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Cost-effectiveness analysis of chlorine-based and alternative disinfection systems for pool waters

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Abstract:	The aim of this study was to ascertain the biocidal efficacy, based on the so-called C·t values, and the usage expenses, of seven disinfection products in recreational waters using Escherichia coli and Pseudomonas aeruginosa as microorganism models of faecal and environmental contamination, respectively. A 250-L indoor fully equipped pool basin was harnessed as a proof-of-concept setup for evaluation of chlorine-based (viz., trichloroisocyanuric acid (trichloro), sodium hypochlorite, sodium hypochlorite + isocyanuric acid, and saline electrolysis) and unconventional (viz., 1-bromo-3-chloro-5,5-dimethylilhidantoine, chlorine dioxide and hydrogen peroxide) biocides at 30 °C and different pH values . The economic losses resulting from human action, mimicked by urea addition, were also contemplated. Experimental results showed that trichloro, chlorine dioxide and sodium hypochlorite were the most effective disinfection agents with a log 3 removal of both organisms in 60 s regardless of the water pH. On the other hand, sodium hypochlorite and trichloro afforded unparalleled cost-effectiveness analysis. Chlorine dioxide exhibits the greatest biocide efficacy, yet its elevated usage costs make it merely applicable in shock treatments to offset high organic loads.
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changed in this current version in the comments box below. You may upload a separate response to reviewers if your comments are extensive. as follow-up to "Was this paper previously declined or withdrawn from this or another ASCE journal? If so, please provide the previous manuscript number and explain what you have changed in this current version in the comments box below. You may upload a separate response to reviewers if your comments are extensive. "	encouraged us to transfer the manuscript to the Journal of Environmental Engineering. Therefore, the manuscript was submitted to this journal with ID EEENG-4866. In the first review process, the reviewers requested several changes to the initial manuscript. We did major revisions, including the reorganization of experimental results. All the reviewers' concerns were addressed, and a fresh, fully revised version was resubmitted according to the Editor's recommendation. A separate point-by-point document is again submitted as "Answers to Reviewers 1.docx". After this resubmission, Reviewer #2 did in fact recognize our efforts to tackle the overall reviewers' remarks in the preparation of a fully revised manuscript. However, he/she raised entirely new critiques on several aspects of the work that were not even mentioned in his first report. Those concerns were not fully justified and, in some cases, his/her criticisms were based on assumptions that were proven not to be accurate and fully applicable to our experimental model. Based on that, we felt that this might be a case of conflict of interest with our work submitted to JCE, and so we did inform the editor by means of a letter that we again attach as "Letter to the EDITOR-EEENG.docx". Finally, the manuscript was declined with encouragement to resubmit. The hereby submitted manuscript includes all the changes performed after two rounds of revision.
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ABSTRACT

The aim of this study was to ascertain the biocidal efficacy, based on the so-called C·t values, and the usage expenses, of seven disinfection products in recreational waters using *Escherichia coli* and *Pseudomonas aeruginosa* as microorganism models of faecal and environmental contamination, respectively. A 250-L indoor fully equipped pool basin was harnessed as a proof-of-concept setup for evaluation of chlorine-based (viz., trichloroisocyanuric acid (trichloro), sodium hypochlorite, sodium hypochlorite + isocyanuric acid, and saline electrolysis) and unconventional (viz., 1-bromo-3-chloro-5,5-dimethylilhidantoine, chlorine dioxide and hydrogen peroxide) biocides at 30 °C and different pH values . The economic losses resulting from human action, mimicked by urea addition, were also contemplated. Experimental results showed that trichloro, chlorine dioxide and sodium hypochlorite were the most effective disinfection agents with a log 3 removal of both organisms in 60 s regardless of the water pH. On the other hand, sodium hypochlorite and trichloro afforded unparalleled cost-effectiveness analysis. Chlorine dioxide exhibits the greatest biocide efficacy, yet its elevated usage costs make it merely applicable in shock treatments to offset high organic loads.

INTRODUCTION

Tourism is the main economic activity in Majorca (Balearic Islands, Spain) since late 80s. There are currently *ca.* 2,600 swimming pools for touristic activities and 250 spas or hot springs in the island. The number of private or community pools is not exactly known, but can be estimated around 60,000 (Govern Balear n.d.; Matas et al. 2013). Recreational waters are however a major source of infection by several pathogenic microorganisms, which in turn might lead to acute gastrointestinal, cutaneous and respiratory illnesses as a result of water swallowing during swimming activity (Dufour et al. 2006; Rice et al. 2012; Suppes et al. 2014). Outbreaks related to recreational waters usually reflect deficient control of the disinfection system (Chowdhury et al. 2014; Doménech-Sánchez et al. 2008; Dziuban et al. 2006). Chlorine is still the most common product for the disinfection of recreational waters on the basis of its recognized biocidal activity, with a current market share of 70% in the Balearic Islands, and more than 10,000 tonnes per year of demand. As a consequence of the generation of potentially harmful chloramines and organic derivatives thereof (Chowdhury et al. 2014; Parrat et al. 2012; Teo et al. 2015), and the fact that the chlor-alkali sector in Europe is progressing towards a phase-out of mercury cell technology, there is a quest of novel manufacturing industry for hypochlorite. Alternative biocide systems include cyanuric acid stabilized chlorine (chlorine isocyanates), bromine, chloride dioxide, UV-irradiation, ozone, saline electrolysis and non-thermal atmospheric plasma (Matas et al. 2013), just to mention a few. Below some of the (potential) disinfection systems for swimming waters are briefly described.

Chlorine isocyanates are triazine compounds used worldwide as chlorine stabilizers in outdoor pools, so as to hinder the loss of chlorine caused by the action of solar ultraviolet irradiation (Dorevitch et al. 2011). There are two commercial formulations: sodium dichloroisocyanuric acid (also known as Dichlor) and trichloroisocyanuric acid (Trichlor). Trichlor contains ca. 90% available chlorine and is usually supplied as crystalline power or tablets. This biocide is not very soluble in water, which makes it more appropriate for direct use in flow injectors or skimmers. It bears a low pH (approximately 3), which in turn may shift the water pH below accepted levels and thus re-adjustment of the pH would be called for. Further, monitoring of cyanuric acid concentration in pools to concentrations levels ≤ 75 mg L^{-1} is mandatory to cope with current legislation (Ministerio de Sanidad Servicios Sociales e Igualdad 2013). This is because chlorine is consumed throughout the redox reaction with organic matter but the isocyanuric acid remains. As a consequence, the concentration of the triazine species might increase to levels >150 mg L^{-1} that would in turn give rise to the so-called chlorine lock phenomenon in pools (Murphy et al. 2015). Chlorine analysis still shows adequate levels of free chlorine, but it rendered inactive toward bacteria and viruses, with the subsequent impact onto the human health.

