly high organic load, such as intensive aquaculture. Moreover, several organic compounds, which are present in aquaculture systems, can be toxic, detrimental or growth inhibiting so that they must be removed together with pathogenic microorganisms [12] in order to grant a safe rearing environment and a low environmental impact. Actually, the contamination of the aquatic environment from aquaculture is a major concern in European [13,14] and Asian [15] inland and marine waters.

Besides compounds from uneaten and spoiled feed, the chemicals of major concern include substances, which are introduced or produced in the common practice of aquaculture, and excreta of aquaculture species. For example, pharmaceuticals, hormones and bromate fall under the first category, whereas metabolites from cyanobacteria (cyanotoxins and off-flavours), polyphenols, such as terpenes, humic and fulvic acids fall mainly under the second one. Interestingly, recent researches demonstrate that photocatalysis and/or ozonation are able to degrade these kinds of compounds [16-28]. Filtration, protein skimming (foam fractionation) and other physical purification methods, along with biological depuration, are the traditional depuration methods as they provide a reasonable purification degree with low operating costs and acceptable side effects. Nevertheless, these methods can be inadequate for the purification levels required in RAS, because they do not eliminate at an adequate rate fine colloidal solids, pathogenic microorganisms and bio-recalcitrant compounds, which in many cases are noxious or detrimental even at low concentration.

Furthermore, the purification processes must minimize the production of unsafe by-products and ensure cost effectiveness and operational simplicity.

Advanced oxidation processes (AOPs), such as photocatalysis (PC) and ozonation (OZ), are promising tools for water purification.

PC is a relatively new technology based on the ability of semiconductors, under light irradiation of suitable wavelength, to generate hydroxyl radicals or active agents, which in turn allow the mineralization of organic compounds and water sterilization [29-32].

OZ is applied in aquaculture [7,16-21], maintenance of aquaria [33,34], and water potabilization plants [35,36], thanks to the sterilization and purification capabilities.

One of the main drawbacks of ozonation is the induced oxidation of bromide to bromate ions, which are the ozonation by-products of main concern (Eq. 1).



On the contrary, in presence of bromide ions, PC does not induce the formation of bromate ions. In fact, the oxidation of bromide by the photocatalytically formed $\cdot\text{OH}$ radicals (Eq. 2-4) produces intermediates such as hypobromite ions which are reconverted to bromide by photogenerated electrons (e^-) according to Eq. 5.



When performed concurrently with ozonation, PC reduces the formed bromate ions to innocuous bromide ions (Eq. 6) at a rate that increases with the concentration of oxidizable compounds (i.e. organic matter) [37,38].



This aspect is of great importance because ozonation of bromide ions (Eq. 5) would produce unacceptable amounts of bromate if carried out without an effective control of their formation. On the other hand, bromide ions are ubiquitous in ground and surface water. In seawater bromide concentration is relatively high (ca. $67 \text{ mg}\cdot\text{L}^{-1}$) [39] and consequently the amount of bromate ions produced may be deleterious for the living aquatic organisms [40] by considering for instance that the suggested exposure safety value to protect aquatic organisms from long term adverse effects is $3 \text{ mg}\cdot\text{L}^{-1}$ [40]. By the way, the United States Environmental Protection Agency (USEPA) and the Drinking Water Commission of the European Union adopt a maximum bromate level in drinking water of $10 \mu\text{g}\cdot\text{L}^{-1}$ [41]. Indeed, the attention on bromate formation by ozonation is so high that some manufacturers of artificial sea salts for aquaria, such as Aquaforest, warn that “The salt contains bromides that are found in natural marine water, so it is not recommended for use in the ozone supported filtration systems.” However, bromide is present as impurity also in the artificial sea salts that do not explicitly include it in their formulation [34] and, on the other hand, bromide seems to be necessary for the health of some marine organisms.

Recently, it has been observed [36,37,42-45] that the rate of purification in photocatalytic ozonation, PO, (i.e. PC and OZ acting concurrently) is higher than the sum of the rates of the single processes, with potential advantages in terms of global efficiency and operating costs for practical applications. In the present work, the potentialities of photocatalytic ozonation in a recirculating system have been explored in a long-term test. In a prolonged experiment, it is possible also to verify the constancy of the depuration performances, to establish the possible delayed onset of negative phenomena and to tune more accurately the operating conditions. To this aim, photocatalytic ozonation and photocatalysis have been continuously applied on a laboratory scale for some years in a coral reef

aquarium with recirculation of the treated water. This system mimics on a small scale a RAS and represents a meaningful test because of (i) the negligible amount of new water, which has been adopted, (ii) the presence of a diversified population of living organisms (corals, fish, molluscs, echinoderms and crustaceans) which are particularly sensitive to water quality, (iii) the relatively high organic load of the system, (iv) the high salinity which strongly puts a strain on the purification system.

2. Materials and Methods

A coral reef aquarium has been utilized in the present study. It has been stocked with a population of soft and hard corals, fishes, starfishes, molluscs and other small marine organisms (see Table S1 of the supplementary material for a list of the organisms), 5 kg of cultured “live rocks” [46] and a 5 cm thick bottom of fine coral sand. Fish are daily fed with: 5 g of frozen brine shrimp (*Artemia salina*), 5 g of frozen krill (*Euphasia pacifica*) or 5 g of frozen lobster eggs (all by Ocean Nutrition), and approximately 1.5 g of a mixing of various dry food for marine aquaria (Formula 1 Marine pellets by Ocean Nutrition, SV1 by Elos and Premium Marine by SHG).

“Artificial” seawater has been prepared by dissolving a commercial mixture of sea salts (Reef Crystal by Aquarium System) in demineralised water to obtain a salinity of $34 \text{ g} \cdot \text{L}^{-1}$.

The system is schematically shown in Fig. 1.

