Intensified Heterogeneous AOPs for the disinfection of *Enterococcus faecalis* in aquaculture water matrices

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Recirculating aquaculture systems (RAS) have risen as an interesting approach towards large-scale fish production. The presence of microbes in fish-inhabited water poses a great hazard to fishes, compromising the whole fish production. In this work, several laboratory-scale advanced oxidation processes featuring UV-C radiation in presence of oxidizing agents and a synthetic semiconductor (La_{1-x}Ti_xFeO₃) is proposed for the inactivation of a common faecal microorganism (*Enterococcus faecalis*) in a simulated aquaculture matrix. The performance of complementary experiments mechanistical considerations and toxicity measurements is expected to be carried out.

Introduction

The steady growth in world population has drawn growing interest to new approaches towards mass food production. Novel aquaculture production methodologies, such as Recirculating Aquaculture Systems (RAS), are proposed a solution to this problem of global concern [1]. However, in a global context of water scarcity with low water usage as a way of reducing water uptake of RAS, the compliance with biological quality criteria and the disinfection of intensively reutilized water must be ensured. To overcome this issue, novel approaches which enhance pathogenic disinfection, yielding suitable effluents for reuse, present great interest: Advanced Oxidation Processes (AOPs), based on the generation of reactive species such as the hydroxyl radical (HO^{\bullet}), the singlet-oxygen (¹O₂), or the sulfate radical (SO4"), which promote matrix disinfection and highquality effluents, emerge as novel tools for intensive water treatment. The development of these processes may involve, among others, the use of oxidizing agents, (nanostructured) catalysts, and electromagnetic radiation in a broad wavelength spectrum [2].

In this work, UV-C treatments are intensified by application of four different oxidants (H_2O_2 , peracetic acid, sodium peroxymonosulfate and sodium persulfate) and a novel catalyst (La_{1-} , xTi_xFeO_3) with the goal of studying their efficiency and synergetic effect on the inactivation of *Enterococcus faecalis* in a typical, simulated aquaculture matrix.

Material and Methods

An UV-C batch system, equipped with a lamp provided by Apria (LED 6W ultraviolet germicidal with a lamp current of 0.16A and a 263-268 nm spectrum) was used. Four different oxidants are employed (namely hydrogen peroxide (H_2O_2), peracetic acid ($C_2H_4O_3$), sodium persulfate ($Na_2S_2O_8$), and potassium peroxymonosulfate (2KHSO $_5$ ·KHSO $_4$ ·K2SO $_4$)) in a concentration range of 0.01-1mM.

Simulated aquaculture water (whose chemical composition is detailed in *Table 1*) is the employed matrix for all the experiments. The matrices were spiked with a commercial strain of *Enterococcus faecalis* (ATCC 29212, Scharlab). The samples collected after treatment were cultured on Slanetz&Bartley agar and incubated at 37 °C and 48 h prior to their processing.

 $La_{1-x}Ti_xFeO_3$ catalysts were synthesised by a modified Pechini sol-gel method, published by García-Muñoz et al [3]. Catalyst was applied in the 0.1-1 g/L range.

Compound	Concentration (mg/L)
Yeast extract	193.3
NH₄Cl	26.23
KH ₂ PO ₄	2.0
NaHCO ₃	0.3
FeC ₆ H ₆ O ₇ • H ₂ O	0.3
MgCl ₂ • 6H ₂ O	50.0
CaCl ₂	55.9
ZnSO ₄ • H ₂ O	0.3

Results and Discussion

Characterization of the catalyst was carried out and published by García-Muñoz et al. [3]. The dark-phase assessment of the disinfection potential of the oxidants (in the 0.5-1 mM range of concentration) and the catalyst (1 g/L of concentration) was performed. All the assessed oxidants, except for PS, achieve the detection limit of 10 CFU/mL in a maximum of 90 minutes. *Figure 1* summarizes the drop in the population of *E. faecalis* during the dark-phase disinfection experiments, comparing the different behaviour of H_2O_2 , PAA, PMS and PS.



Figure 1: Comparison of the *E.faecalis* inactivation in aquaculture matrix by the combined system $Oxidant/1 g/L La_1 Ti_F EO_3$.

PAA appears to be an effective oxidant towards *E. faecalis* disinfection. Disinfection experiments in presence and absence of the photocatalyst are performed to assess the influence of the interaction between oxidant and catalyst. Catalyst concentrations of 1 g/L and oxidant concentration of 0.5-1.0mM are studied. A synergic effect is seen for both oxidant concentrations, as it can be seen in *Figure 2*.

Figure 2: Comparison of the disinfection effect for PAA in presence and absence of photocatalyst.

After the inspection of the dark-phase disinfection of *E. faecalis*, UV-C radiation experiments have also been carried out. The influence of the concentration of the catalyst (0.1-0.5 g/L) under 0.01 mM PAA and 1 W/m² UV-C was studied. A decrease in the disinfection efficiency as the concentration of the catalyst rises, possibly attributed to the lower radiation disposability of the system and turbidity increase, is observed. The evolution of the concentration of *E. faecalis* for each set of conditions is presented in *Figure 3*.



Figure 3: Evolution of the disinfection process in presence of the La_{1×}Ti_xFeO₃ catalyst under fixed conditions of PAA and UV-C radiation.



Conclusions

The application of UV-C radiation in presence of common oxidants and $La_{1-x}Ti_kFeO_3$ catalysts is employed for the disinfection of pathogens in aquaculture water matrices. Application of UV-C radiation enhances water disinfection, greatly shortening disinfection time. A low (0.1 g/L) concentration of catalyst is the most suitable towards water disinfection. Experiments considering the synergetic effect of common oxidants and catalysts, studies of toxicity evaluation after the application of such techniques and mechanistic considerations (*via* scavenger studies) are expected to be performed.

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