Saline electrolysis (salt water chlorination) is another yet disinfection system which has grown in popularity over the past decade in small pools and touristic resorts (Matas et al. 2013). The investment costs in electrolytic equipment have been significantly minimized in yesteryears, with the subsequent decrease in the overall depreciation expenses. On the other hand, disinfection is still based on chlorine oxidation, and hazards for workers and swimmers health, derived from disinfection by products in inner pools and spas, have been described (Jacobs et al. 2007; Rosenman et al. 2015; Rotman et al. 1983). To tackle this issue, non-chlorine biocides, such as bromine, have been recently regulated (Ministerio de Sanidad Servicios Sociales e Igualdad 2013). The oxidizing species of bromine-treated water is hypobromous acid (HBrO). Free bromine is deemed to bear comparable disinfectant properties than those of chlorine, but in the swimming pool framework its biocide efficiency does seem superior. As indicated above, combined chlorine or chloramines in chlorinated water might cause eye irritation to swimmers, and occasionally offensive odours. In bromine-treated pools, though combined bromine or bromamines (poorly stable in water (Heeb et al. 2017)) might be also be generated, ocular irritation is almost non-existent. The use of elemental bromine however is not widespread, because it is available as a corrosive dark red liquid, which might evolve harmful volatile compounds. The organic compound 1-bromo-3-chloro-5,5- dimethylhydantoin (BCDMH) bearing both elemental chlorine and bromide is deemed a suitable alternative to bromine in pools and spas. It is usually supplied as tablets and contains 61% of available bromine and 27% of available chlorine. BCDMH dissolves in water to release both free bromine and free chlorine (hypochlorous acid/hypochlorite ions) slowly. Though the latter is also operating as a disinfectant the primary antimicrobial agent in a long-term treated with BCDMH pool is the hypobromous acid. In fact, bromide ions are oxidized by hypochlorous acid to generate more hypobromous acid. The main asset of BCDMH capitalizes upon its superior chemical stability as opposed to chlorine species and does not require special storage conditions, beyond controlled temperature and moisture.

Other systems which enable greater innovation, such as chlorine dioxide (Junli et al. 1997) and other activating compounds, including hydrogen peroxide, are basically unknown by pool owners and maintenance staff, and their use is not regulated in many countries (Ministerio de Sanidad Servicios Sociales e Igualdad 2013). Notwithstanding the fact that chlorine dioxide is less reactive than chlorine compounds (Rice and Gomez-Taylor 1986), it features unique selectivity by acting as oxidizing species upon electron-rich centers of organic molecules by one-electron exchange mechanism (Dietrich et al. 1992). Not the least, chlorine dioxide efficiently removes organic species that provide colour or are precursors of unwanted organic haloforms, which would otherwise be generated via traditional chlorination procedures. However, the major shortcomings related to the use of chlorine dioxide are (i) the hazardous nature of the vapour species and its precursor, and (ii) the volatility of aqueous solutions that calls for stringent requirements for manipulation and design of feeding equipment. Hydrogen peroxide in recreational waters is a very novel and non-aggressive system for users. Its biocide mechanism focuses on the oxidation of some proteins, altering their functions and inhibiting metabolic processes in microorganisms (Borgmann-Strahsen 2003). The great advantage of this procedure is that it leaves no residual biocide, only water and oxygen. The disadvantages however are related to cost, manipulation, easy degradability with temperature or moisture, and questionable disinfectant efficiency in pool waters (Borgmann-Strahsen 2003).

It is important to stress the fact that previous works dealing with the comparative evaluation of biocides in recreational waters only used artificial pool water with test assays in a 0.35-1.0-L beaker format under magnetic agitation (Borgmann-Strahsen 2003; Korich et al. 1990). However, biocide and microorganism dispersion and pool dynamics are more complex than those simulated by bench analysis. Therefore, extrapolation to real-life situations is actually debatable. To solve this gap, in the present work, we have used a more realistic approach. Our experimental model includes a 250-L indoor pool basin with a pumping system for water recirculation and real-time monitoring of pH, temperature and redox potential. To our knowledge, this is the first time that this model has been exploited, and is clearly more representative of real-life scenarios than those carried out in test tubes.

The aim of this work is to present a novel approach for in-depth investigation of the biocidal activity and the cost-effectiveness of a number of unconventional disinfection systems (*namely,* chlorine dioxide, hydrogen peroxide, BCDMH) in recreational waters without harmful by-product formation against conventional chlorine-laden products (hypochlorite, hypochlorite + isocyanate, tri-chloro and saline electrolysis).

METHODS

Experimental design

The pilot experimental setup consisted of a 250-liter whirlpool vessel (herein after referred to as pool) and accessories thereof including the pumping system and on-site monitoring devices (without filtration systems) that allowed the assessment of the biocide efficacy of various disinfection chemicals (see Online Resource 1). Distilled water was used for preparation of stock solutions throughout. Chlorine-based products were standardized pending use by redox titration against sodium thiosulphate using potassium iodate as a primary standard.

In every individual experiment, the pool was filled with chlorine-free water by in-line filtration through activated carbon, and heated and stabilized at 30°C (so-called preparation phase). Thereafter, throughout the so-called initial phase, a given disinfection system was applied and monitored until attaining the prescribed concentration level of biocide (see Table 1), followed by adjustment of the water pH to the appropriate pre-set value (7.2 or 8.0) inasmuch as pH changes are expected upon addition of the oxidizing chemicals. At this time, and to reproduce real conditions of use, organic load was simulated by urea addition. Urea is commonly occurring in recreational waters because it is an endogenous component of the human saliva, sweat and urine (Afifi and Blatchley 2016; Yang et al. 2018). In this work, urea concentration was fixed to 1.2 mg L^{-1} on account of the average concentration reported in a recent study of 50 pools in use (De Laat et al. 2011). In the subsequent stabilization phase, the evolution of the disinfectant concentration was monitored at real time so as to offset possible fluctuations due to evaporation over time.

This was followed by the microbiological contamination phase consisting of the addition of microorganisms, namely *Escherichia coli* (*E. coli)* and *Pseudomonas aeruginosa* (*P. aeruginosa*), into the experimental pool, with the subsequent homogenization and collection of water aliquots over time to elucidate the actual biocide activity of the tested chemicals and systems. Upon finalization of the sampling stage, the possible remnants of microorganisms were eliminated by water hyperchlorination with 20 mg L^{-1} chlorine for at least 1 h. Surplus of chlorine was neutralized with 33 mg L^{-1} sodium thiosulfate, and after corroboration that the chlorine level was down to 3 mg L^{-1}, the pool was completely emptied. To make it ready for the next experiment, the vessel was cleansed with 10 L of chlorine-free water, and the inner walls were sprayed with 100 mL of isopropyl alcohol, which was allowed to evaporate until the onset of the ensuing assay.

Experimental conditions

Seven types of disinfection chemicals were evaluated in this work, including extensively used chlorine-based products and others uncommon in swimming pool disinfection (see Table 1). Experimental conditions were selected based on current legislation and manufacturers' recommendations. For chlorine, four different forms and/or formulations were applied: chlorinated isocyanates (stabilized chlorine, solid), liquid chlorine (sodium hypochlorite), liquid chlorine + isocyanates, and saline electrolysis.

Temperature and pH are two parameters that greatly affect the efficiency of biocides. To this end, the water temperature was maintained at 30 ± 1°C to simulate indoor pools, as an intermediate situation between those of outdoor pools and whirlpools. In addition, two pH values, 7.2 and 8.0, were assessed so as to cope with the entire pH range endorsed by current regulations (Ministerio de Sanidad Servicios Sociales e Igualdad. 2013). The temperature was held constant by an in-line electric heating system (Electric Heat Exchanger, Astral, Spain) and the pH was monitored continuously by a flow-through combined pH electrode placed within the water recirculation system. To adjust and maintain the water pH, minute volumes of 1 mol L^{-1} HCl or 1 mol L^{-1} NaOH were used. All experiments were carried out in triplicate.