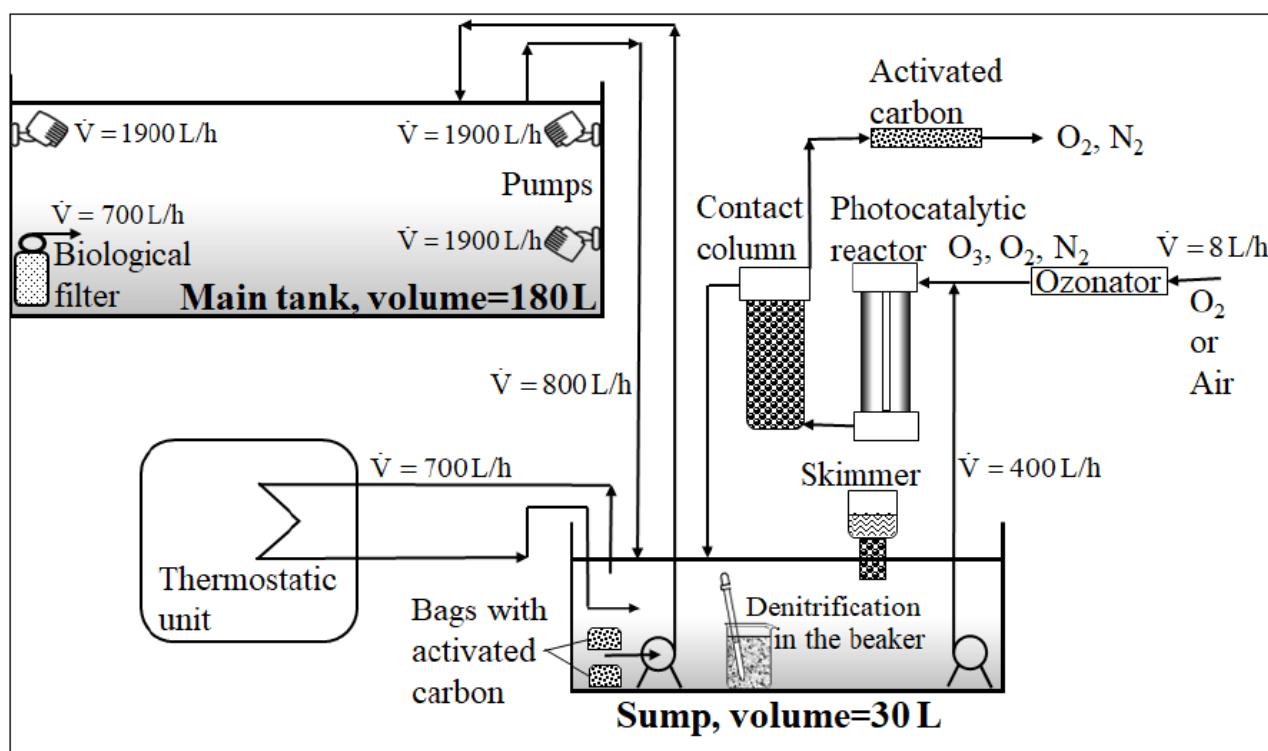


Fig. 1. Scheme of the system.

Water was continuously discharged from the 180 L aquarium tank (main tank) to a 30 L sump where it was treated and recycled to the tank by means of an immersed centrifugal pump (flow rate equal to ca. $800 \text{ L} \cdot \text{h}^{-1}$). The water temperature was maintained in the $25\text{--}27^\circ \text{C}$ range by a thermostatic unit (Teco TK500) which recirculates the water in the sump at $700 \text{ L} \cdot \text{h}^{-1}$. Effective mixing in the main tank was obtained using three circulation pumps with a flow rate of $1900 \text{ L} \cdot \text{h}^{-1}$ each. Effective mixing in the sump was guaranteed by the flow of the water exchanged with the main tank ($800 \text{ L} \cdot \text{h}^{-1}$), with the photocatalytic reactor ($400 \text{ L} \cdot \text{h}^{-1}$) and with the thermostatic unit ($700 \text{ L} \cdot \text{h}^{-1}$).

The following elements were used for water purification:

- Protein skimmer (Tunze skimmer 9002)
- Biological filter (Askoll PH 402 pump, flow rate $700 \text{ L} \cdot \text{h}^{-1}$, with a canister containing approximately 400 mL of synthetic sponge for biochemical depuration)
- A basic denitrification device (a 1 L beaker immersed in the water of the sump filled with fine coral sand and fed at the bottom with $5 \text{ mL} \cdot \text{d}^{-1}$ of an ethyl alcohol, 60 % vol., - acetic acid, 40 % vol., mixture)
- Adsorption on activated carbon (two filter bags filled with 800 g of Carbolit by Aqua Medic were positioned close to the suction of the pump that returns water from the sump to the main tank. Note that these bags were never replaced.)
- Photocatalytic reactor
- Ozonator

The photocatalytic reactor (see Fig. 2 for a picture of the reactor) consisted of a fixed bed of packed glass beads, covered by a TiO_2 film, in the space between two coaxial borosilicate tubes. The dimensions of the annular space, where the illuminated glass beads are present, are: inner diameter = 24 mm, outer diameter = 47 mm and length = 265 mm. A linear fluorescent blacklight lamp (Philips 8 W TL/08) is placed on the axis of the reactor emitting UVA radiation from 350 to 400 nm with 82% of the emitted radiant power in the range 360 to 380 nm and with an emission peak at 365 nm. A radiation intensity of about $24 \text{ W} \cdot \text{m}^{-2}$ was measured by a Delta Ohm HD9021 radiometer with a LP9021 UVA probe at the inner radius of the annulus. The transmitted radiant intensity at the external of the outer tube was $0.2 \text{ W} \cdot \text{m}^{-2}$. The titania film on the glass beads (diameter 4 mm) was prepared according to the following procedure. All the chemicals were used for the deposition of the film as received: titanium tetraisopropoxide (TTIP) (97%, Sigma-Aldrich), 2-propanol (2-PrOH) (99%, Aldrich), diethanolamine (DEA) (99%, Riedel de Haën), TiO_2 P25 Evonik Aeroxide, TiO_2 Hombikat UV 100. Initially 60 g of TTIP and DEA in molar ratio 1:4 were dissolved in 270 g of 2-PrOH. This solution was stirred for 2 h at room temperature. Thereafter demineralized water was slowly added to reach a molar ratio $(\text{H}_2\text{O})/(\text{TTIP}) = 2$ in order to induce hydrolysis of the titanium oxide precursor. Equal amounts ($20 \text{ g} \cdot \text{L}^{-1}$) of P25 Aeroxide and of Hombikat TiO_2 were added to the solution and the resulting suspension was ultrasonicated for 15 minutes and vigorously stirred during 12 h. This solution with TiO_2 powders in suspension was utilized for the sol-gel deposition of all the six TiO_2 layers, which compose the film. Before the deposition of the first layer, the glass beads (650 g) were kept for 24 h in a 8% v/v HF solution, rinsed with distilled water and dried. This chemical attack of the glass by the acid makes rougher the surfaces of the beads and allows for a stronger adhesion of the film. For the deposition of each layer the beads were dipped into 500 mL of the suspension for 30 minutes. Then they were let to drain the solution in excess for 2 min and placed in a Pyrex tube at 150°C for 2.5 h in the presence of a slow air flow. The last step of the formation of a layer was the calcination at 550°C for 3.5 h. This procedure was repeated for each layer in order to increase the total thickness of the film and to increase its mechanical stability. It was observed that with six layers the rate of photocatalytic mineralization of formic acid (tested as described at the end of the present section) was almost equal to the one obtained with 5 layers, so no additional layers were added, because no improvement of the rate is expected [47].

The resulting thickness of the film ($13 \mu\text{m}$) was measured by means of a SEM-EDAX mapping (FEI ESEM Quanta 200 FEG microscope equipped with an EDAX probe) carried out on samples prepared by embedding the beads in a polystyrene resin and then smoothing in order to evidence the beads cross section.

A peristaltic pump, activated by a level controller, replaces with “kalkwasser” (a saturated solution of CaOH in demineralized water) the water, which evaporates from the main tank (about $2.5 \text{ L} \cdot \text{d}^{-1}$). In this way the concentration of the calcium ions, which are consumed by the growth of hard corals, is maintained at a suitable level. Oligoelements useful for the growth of corals (Marin trace elements by Sera including: Mo, B, I, Br, F, V, Sr, Ni, Fe, Mn, Cu, Co, Cr, Rb and Zn) are weekly supplemented according to the instructions of the manufacturer. Before starting the PC and PO tests, 5 % of the water was changed every two weeks, while the water changes were reduced to 1 %

every three months during the tests.

Water is pumped by a centrifugal pump from the sump through a synthetic sponge filter (Eheim prefilter 4004320) and enters the photocatalytic reactor with a flow rate of $400 \text{ L}\cdot\text{h}^{-1}$. A picture of the photocatalytic fixed bed reactor is displayed in Fig. 2.