Determination of concentrations of chemical disinfectants

For the determination of chlorine levels throughout the experimental phases (re. above), the N,N-diethyl-p-phenylenediamine (DPD) colorimetric 4500-G Cl method (APHA, AWWA & WEF 2017) was deemed most appropriate. In the absence of iodide, the available free chlorine reacts instantaneously with DPD at pH ca. 6 to generate an oxidized conjugated organic compound that can be measured spectrophotometrically at 515 nm. Subsequently, iodide is added, which is oxidized by chloramines into iodine. The latter reacts with the surplus of DPD, thus serving for determination of combined chlorine. A linear calibration graph (Absorbance at 515 nm vs chlorine concentration) with chlorine standard solutions ranging from 0.05 to 4 mg/L was used throughout.

Isocyanuric acid concentration in the pool was estimated on the basis of a turbidimetric method in which turbidity of the reaction product of isocyanuric acid with melanin is monitored (Downes et al. 1984).

As for bromine, as is the case with free chlorine, total bromine reacts with DPD at pH 5-6 forming the red-violet oxidized DPD dye (Sollo et al. 1971). This method is thus an extension of the DPD method described above. The experimental data obtained by interpolation of the hypochlorite calibration graph were multiplied by 2.25 based on the molecular mass ratio of bromine to chlorine. It should be noted that, unlike chlorine, DPD does not react with free bromine but total bromine. In any case, the combined bromine is a very good disinfectant without harmful effects. Therefore, only total bromine is herein determined.

The concentration levels of chlorine dioxide were determined by standard recommended method (APHA, AWWA & WEF 2017). It is an extension of the DPD method abovementioned. If the sample is first acidified in the presence of iodide and then brought to a near-neutral pH by addition of sodium hydrogen carbonate, chlorine dioxide behaves alike the total available chlorine content. The experimental procedure was akin to the determination of free chlorine using normalized hypochlorite standards, yet the results were multiplied by 1.9 based on the chlorine to chlorine dioxide stoichiometric ratio, and molecular weights of both species.

The quantification of hydrogen peroxide was also relied upon the spectrophotometric DPD method. Unlike sodium hypochlorite, prior activation of hydrogen peroxide with potassium iodide and ammonium molybdate was called for. Molybdate catalyzes the oxidation of iodide by hydrogen peroxide into iodine which is amenable to oxidizing the DPD compound. In the determination of hydrogen peroxide, 150 µL of 20% (w:v) potassium iodide and 150 µL of 90 g L^{-1} ammonium molybdate were added to 9 mL of probed pool water. The mixture was stirred vigorously and allowed to react for 6 min. Then, 0.5 mL of 0.5 mol L^{-1} dihydrogenphosphate/hydrogen phosphate buffer at pH= 7.5 followed by 0.5 mL of DPD were added, and the absorbance of the mixture was measured at 515 nm (APHA, AWWA & WEF 2017).

Preparation of inocula and contamination of the pool

The microorganisms selected as model pathogens to contaminate the pool were *Escherichia coli* ATCC 11775 *(E. coli)* and *Pseudomonas aeruginosa* ATCC 25668 *(P. aeruginosa).* These species were selected because they are endorsed in our current legislation (Ministerio de Sanidad

Servicios Sociales e Igualdad. 2013). To this end, two independent inocula, one per microorganism, were prepared. In both cases, 1-2 colonies of the microorganisms to be studied were inoculated into 5 mL of Tryptic Soy Broth (TSB) (Sharlab, Barcelona, Spain) and incubated overnight at 37 °C and 180 rpm in an orbital shaker (Unitron Plus AJ252, Infors AG, Bottmingen, Switzerland). Subsequently, 250 μL of every culture were added separately to 30 mL of TSB and incubated again under the same conditions for 1.5-2 hours to foster bacterial growth, and obtain a nominal concentration as per the standard operational protocol of about 10^9 CFU mL^{-1} (APHA et al. 2017). The exact concentration was calculated by preparing decimal serial dilutions in tryptone water (Sharlab, Barcelona, Spain) and plating onto the appropriate medium: Tryptone Bile Glucuronic Agar (TBX) for *E. coli* and cetrimide agar for *P. aeruginosa* (Sharlab, Barcelona, Spain).

Whenever steady-state conditions were identified for the physicochemical parameters of the pool (pH, temperature, redox potential) upon biocide application, 25 mL of each culture were simultaneously added to the 250 L pool water to simulate faecal and environmental contamination of water, thus obtaining a nominal concentration of 10^5 CFU mL^{-1} (log CFU mL^{-1} = 5) of every individual microorganism. The real concentration added to the pool was calculated as indicated above. Both inoculation and subsequent sampling steps were manually performed at the central zone of the pool after water homogenization. At pre-set incubation timeframes, viz., 0 s, 20 s, 40 s, 60 s, 120 s, 300 s, 600 s and 900 s, a metered volume of 100 mL water was sampled in a sterile vial containing 2 mg of solid sodium thiosulfate to eliminate the oxidant (disinfectant) surplus. Immediately after collection, samples were placed on ice and transported refrigerated to the laboratory. To determine the bacterial concentration, 10-fold dilution aliquots were prepared in 9 mL tubes of tryptone water (Sharlab, Barcelona, Spain) and 0.1 mL aliquot of every tube was plated in the selective media for the analyzed microorganisms: TBX (for *E. coli)* and cetrimide agar (for *P. aeruginosa)*. The idea behind this procedure is to attain plates within a final range of 30-300 CFU after incubation for 24 h at 37°C for proper bacterial quantification.

Data analysis

Three replicate experiments (addition of both test organisms) were performed per individual biocide and pH value (7.2 or 8.0). In nature, microorganisms do not follow a normal distribution because they tend to aggregate. For this reason, prior to statistical analysis the microbiological counts were transformed to $\log_{10}$ values for normalization. Mean and standard deviation values were calculated for every disinfectant and time of operation.

Our response variable is the so-called C·t value (Hoff 1986; Korich et al. 1990), which is herein defined as the product of the nominal concentration of disinfectant added to the pool (see Table 1) (given as mg L^{-1} or µmol L^{-1}, see Results and Discussion) by the time (in minutes) to attain a given degree of disinfection at either pH 7.2 or 8.0. Conditions including 90%, 99%, 99.9%, and 99.99% elimination of the target microorganism (or the equivalent 1 log , 2 log , 3 log, 4 log reduction, respectively) have been used in previous studies (Ding et al. 2012; Hoff 1986; Korich et al. 1990; Li et al. 2017; Lim et al. 2010; Oh et al. 2014; Pak et al. 2016; Thurston-Enriquez et al. 2005). Our criterion was to set a 3 log reduction of the target microorganisms equating to a 99.9% of elimination. Average Ct values (calculated from the values obtained in the individual experiments for the same disinfectant and condition) were estimated in this work for individual disinfectant agents and pH values at a given disinfectant concentration to evaluate biocide efficiency and the expenses of the disinfection procedure.