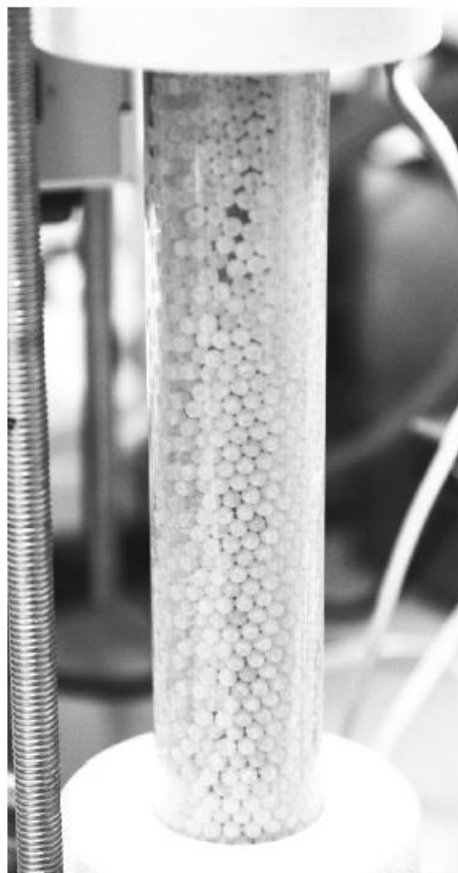


Fig. 2. External view of the photocatalytic reactor with the fixed bed of glass beads covered by the photocatalytic TiO_2 film.

For the production of ozone, pure oxygen with a flow rate of $8 \text{ L}\cdot\text{h}^{-1}$ (at 25°C and atmospheric pressure) is fed to a corona discharge ozonator (Certizon C100 by Sanders), which supplies about 90 mg/h of ozone. The resulting gaseous mixture (ozone and oxygen) is continuously injected through a T joint into the tube, which feeds the photocatalytic reactor (see Fig. S1 of the supplementary material). So, inside the reactor, the gaseous mixture is present in the form of dispersed bubbles, which are entrained by the flowing water and come in contact with the photocatalyst in the fixed bed. In this way, when the ozonator is turned on, photocatalysis and ozonation act concurrently (PO) inside the reactor. In order to complete the dissolution of the residual ozone, the water that leaves the reactor, passes through a needle wheel impeller pump (see Fig. S2 of the supplementary material), which creates a mixture of fine bubbles (estimated average bubble size = 0.4 mm) inside a bubble contact column (height 20.5 cm , diameter 8 cm , with no packing materials) before returning to the sump. The gas exiting the contact column passes through a tube containing 100 g of activated carbon in pellets before being conveyed outside the laboratory. Video S1 in the supplementary material shows the reactor and the contact column in operation. After a series of preliminary tests, it was chosen to operate photocatalysis continuously, whereas

photocatalytic ozonation only for 90 min per day. In the rest of the time, i.e. during the shutdown of the ozonator, air is injected into the photocatalytic reactor in substitution of pure oxygen.

Water quality was assessed through the evaluation of different properties. The oxidation reduction potential (ORP) and the pH were detected by means of a Hanna Instruments HI3220 pH/ORP meter. Samples of the water in the main tank and in the sump were collected at a depth of about 10 cm. Anyhow, the values of any water parameter were practically uniform inside the main tank and inside the sump thanks to the very effective mixing in both of them. Water hardness and calcium concentration were measured by Salifert test kits. Hanna HI 83200 was utilized to measure ammonia, nitrites, nitrates and free bromine. Ozone concentration in the liquid phase was estimated by measuring absorbance at 258 nm by means of a Shimadzu UV-1601 spectrophotometer. Bromate concentration was measured according to the method reported by Brookman et al. [48]. The concentration of total organic carbon (TOC) was measured by means of a Shimadzu TOC 5000A analyzer. Absorbance at 254 nm was regularly measured by the Shimadzu spectrophotometer, as an indication of the amount of dissolved organic compounds (DOC) accordingly to relevant literature [49-51]. It was verified that the absorbance at 254 nm correlates satisfactorily with the concentration of TOC (see Fig. S3 of the supplementary material). On the contrary, the exponential variation of the absorbance with the wavelength in the 250-360 nm range observed in a natural seawater [52] does not hold for the water treated by photocatalytic ozonation (see Fig. S4 in the supplementary material).

The photocatalytic activity of the fixed bed and some blank experiments were carried out with the same photocatalytic reactor, but in a separate plant. The artificial seawater (1 L) containing formic acid (98%, Sigma-Aldrich) and/or potassium bromate (p.a., Sigma-Aldrich) was recirculated by means of a peristaltic pump (flow rate $2 \text{ L} \cdot \text{min}^{-1}$) through the photocatalytic reactor. The solution was kept recirculating during 30 minutes in the dark to ensure adsorption equilibrium conditions before turning on the lamp. Samples were taken at fixed intervals of times. Formic acid concentration was measured by means of the TOC analyzer.

3. Results and discussion

The effects of photocatalysis and photocatalytic ozonation on the quality of water of the aquarium have been monitored in a quite long period during which the single technologies were in turn applied. Initially, the seawater aquarium operated without photocatalysis and ozonation for 11 months. In the first 5 months only rocks and coral sand were introduced. Then, along the following six months the fish and the corals were progressively added (see the list of the final population in the supplementary material). This usual procedure of “tank maturation” allows to approach at some extent a biological equilibrium with a sufficiently numerous and diversified bacterial population. Some operating conditions were established by a trial and error approach. For instance, it was decided to utilize pure oxygen instead of air when the ozonator is switched on, because it was observed that in this way the production of ozone is much more efficient, there is no need of dehumidifying the gas stream and the possibility of nitrate formation from gaseous nitrogen is excluded [38]. On the other hand, it was observed that a gaseous stream (air or oxygen) should be maintained all the time in the fixed bed to avoid fouling of the photocatalytic film. After some preliminary tests, it was established to operate photocatalytic ozonation only for 90 min per day from 2 p.m. to 3.30 p.m. whereas photocatalysis alone was applied in the rest of the whole time. In this way, it was possible to obtain adequate purification rates while avoiding the build-up of bromate ion. Indeed, it is known that, in the case that some amount of bromate ions are produced, photocatalytically generated electrons are able to reduce them to innocuous bromide ions (Eq. 5-6). Actually, photocatalytic ozonation does not produce bromate if organic compounds are present [37,38], as it is usually the case of aquarium water, but the possible formation of small amounts of bromate could not be excluded inside the reactor. Furthermore, photocatalytic ozonation affords purification yields higher than the ones obtained with the two processes working in series [38].

Typical results obtained operating with the different processes are reported in Fig. 3.

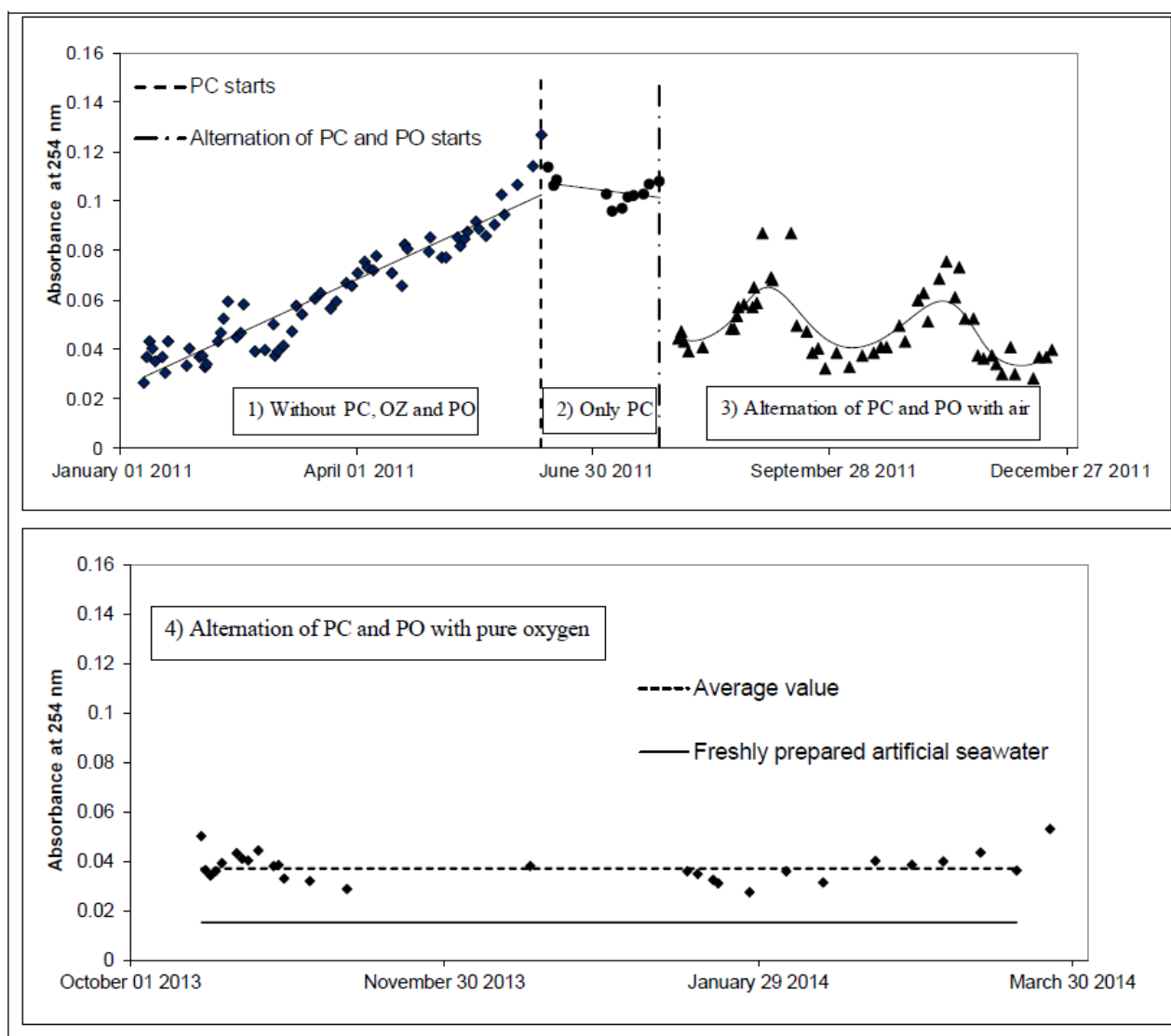


Fig. 3. Absorbance of the water in the main tank at 254 nm vs. time. 1) without PC, OZ and PO; 2) Only PC; 3) PO with dehumidified air (90 min every day) and PC for the rest of the day; 4) as in 3 except that pure oxygen is used for PO instead of air.

It is possible to observe that in the absence of PC or PO (see region 1 of Fig. 3) accumulation of organic compounds occurred. Indeed, the absorbance at 254 nm increased in the first period of observation and a yellowish appearance became progressively evident. The presence of “gelbstoff” in aquarium or aquaculture water is a well-known negative phenomenon [53] caused by the formation or introduction of humic and fulvic acids and other persistent organic compounds that neither skimming [54] nor biological treatment are able to remove. These persistent compounds would include also noxious and potentially hazardous compounds [12,55,56]. Adsorption on activated carbon could reduce their presence, but, at the time of the beginning of the monitoring of the absorbance, it is likely that the activated carbon was already saturated since it was introduced 5 months before and never changed. In fact, in absence of PC or PO the absorbance at 254 nm increases almost linearly with time (region 1 of Fig. 3). Conversely, when PC or PO were applied the transparency and the absence of colours were recovered, indicating that both PC and especially

PO were able to limit or reduce the concentration of dissolved organics (regions 2 and 3 of Fig. 3) and to avoid their accumulation. Furthermore, it is clear that when PO was carried out with pure oxygen (region 4) the concentration of the dissolved organics of concern was even a bit lower and much more stable. With pure oxygen the absorbance is only slightly higher than the one measured in the artificial seawater freshly prepared using demineralized water.

Notably, during the period when ozonation was carried out, no fouling of the photocatalytic fixed bed was observed because ozone is able to oxidize the organic matter adsorbed on the TiO_2 film and to restore its active sites.

The rate at which TOC is degraded during PO can be appreciated from Fig. 4.

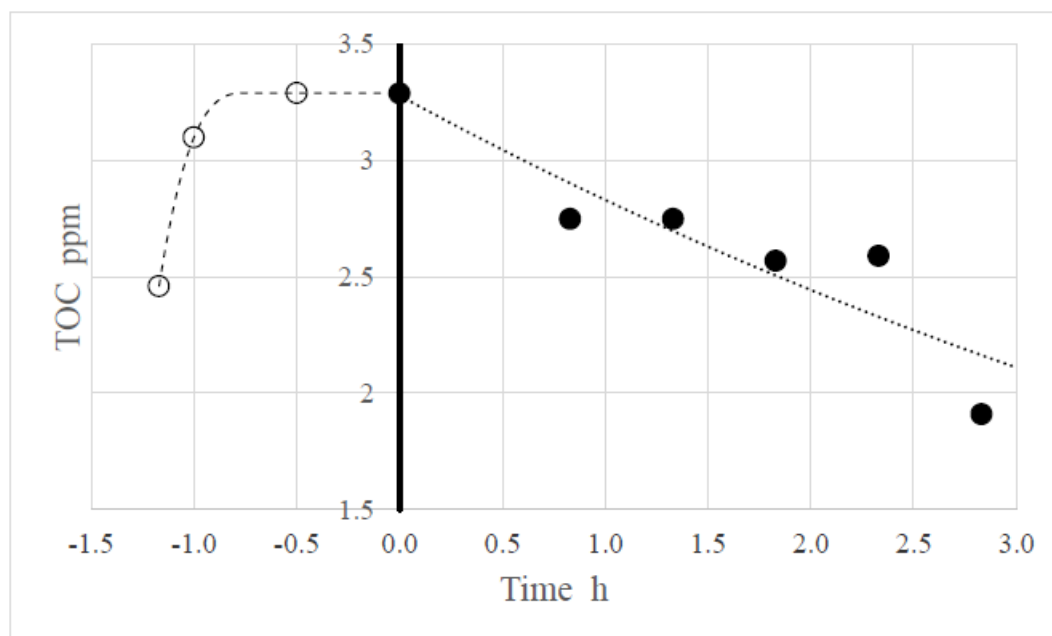


Fig. 4. Time progression of TOC concentration in the aquarium (main tank) after feeding (empty symbols) at 9 a.m. followed by PO (filled symbols), which was turned on at 10:20 a.m. (time =0).

In Fig. 4 an initial increase of the TOC concentration after feeding is observed towards an almost constant value, followed by a decay, which starts when PO is turned on. Even with the adopted small photocatalytic reactor and the relatively low rate of ozone production, PO is able to destroy more than 100 mg/h of TOC in the investigated operating conditions with an estimated TOC half-life of 4.7 h.

Photocatalysis produces hydroxyl radicals, which have a very short life [57], and, therefore, cannot exit the reactor. On the contrary, during PO it is possible that some oxidizing agents, produced by ozonation, arrive to the sump. If they arrive also to the main tank, they could cause problems to the living organisms. During ozonation the ORP, which increases when oxidizing agents are present, is usually monitored to interrupt ozonation if its value exceeds a safe level [17]. The progression of ORP in the sump and in the main tank after the start of PO is shown in Fig. 5.