RESULTS AND DISCUSSION

Consumption and stabilization of the concentration of chemical disinfectants

The first part of our study focused on the analysis of the consumption of biocides associated to the stabilization of the levels of disinfectant in the pool. As an example, a diagrammatic description of the variation of sodium hypochlorite at pH 7.2 throughout the diverse experimental phases is shown in Fig. 1. As indicated above, the exploration of the biocide performance for conventional and unusual disinfection products in our pilot setup was initiated by adjustment of the biocide concentration to a pre-set concentration, as indicated in Table 1, at 30 ± 1 °C for pH 7.2 or 8.0, followed by a final pH tuning of the pool water (see Fig. 1). This preparation phase lasted approximately 35-40 min. Once the target concentration was reached, organic contamination of human origin was simulated by the addition of urea at the 1.2 mg L^{-1} level. After the organic load and during the stabilization phase, an ancillary step of addition of the biocide was in some instances deemed necessary because of either the decomposition of urea or the volatility of the disinfectants, e.g., chloride dioxide. In fact, the influence of the organic load was noticeable for some chlorine based biocidals, in particular, in the course of the saline electrolysis experiments inasmuch as the steady value of 2 mg L^{-1} free chlorine was difficult to reach by temporal on/off activation of the electrolysis setup. In all cases, once the desired concentration of biocide was reached, the water pH was adjusted to either 7.2 or 8.0 and the pool water was sampled and analyzed every 30 minutes during the so-called stabilization phase, which lasted approximately 120 min. Whenever required, the concentration of biocide and the pH of the pool were re-adjusted to initial conditions. Significant addition of biocide was deemed necessary for the most volatile species (viz., chloride dioxide).

Altogether, this study allows us to estimate the amount of biocide needed to stabilize the disinfectant levels at endorsed/recommended concentrations for the experimental pool (see Table 2). Throughout these experiments, the consumption of reagents for pH stabilization is also estimated for proper evaluation of the cost-efficacy of the distinct biocides as discussed in the following sections.

Investigation of the effectiveness of the disinfection products

To evaluate the bactericidal efficiency of the disinfectants after the microbial contamination of the pool, water aliquots were taken at different incubation times, from 20 to 900 s, followed by determination of *E. coli* and *P. aeruginosa* levels after plating as explained above. The percentages of removal of both bacteria for every individual biocide at pH values of 7.2 and 8.0 for 20, 60, 300, and 600 s are shown in Fig. 2 and Fig. 3, as no statistically significant changes were observed in aliquots probed from 600 s onwards even for low-efficacy biocides, such as hydrogen peroxide (see Fig. 2).

Chlorine dioxide averagely afforded a >3 log reduction of *E. coli* in a mere 20 s, virtually instantaneously and regardless of the pH (see Fig. 2). Detection limit for *E. coli* was 5.0 ± 0.4 log reduction. This range was a consequence of the variability in the actual amount of microorganisms added to the pool in individual experiments. Trichloroisocyanuric acid and sodium hypochlorite also afforded a >3 log reduction of *E. coli* in 20 s at pH 7.2, whereas at this time and pH 8 removal ranged between 2 and 3 log. This is in good agreement with previous observations by Hoff (Hoff 1986). Results also show that the removal efficiency of E. coli for 20 s by sodium hypochlorite in combination with 75 mg L-1 isocyanuric acid was lower, with a 1 log reduction of the microorganism at both pH values. Similar values were obtained for the electrolysis based system.

Regarding bromine and hydrogen peroxide the biocidal effect at both pHs decreased down to 0.6 and 0.1 log reduction, respectively, for a disinfection time of 20 s, in comparison with the biocidal efficiencies (> 3 log elimination) of chlorine dioxide, trichloroisocyanuric acid and sodium hypoclorite.

P. aeruginosa is a more resistant microorganism than *E. coli* (Sánchez-Diener et al. 2017). This is consistent with our experimental results (see Fig. 3), which indicated that the removal percentage for all of the disinfectants evaluated was inferior to that of *E. coli.* In this case, the detection limit was 4.0 ± 0.5 log reduction. Still, similar trends were observed*:* trichloroisocyanuric acid, sodium hypochlorite and chlorine dioxide were the most efficient disinfectants. For *P. aeruginosa*, the unfavourable effects of isocyanuric acid in combination with sodium hypochlorite in terms of expeditious disinfection were more evident, inasmuch as just a 0.2 log reduction was detected at pH 7.2 and no biocide performance after 20 s was identified at pH 8.0. To note, bactericidal effects were observed for bromine at this pH neither.

As expected, killing rates for *E. coli* and *P. aeruginosa* increased with time (Fig. 2 and Fig. 3). In fact, all chlorine-based and unconventional disinfectants but hydrogen peroxide achieved a >3 log reduction of *E. coli* regardless of pH after 60 s of treatment. For *P. aeruginosa,* the removal rate was still lower than for *E. coli,* remaining negligible for hydrogen peroxide at either pH. Sodium hypochlorite combined with 75 mg L^{-1} isocyanuric acid afforded average removal values of 0.23 and 0.01 log reduction for pH 7.2 and 8.0, respectively, for *P. aeruginosa* at 60 s. It should be noted that all disinfectants but hydrogen peroxide achieved >99.9% elimination (> 3 logarithmic reduction) of both microorganisms for contact times from 10 min onwards.

To evaluate and normalize the biocide effectiveness, the C·t (thereafter called Ct) value is herein used as a standard parameter. We herein propose a paradigm change in the calculation of Ct by reporting the biocide concentration in µmol L^{-1} rather than mg L^{-1} based on the stoichiometric laws of the redox reactions involved (see Fig. 4). This will foster a more reliable comparison of the biocidal efficiency of distinct chemical species as compared to current data. It should be however born in mind that current legislation copes with the molecular weight of classical biocides, e.g., chlorine vs bromine, by endorsing twice as much as bromine concentration (in mg L^{-1}) in pools than chlorine. Based on this parameter, the greater the Ct value the lower the disinfectant efficiency is. For all of the chlorine-based disinfectants, Ct values at pH 8.0 were higher than those at pH 7.2 for *E. coli*, on account of the superior disinfection efficiency of hypochlorous acid (pKa ~ 7.5) (Fig. 4). For *P. aeruginosa* this also held true except for sodium hypochlorite combined with isocyanates and chlorine dioxide, though results at both pH were quite similar.

As for the individual treatments, chlorine dioxide was proven the most effective biocide with Ct values spanning from 0.2-0.3 (in mg min L^{-1}). Critical comparison of our Ct values (3 log decrease) with those previously reported in the literature is not straightforward, because distinct experimental conditions regarding temperature, pH, disinfectant concentration or percentage of elimination apply. In any case, our values are on a par with the Ct value of 0.15 (in mg min L^{-1}) endorsed by the United States Environmental Protection Agency (USEPA) for *E. coli* (Symons et al. 1981), and half as much the Ct value of 0.6 reported by Hoff (Hoff 1986), in all cases for a 2 log bacterial decrease. Further, our results are notably better than the Ct value of 30 recently estimated by Ofori *et al.* for the same concentration of chlorine dioxide but for a mere 1 log bacterial reduction (Ofori et al. 2017). Hypochlorite is the biocide in the second place as for its effectiveness, with values in our system from 0.7 to 2.2 (in mg min L^{-1}) for both microorganisms, which are higher than those reported for *E. coli* by Hoff (0.05) and the USEPA (0.3) but for a 2 log reduction (Hoff 1986; Symons et al. 1981), but better to those reported by Oh et al. (Oh et al. 2014), amounting to as much as 30 mg min L^{-1} for 1 log reduction.

Among the chlorine derivatives, sodium hypochlorite was the most active biocide against *E. coli*, though solid chlorinated isocyanates showed better results than liquid sodium hypochlorite when combined with isocyanates, with Ct ranging from 1-2 against 2.0 (in mg min L^{-1}), respectively. Electrolysis results for *E. coli* were similar to chlorinated isocyanates at pH 7.2, and even better at pH 8.0, with Ct values of 1.5 and 1.3, respectively against ≤2 (in mg min L^{-1}). For *P. aeruginosa* elimination, significantly superior biocide efficiencies were observed for liquid hypochlorite and solid isocyanates against saline electrolysis and hypochlorite + isocyanuric acid, regardless of the pH, with Ct values ranging from 1.3 to 2.2 mg min L^{-1} for the former two biocides against Ct values of 13-20mg min L^{-1} for the latter.

Ct values of BCDMH were higher than those of chlorine derivatives regardless of pH conditions and microorganisms, with values ranging from 3-27 in mg min L^{-1}. Strikingly, the high Ct values of hydrogen peroxide for both microorganisms (> 150 mg min L^{-1}) indicated null bactericidal action under the experimental conditions set in this pilot study. This behaviour was also observed in a previous report (Borgmann-Strahsen 2003), where a Ct value of 4500 mg min L^{-1} was reported for a mere 0.16 and 0.13 log reduction of *P. aeruginosa* and *E. coli*, respectively.

Evaluation of costs related to the varied disinfection systems

We have resorted to the average costs of products available at the Balearic Islands in 2016 for calculation of the expenses of the usage of the distinct disinfectants, yet the consumption of reagents throughout the stabilization phase in our experimental system was also considered (see Table 2). This is in contrast to previous studies, in which estimation is merely done at different concentration levels of biocides (Symons et al. 1981). Our model was translated to a realistic 500 m^3 swimming pool with a daily 5% water renewal, i.e., 25 m^3 day^{-1}. For isocyanuric acid-laden biocides, the water renewal was increased to 37 m^3 day^{-1} so as to maintain isocyanuric acid concentration down to 75 mg L^{-1}according to legislation requirements (Ministerio de Sanidad Servicios Sociales e Igualdad. 2013). In our calculation, the cost of € 2.52 water/m^3 stands for the average price at the Balearic Islands in 2016, excluding local taxes (Organización de Consumidores y Usuarios 2016). The main parameters for the calculation of the biocide expenses were as follows: a) disinfectant consumed to maintain the prescribed concentration of biocide for an operational timeframe of the swimming pool of 12 hours, with a total number of six human-like contamination (1.2 mg L^{-1} urea), once every 2 hours; b) pH control with addition of minute volumes of NaOH or HCl; c) water renewal cost; d) for electrolysis: a standard electrolyser generating ca. 500 g chlorine h^{-1} with a daily average performance of 11 h, including energy consumption (4 kW at 0.08 kW h^{-1}). A saline electrolysis equipment for a 500 m^3 pool might amount to 30,000 € with a replacement price of electrodes equating to 1 €/operational hour. A depreciation time of 10 years has been deemed appropriate for the electrolysis installation. Our estimations apply to facilities for which infrastructure is already available, and only refer to operational and maintenance costs (without personnel). Construction costs for adaptation of facilities included in other studies (Gumerman et al. 1979), are not herein considered. Table 3 lists the costs associated with each of the disinfection systems evaluated according to the criteria mentioned above.

Sodium hypochlorite was the most economically viable system for disinfecting recreational waters at both pH values (7.2 and 8.0) (see Table 3). Note that the action of solar UV radiation was not considered in this pilot study. Solar radiation plays a very important role in the evaporation and decomposition of chlorine, which would most likely lead to increased consumption of disinfectant. In terms of economic viability, trichloroisocyanuric acid and sodium hypochlorite combined with isocyanuric acid were equally ranked second (see Table 3). The need for water renewal increases the cost of these treatments relative to sodium hypochlorite. Saline electrolysis was the most expensive treatment based on chlorine, amounting to 124 and 138 €/day at pH 8.0 and 7.2, respectively (see Table 3). BCDMH occupied the fourth place in price, followed by hydrogen peroxide. We must emphasize that further studies combining hydrogen peroxide with catalysts are needed, because of the negligible bactericidal efficiency of hydrogen peroxide for both microorganisms using our conditions of biocide concentration, pH and temperature, with killing efficacies down to 50% in all instances.

Notwithstanding its superior biocidal activity on a short notice (Ofori et al. 2017), chlorine dioxide is not an economical viable option (see Table 3), when used alone in public and private pools, as a consequence of the elevated expenses of the raw materials and manufacturing process along with its high volatility. On the other hand, it might be deemed attractive for shock treatment in emergency situations of excessive organic load in pools, or in facilities, such as summer resorts, for which penalties may apply when the pool is not available to guests.

Cost-effectiveness of chlorine-based and unconventional disinfection products

The cost-effectiveness (CE) of the varied chlorine and non-chlorine based disinfection products and systems assessed in this work was normalized as the product of the daily cost of biocide by the Ct value (CE= €·Ct). Values were estimated against each of the pathogenic microorganisms for a 3 log reduction at both pH values (Fig. 5). Regardless of pH, the most cost-effective disinfectant agent was sodium hypochlorite whereas dioxide chloride was again deemed impracticable with CE values > 1000 against CE values < 200 for hypochlorite. The experimental results revealed that the pH did yield to a significant variation of CE for *E. coli* for all of assayed biocide agents, the lower the pH the better the CE value was. Increased consumption of disinfectants at pH 8.0 in chlorine-based treatments was however partially offset by the need of increasing the amount of HCl to maintain pH at 7.2 in alkaline waters as those used in this study (pH tap water= 8.1) due to the presence of limestone, which causes carbonation of water aquifers. On the contrary, there was not a dependence of pH upon CE for *P. aeruginosa*, yet a 2-8 fold increase in CE values were observed for the overall suite of biocide agents against *E. coli*, except for the chlorine dioxide where the CE values for both microorganisms were virtually identical due to the superior bactericidal power of this biocide.

CONCLUSIONS

The main findings of the research conducted within the framework of this study are summarized as follows:

- The lowest Ct, namely, the higher disinfection efficiency, was obtained for chlorine dioxide, without significant biocidal differences for either *E. coli* or *P. aeruginosa*, regardless of the poorer inherent susceptibility of the latter to biocides. However, it is advisable to use ClO_2 alongside other disinfectants because the elevated manufacturing costs and high CE values make its use as a sole biocide in pools impracticable. Chlorine dioxide is however deemed appropriate in shock treatments for emergency scenarios. Due to its lower redox potential compared to chlorine-laden biocides, no chloramines or by-products potentially harmful to human health are generated.

- The most affordable disinfectant agent in our pilot setup was sodium hypochlorite, though degradation by UV radiation was not contemplated in the experimental settings.
- The price difference was negligible for pool disinfection with trichloroisocyanuric acid (solid) against hypochlorite combined with isocyanuric acid at the level of 75 mg L^{-1}.
- The lowest Ct for all of the chlorine and bromine-containing disinfectants was obtained at pH 7.2.
- Hydrogen peroxide was not effective as a biocide under the experimental conditions assayed.
- Biocide and microorganism dispersion and pool dynamics are more complex than those simulated in bench analysis. Our model is thus more representative of real-life scenarios than those carried out in test tubes.
- A recommendation is given to report Ct of novel chemical biocides as concentration in μmol L^{-1} times time rather than the usage of the conventional mg L^{-1} times time units.