The main possible oxidizing agents are the same ozone and free bromine. Ozone concentration was found to be below detectable values both in the main tank and in the sump. In fact, the values of the absorbance at 258 nm of samples collected from the sump or from the tank after 90 min of PO remained stable when continuously measured from 5 s after the collection (the minimum time necessary to carry out the spectrophotometric measurement) to 120 s (a time at which all ozone is surely decayed). It is likely that ozone is completely decomposed inside the photocatalytic reactor and inside the bubble contact column. On the other hand, it is known that the kinetics of ozone

decay in seawater (i.e. at relatively high values of pH and salinity) is fast [58] with a typical halflife of just 5 s [59]. Also the mass balances of ozone in the sump and in the main tank confirm that with such a fast kinetics ozone can be present only in traces in the sump and completely absent in the main tank. Moreover, the mass balance in the sump demonstrates that an asymptotic very low value of its concentration in the sump is approached in less than 1 min, a time which is not compatible with the observed transient behaviour of the ORP, which exhibits a much longer time for the built-up of the oxidizing agents in the sump (see Fig. 5).

Free bromine (mostly as hypobromous acid) increases till 2 ppm (as Br₂) in the sump and to 0.04 ppm in the main tank after 90 min of PO, but 15-20 min after the termination of PO free bromine vanishes from the whole system.

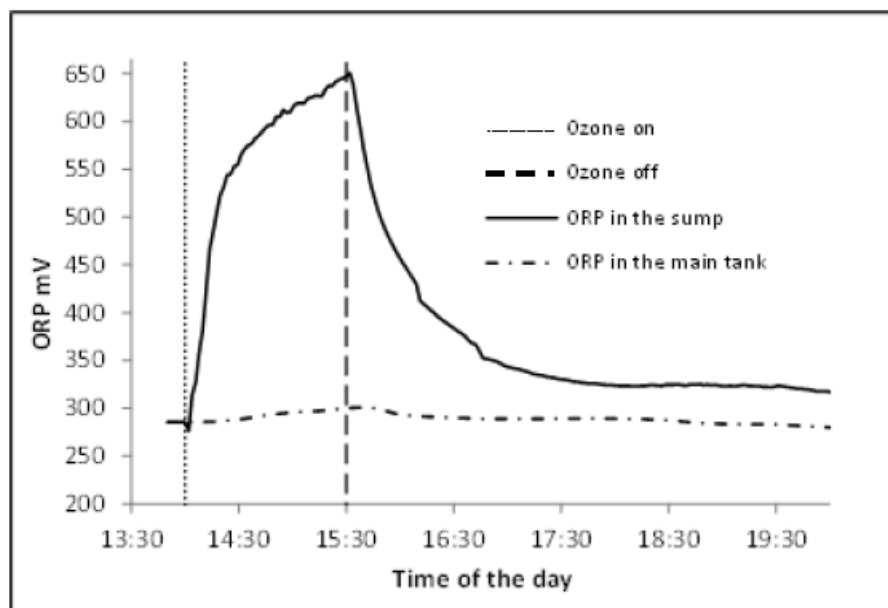


Fig. 5. ORP vs. time with photocatalytic ozonation acting from 2:00 p.m. to 3:30 p.m.

As previously discussed, the observed increase of the ORP should be mainly related to the increase of free bromine and it is much higher in the sump than in the main tank. This difference is probably due also to the adsorption of free bromine onto the activated carbon positioned in the sump close to the suction of the recirculation pump and the subsequent reaction with adsorbed organic compounds [60]. In this way, only a small fraction of free bromine can pass to the main tank with the water recirculated from the sump. On the other hand, it was observed that fishes in the main tank do not show signs of distress, such as accelerated breathing, if PO lasts less than 100 min.

Fig. 6 shows the results obtained in another test in which ORP and bromate ions concentration were measured in the sump. Only OZ was applied in the first 180 minutes, then the ozonator was turned off and the lamp of the photocatalytic reactor was switched on.

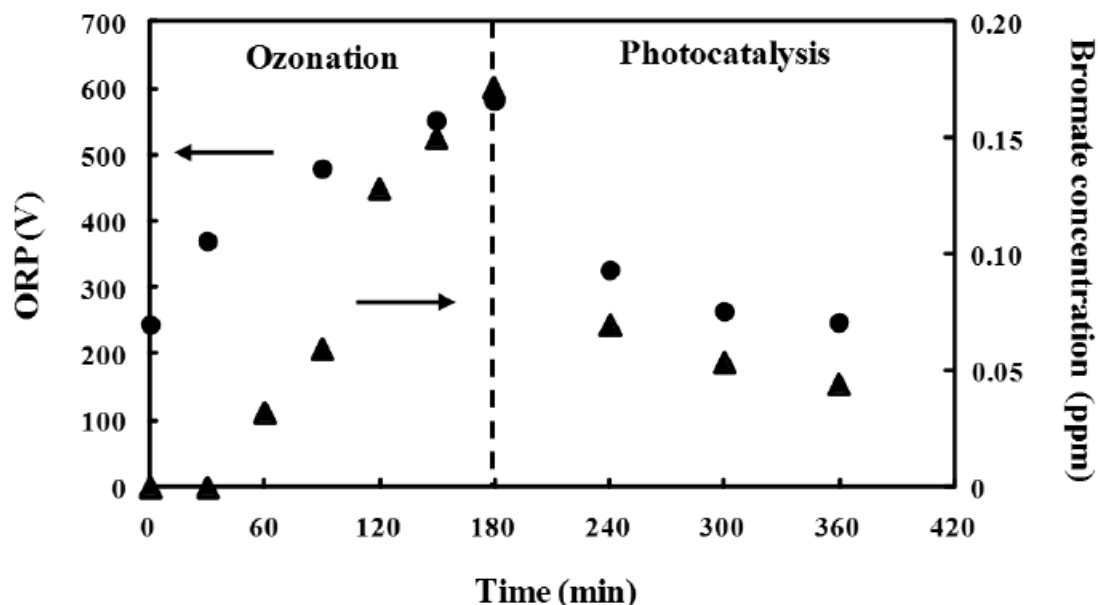


Fig. 6. ORP values (circles) and bromate concentration (triangles) vs. reaction time in the sump for a run with ozonation and photocatalysis applied in sequence.

In the presence of ozone without PC both the bromate concentration and the ORP values increased. On the other hand, when ozonator was switched off and photocatalysis was applied both values quickly decreased.

However, in the experimental conditions used it is possible that the decrease, that was observed when the ozonator was turned off, was also a consequence of the dilution in the sump caused by the water continuously spilled from the main tank, where the bromate remained at low values.

Therefore, additional experiments were performed in a separate configuration with smaller flow rates and lower effluent volumes as described at the end of the previous section. In detail, 1 L of a seawater, containing ca. 50 ppm and 18 ppm of formic acid (as a model organic pollutant) and of bromate ions, respectively, was recirculated in the photocatalytic reactor. The concentration of formic acid and of bromate ions is reported versus time in Fig. 7.

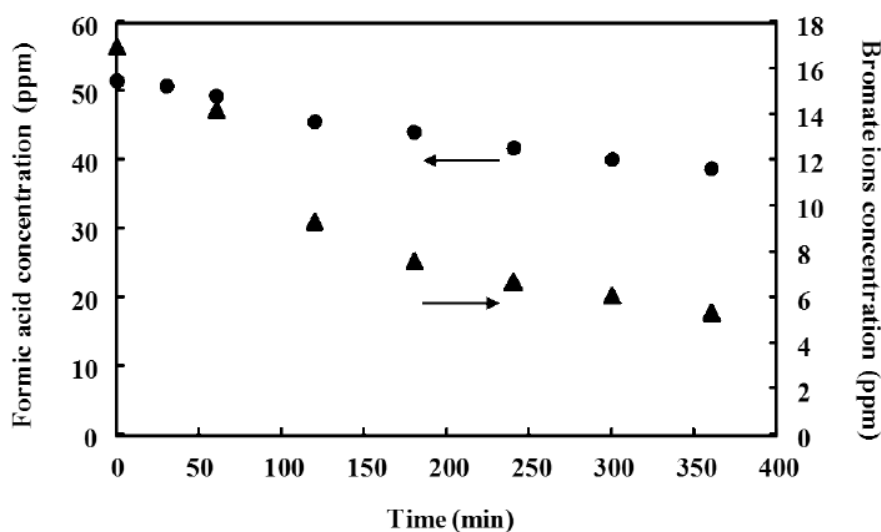


Fig. 7. Formic acid (circles) and bromate ion (triangles) concentrations for a photocatalytic run carried out on a 1 L seawater solution.