DATA AVAILABILITY

All data, models, or code generated or used during the study are available from the corresponding author by request.

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DISCLAIMER

The authors declare that they have no conflict of interest.

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Figure Captions

Fig. 1 Time-course variation of the concentration of sodium hypochlorite at pH 7.2

Fig. 2 Biocidal effect (logarithmic reduction) of *Escherichia coli* by the action of different disinfection systems at pH 7.2 and 8.0. TRI, trichloroisocyanuric acid; HYP, sodium hypochlorite; HYP + CYA, sodium hypochlorite combined with isocyanuric acid; ELEC, saline electrolysis; ClO_2, chlorine dioxide; BR, BCDMH; H_2O_2, hydrogen peroxide. Red line stands for 3 log reduction. Grey bar indicates the range of detection limits.

Fig. 3 Biocidal effect (logarithmic reduction)of *Pseudomonas aeruginosa* by the action of different disinfection systems at pH 7.2 and 8.0. Red line stands for 3 log reduction. Grey bar indicates the range of detection limits.

Fig. 4 Average Ct values (A) (mg min L^{-1}) and (B) (µmol min L^{-1}) of the disinfectants tested against *Escherichia coli* and *Pseudomonas aeruginosa*.

Fig. 5 Cost-effectiveness analysis of chlorine-based and unconventional disinfectants.

Table 1 Disinfection systems and nominal concentrations of biocides used in this study

System	Concentration	Supplier
Trichloroisocyanuric acid (TRI)	2 mg L^{-1} free chlorine	CTX-300 ClorLent, CTX Professional, Barcelona, Spain
Sodium hypochlorite	2 mg L^{-1} free chlorine	Commercial solution (37% (w/w), Jabones Puig, Palma, Spain
Sodium hypochlorite + isocyanuric acid (CYA)	2 mg L^{-1} free chlorine + 75 mg L^{-1} CYA	Commercial solution, Jabones Puig; CTX-400, CTX professional
Saline electrolysis	2 mg L^{-1} free chlorine	Salt: Jabones Puig
Chlorine dioxide	0.5 mg L^{-1} ClO_2	Dioxpure (0.75% chloride dioxide), purity of 99.9%, Eminfor S.L, Barcelona, Spain
BCDMH	4 mg L^{-1} bromine	CTX-130, CTX professional, Spain
Hydrogen peroxide	10 mg L^{-1} H_2O_2	Commercial solution (50% (v:v) hydrogen peroxide), Jabones Puig

Table 2 Biocide consumption in the course of the several phases of the experimental procedure

pH	Phase	Disinfection system[a]						
		TRI	HYP	HYP + CYA	ELEC	ClO_2	BR	H_2O_2
7.2	Preparation[b]	0.9 ± 0.3 (g)	19 ± 1 (mL)	17.5 ± 0.9 (mL HYP) 18.9 ± 0.06 (g CYA)	1250 (g salt)	85 ± 7 (mL)	2.6 ± 0.2 (g)	5.7 ± 0.1 (mL)
	Stabilization /h	0.044 ± 0.004 (g)	1.47 ± 0.03(mL)	0.5 ± 0.1 (mL HYP)	------	52.5 ± 3.5(mL)	0.065 ± 0.002 (g)	0.25± 0.05(mL)
8.0	Preparation[b]	0.74 ± 0.08 (g)	17.7 ± 0.2 (mL)	19 ± 4 (mL HYP) 18.90 ± 0.01 (g CYA)	1250 (g salt)	91 ± 13 (mL)	2.4 ± 0.1 (g)	5.7 ± 0.2 (mL)
	Stabilization/h	0.06 ± 0.03 (g)	1.1 ± 0.4 (mL)	0.6 ± 0.2 (mL HYP)	-------	46.2 ± 1.7(mL)	0.20 ± 0.01 (g)	0.40± 0.05(mL)

[a] TRI, trichloroisocyanuric acid; HYP, sodium hypochlorite; HYP + CYA, sodium hypochlorite combined with isocyanuric acid; ELEC, saline electrolysis; ClO_2, chlorine dioxide; BR, BCDMH; H_2O_2, hydrogen peroxide

[b] Replicate experimental values (n=3) to obtain the prescribed disinfection level

Table 3 Daily cost (€) for the usage of chemical disinfection systems evaluated in this pilot study at pH 7.2 and 8.0

pH	Concept	Disinfection system [a]						
		TRI	HYP	HYP + CYA	ELEC	ClO_2	BR	H_2O_2
7.2	Chemicals	8.4	9.5	6.5	39.2	5,720	55.4	66.4
	Equipment	-	-	-	17.2	-	-	-
	Power supply	-	-	-	2.9	-	-	-
	pH control	11.6	11.5	13.6	15.9	9.9	17.7	18.8
	Water renewal	93.2	63.0	93.2	63.0	63.0	63.0	63.0
	Daily cost	**113.3**	**83.9**	**113.3**	**138.2**	**5,793**	**136.1**	**148.2**
8.0	Chemicals	9.3	7.9	8.6	39.2	5,171	80.0	80.8
	Equipment	-	-	-	17.2	-	-	-
	Power supply	-	-	-	2.9	-	-	-
	pH control	2.3	2.5	10.0	1.6	3.34	1.62	4.21
	Water renewal	93.2	63.0	93.2	63.0	63.0	63.0	63.0
	Daily cost	**104.8**	**73.4**	**111.9**	**123.9**	**5237**	**144.6**	**148.0**

[a] TRI, trichloroisocyanuric acid; HYP, sodium hypochlorite; HYP + CYA, sodium hypochlorite combined with isocyanuric acid; ELEC, saline electrolysis; ClO_2, chlorine dioxide; BR, BCDMH; H_2O_2, hydrogen peroxide

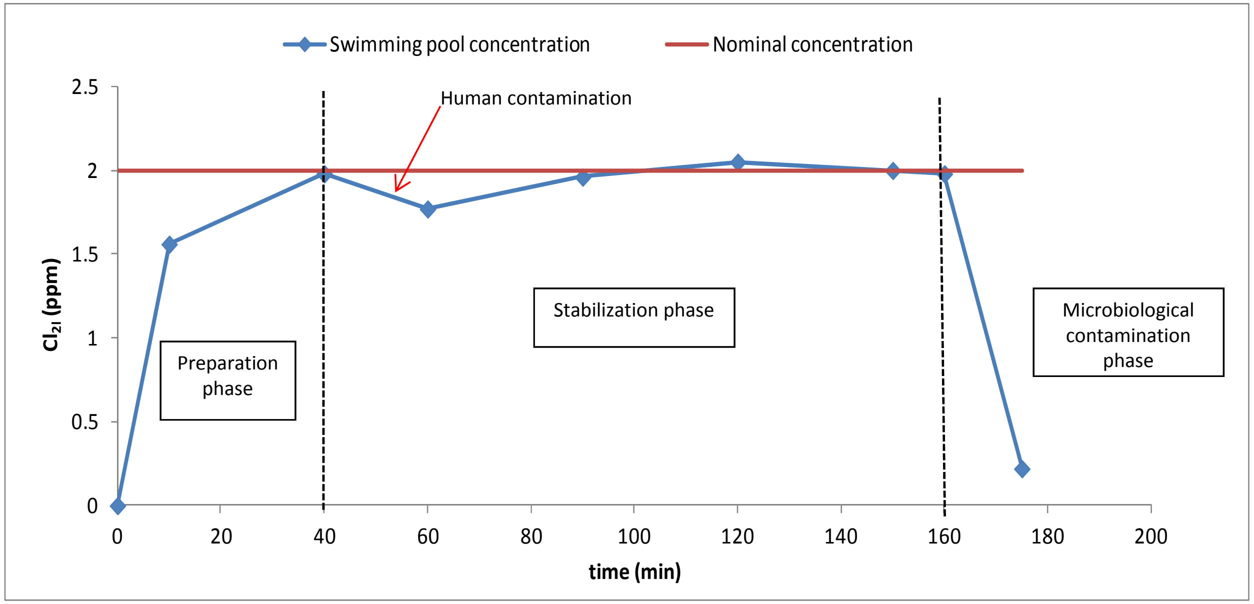

Swimming pool concentration
Nominal concentration
Human contamination
2.5
2
1.5
1
0.5
0
Cl_{2l} (ppm)
0
20
40
60
80
100
120
140
160
180
200
time (min)
Preparation phase
Stabilization phase
Microbiological contamination phase

Figure 2

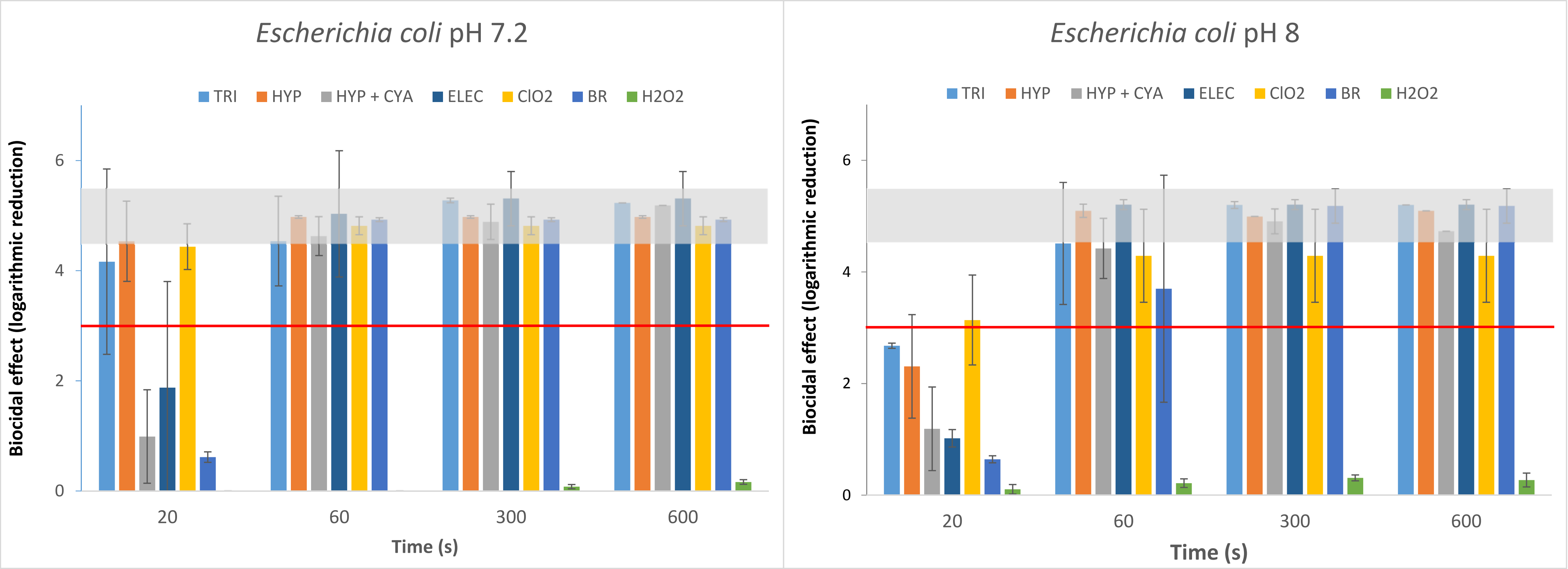
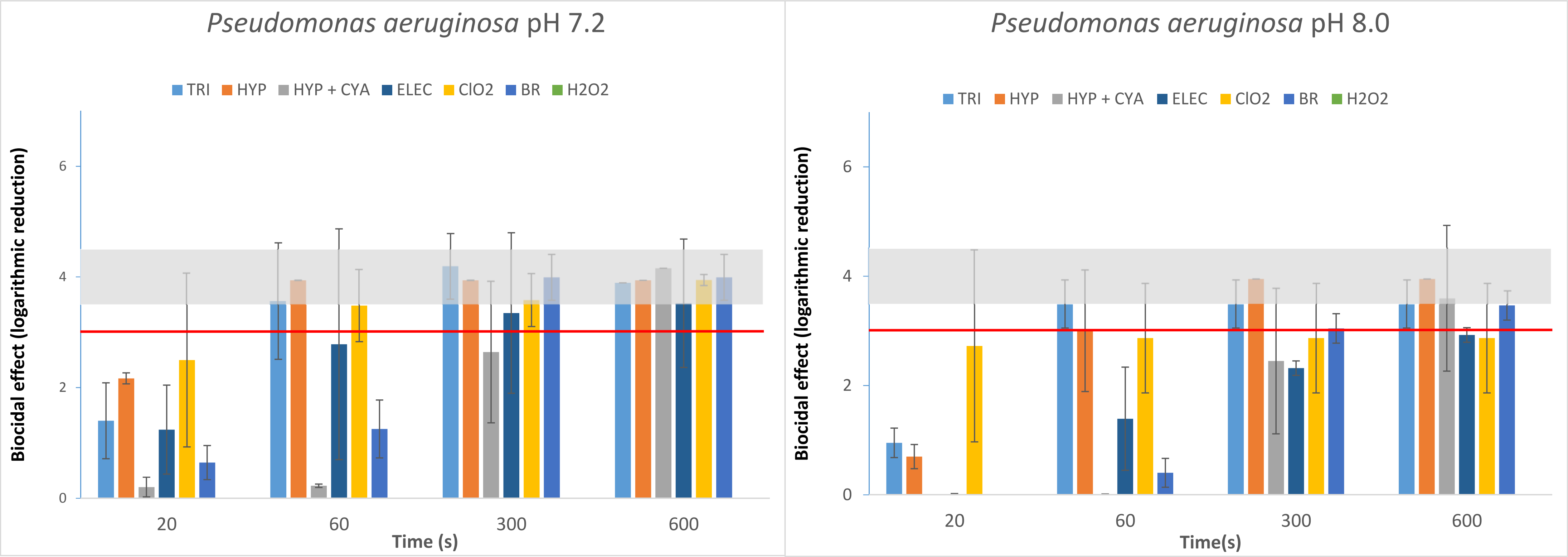

Escherichia coli pH 7.2
TRI
HYP
HYP + CYA
ELEC
ClO2
BR
H2O2
Biocidal effect (logarithmic reduction)
0
2
4
6
20
60
300
600
Time (s)
Escherichia coli pH 8
TRI
HYP
HYP + CYA
ELEC
ClO2
BR
H2O2
Biocidal effect (logarithmic reduction)
0
2
4
6
20
60
300
600
Time (s)