It is apparent that the sole PC gives an efficient bromate reduction and a decrease of the concentration of the organic compound. This demonstrates that the utilization of photocatalysis enables efficient bromate reduction even in the real conditions of a coral reef aquarium. The values of all the water parameters (Ca, Mg, ammonia, NO₂, NO₃, salinity, KH, pH in Table 1 and in Fig. S3 of the supplementary material) measured in the main tank are in the recommended range for healthy seawater aquarium and the utilization of PC and PO had no negative side effects on them.

Table 1. Values of various water parameters measured in the tank during fifteen months.
Parameter Units Average value Standard deviation

Parameter	Units	Average value	Standard deviation
Salinity	g·L ⁻¹	34	± 1.2
Calcium	mg·L ⁻¹	462	± 20
Magnesium	mg·L ⁻¹	1245	± 68
Ammonia and nitrite	--	Below detectable limits	--
Nitrate	mg·L ⁻¹	1.3	± 1.0
Carbonate hardness	dKH	7.4	± 1.5
pH	--	8.1	± 0.4

Furthermore, it was observed that PC and PO contribute also to accelerate the nitrification process and it is likely that the abatement of organic acids by these oxidation processes promote the stability of pH and alkalinity.

The decay of the performances of the photocatalyst was assessed by comparing the initial rate of degradation of formic acid with the one after 18 months of usage. The photocatalytic activity of the photocatalyst resulted to be ca. 60% of the initial one.

Fig. 8 shows SEM images of the TiO₂ film covering the glass beads after 18 months of usage along with their EDAX mapping. Calcium (green points) and silicon (red points) atoms are present in the glass beads whereas the layer on their top presents, as expected, mainly titanium atoms (blue points) as shown in the insert A. The SEM image (Fig. 8B) shows a ca. 13 µm thick TiO₂ film covering the glass bead. It is worth noting that after 18 months this thickness of the film is the same measured before the first utilization. Traces of other elements are present in the TiO₂ layer such as calcium, magnesium, iron, zinc, sodium, chlorine, potassium and aluminium (Fig. 8C) mainly deriving from the artificial seawater.

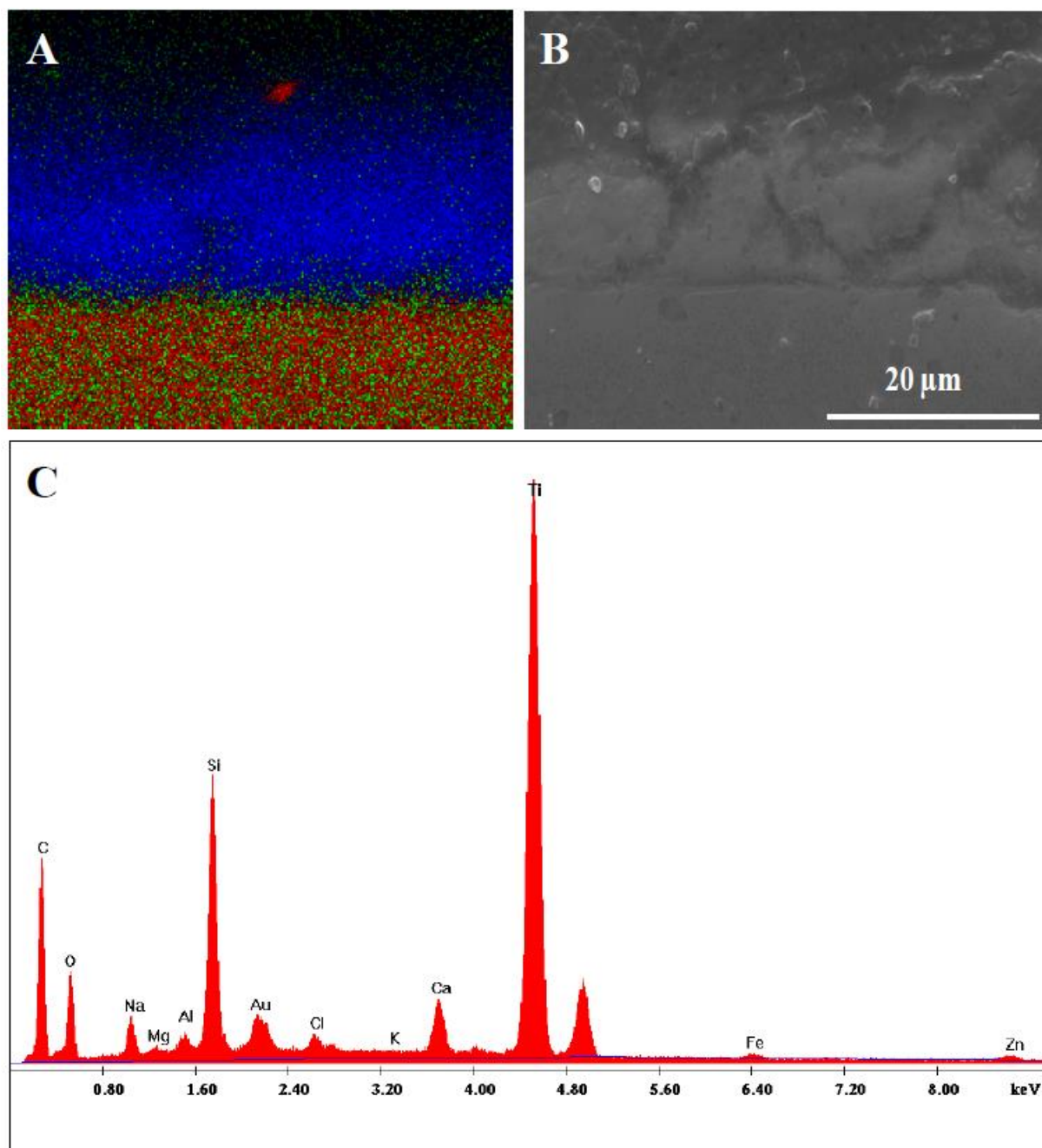


Fig. 8. SEM-EDAX analysis of the glass beads with TiO₂ film used in the fixed bed of the reactor. A: mapping of titanium (blue points), silicon (red points) and calcium (green points) atoms; B: SEM image of a portion of TiO₂ covered bead; C: elements present in the region shown in B.

Notably, the activity of the photocatalytic bed was completely restored after washing with a diluted HCl (5 % vol) aqueous solution followed by 2 h of OZ in demineralized water.

Finally, the living organisms in the aquarium never showed any sign of distress during the years of experimentation with PC and PO, whereas limited growth of the corals and some anomalous behaviours of the fishes, such as loss of appetite, were observed when PC and PO were not utilized and, without adequate water changes, the “gelbstoff” accumulated. On the contrary, during the utilization of PC and PO fishes were always healthy and corals appeared with expanded polyps for most of the time. In particular, the mixing of soft and hard corals was not problematic, even if

separate tanks are usually suggested [61] because of the emission of allelopathic persistent substances [12,55,56], which are hazardous for unrelated species. Actually, the control of such chemicals by PO enabled satisfactory growth rates of fishes and corals (see e.g. Figs. S6 and S7 in the supplementary material, where the growth of corals is apparent).

On the basis of the results obtained at the laboratory scale it is premature to make an economic evaluation for an industrial application of PO in aquaculture, but it is possible to make some preliminary observations. As observed in the introduction, ozonation is increasingly applied in RAS and a preliminary cost-benefit analysis of PO may start from the comparison with the sole ozonation process. If ozone is already utilized in an aquaculture plant the coupling with photocatalysis for PO would infer only limited additional costs. In fact, the capital costs related to the photocatalytic material and the reactor are small, because titanium dioxide is cheap and long lasting and the apparatus is simple and works at mild conditions. Moreover, the same photocatalytic reactor is used to effectively dissolve and utilize ozone and PC is an advanced oxidation technology which requires minimum labour. The electricity for the light source is the principal operating cost, however it is worth noting that the maximum synergy between ozonation and photocatalysis should take place at a rate of photocatalysis which is just a small fraction of the rate of ozonation [38]. This feature contributes to keep the costs low. Moreover, it is possible to activate the photocatalytic reaction using the solar radiation, thus further reducing the costs for the energy. On the other hand, the expected benefits are significant: a higher rate of purification with a more efficient utilization of the produced ozone and the possibility to ensure a safer environment in a RAS by controlling the presence of bromate. These advantages might make convenient the utilization of ozonation also in cases where currently, without photocatalysis, it is not.

4. Conclusions

The adopted process, which couples ozonation with photocatalysis (photocatalytic ozonation), was able to preserve a high quality of the water in a coral reef aquarium even with a very limited water exchange and without the substitution of the photocatalytic material. The same quality was not obtained in absence of photocatalytic ozonation due to the accumulation of biorecalcitrant dissolved organic compounds. A careful organization of the ozonation cycles avoided the build-up of bromate, a toxic disinfection by-product of major concern. Moreover, adopting these periodic ozonation treatments no adverse effects have been observed on the aquarium population, which was constituted by different fishes and invertebrates. The coupling of ozonation with photocatalysis prevents also the fouling and the poisoning of the photocatalytic material, which only after several months of utilization shows some activity decay. Anyway, a simple procedure was established for restoring the original photocatalytic activity of the deposited film, whose mechanical resistance too was adequate for a long utilization in a fixed bed.

On the basis of the obtained results, it can be stated that the adopted system of water purification is promising also for recirculating aquaculture systems. The benefits can be important and justify the low incremental costs which are expected with respect to an ozonation process. For instance, the rate of depuration is higher than the sum of the rates of the individual processes and there is the possibility of controlling bromate, the ozonation by-product of main concern. Moreover, photocatalytic ozonation is simple to operate and photocatalysis is a modular process, which infers flexibility and straightforward scale up. Therefore, it would be worth continuing the investigation to confirm the present results also on a larger scale while extending the study also to the effects of PO on the disinfection of the water in the recirculating system, because it is expected that PO can be beneficial also for the destruction of harmful microorganisms.

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SUPPLEMENTARY INFORMATION

Photocatalytic ozonation for a sustainable aquaculture: a long-term test in a seawater aquarium

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Table S1. List of population of the aquarium

Fishes	Corals	Other invertebrates introduced with the live rocks
2 <i>Acreichthys tomentosus</i>	<i>Sinularia</i> sp.	Amphipods
2 <i>Grama loreto</i>	<i>Sarcophyton</i> sp.	Sponges
1 <i>Centropyge eibli</i>	<i>Seriatopora hystrix</i>	Starfish
1 <i>Synchiropus splendidus</i>	<i>Pocillopora</i> sp.	Snails
2 <i>Gobiodon okinawae</i>		Polychaete worms



Figure S1. Picture of the zone where air is injected into the tube that feeds water to the photocatalytic reactor.



Figure S2. Picture of the needle wheel impeller used to produce the bubbles inside the bubble contact column.

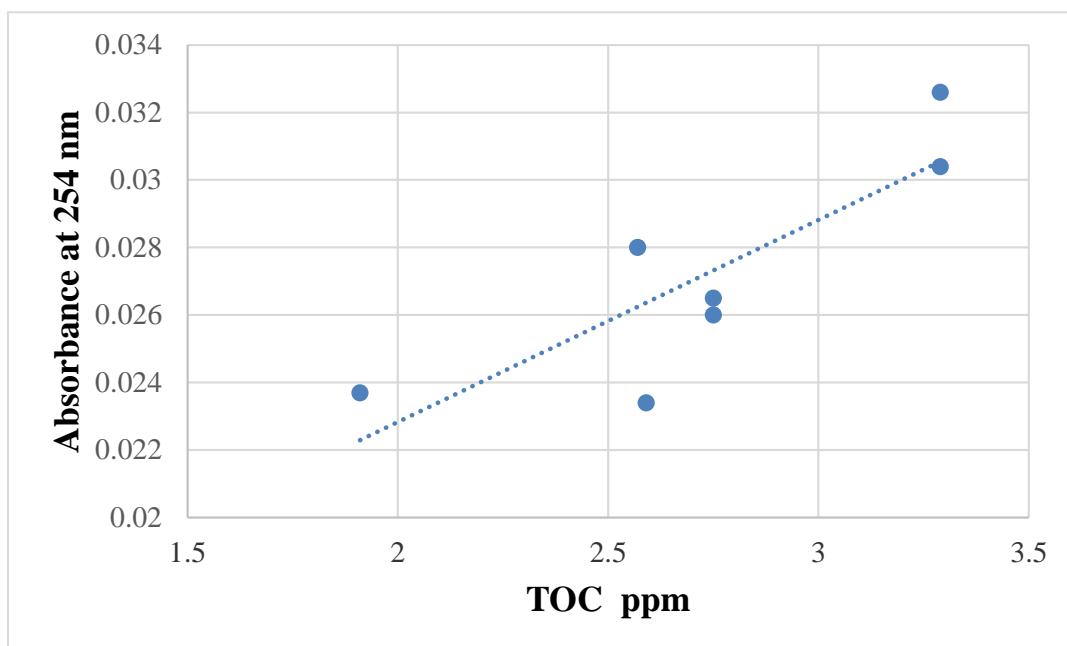


Figure S3. Correlation between absorbance at 254 nm and TOC in the aquarium seawater.

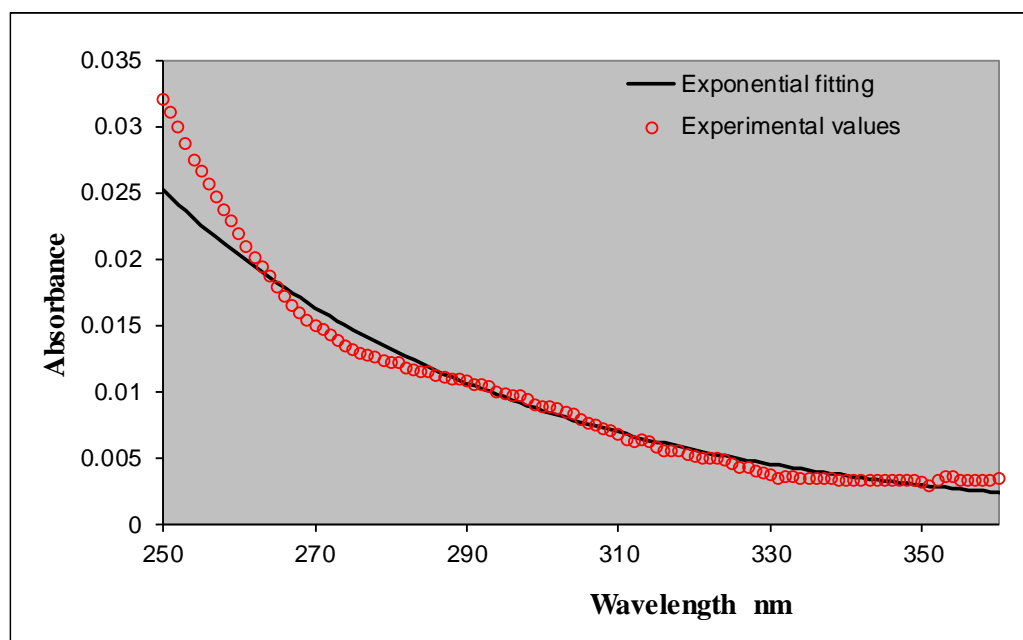


Figure S4. Typical behavior (January, 27th 2014, 8 a.m.) of the absorbance in the range 250-360 nm of the water in the main tank, compared with the best exponential fit of the data. It is evident that the absorbance vs. wavelength does not follows the exponential law usually observed in natural seawater.

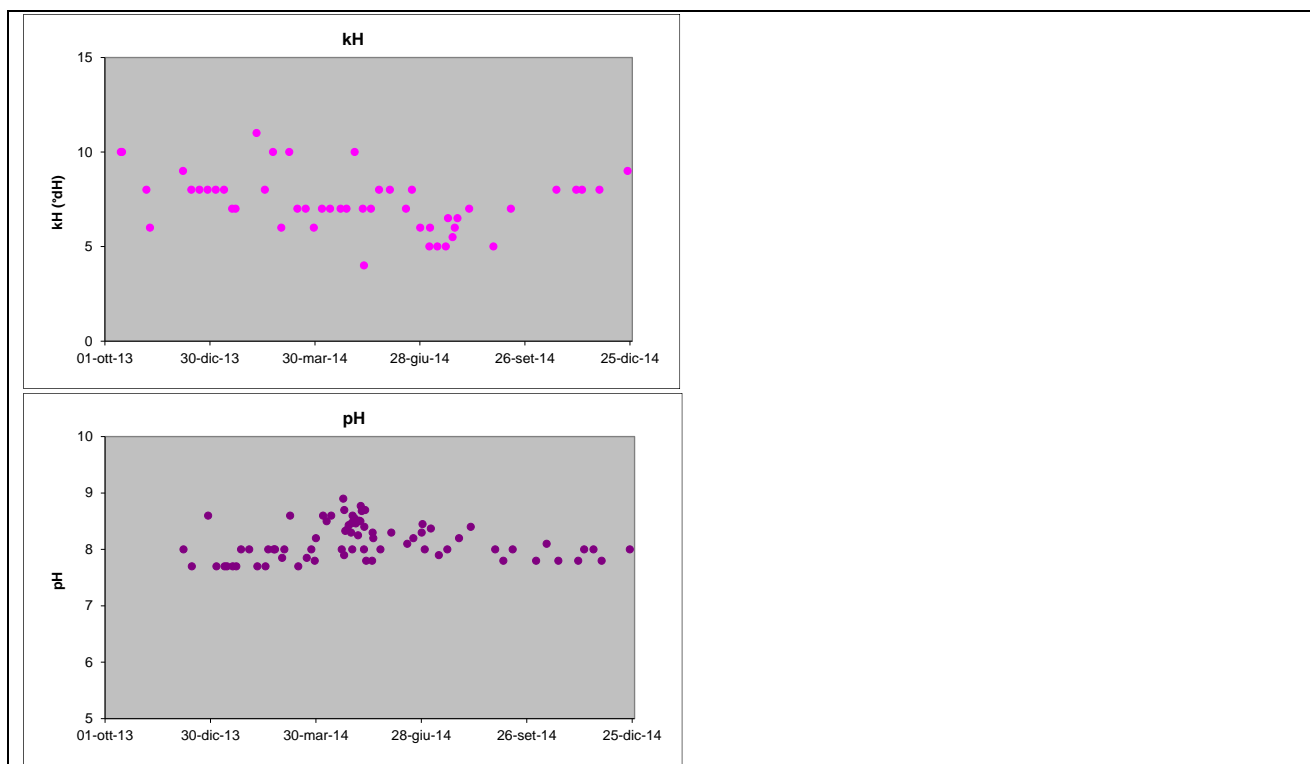


Figure S5. Some parameters, which assess water quality: Calcium, Magnesium, Nitrate, Salinity, KH, and pH vs. time in the main tank.



Figure S6. Pictures of the aquarium before and after three years of purification with photocatalysis and photocatalytic ozonation. It can be noticed the growth of the hard and soft corals and the disappearance of most of cyanobacteria from the rear wall.

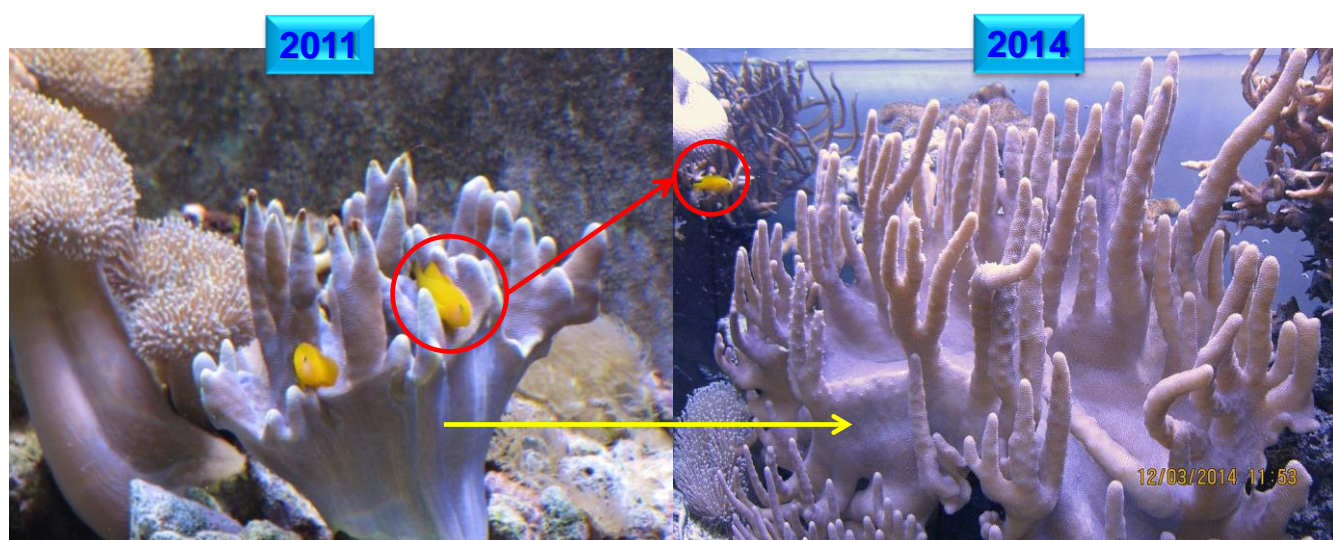
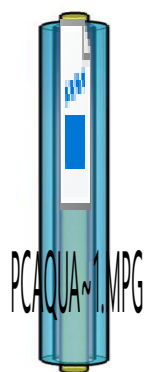


Figure S7. Pictures of some details of the aquarium before and after three years of purification with photocatalysis and photocatalytic ozonation. The growth of the soft coral (*Sinularia* sp.) is evident.



Video S1. Video showing the photocatalytic reactor (0-10 s) and the contact column (11-18 s) in operation.