Figure 3


Pseudomonas aeruginosa pH 7.2

Pseudomonas aeruginosa pH 8.0

Figure 4

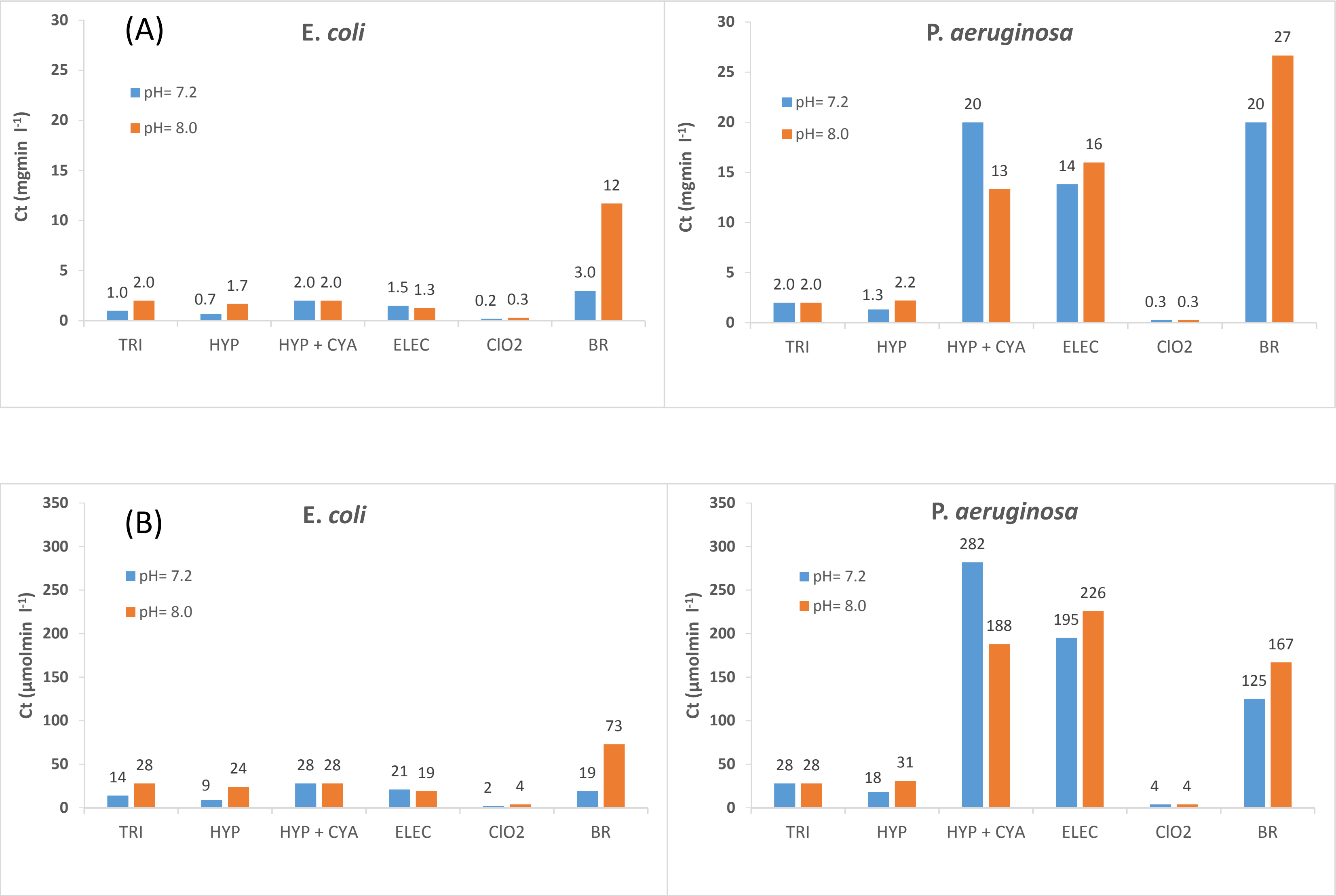
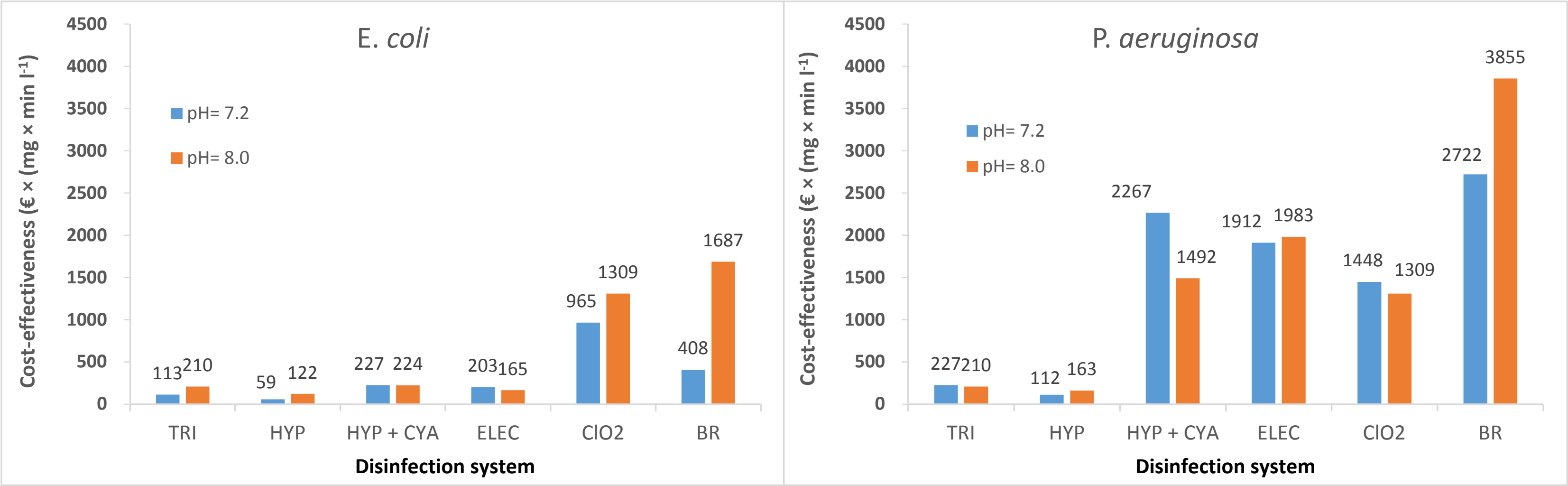

(A)
E. coli
Ct (mgmin l-1)
30
25
20
15
10
5
0
■ pH= 7.2
■ pH= 8.0
1.0
2.0
0.7
1.7
2.0
2.0
1.5
1.3
0.2
0.3
3.0
12
TRI
HYP
HYP + CYA
ELEC
ClO2
BR
P. aeruginosa
Ct (mgmin l-1)
30
25
20
15
10
5
0
■ pH= 7.2
■ pH= 8.0
2.0
2.0
1.3
2.2
20
13
14
16
0.3
0.3
20
27
TRI
HYP
HYP + CYA
ELEC
ClO2
BR
(B)
E. coli
Ct (µmolmin l-1)
350
300
250
200
150
100
50
0
■ pH= 7.2
■ pH= 8.0
14
28
9
24
28
28
21
19
2
4
19
73
TRI
HYP
HYP + CYA
ELEC
ClO2
BR
P. aeruginosa
Ct (µmolmin l-1)
350
300
250
200
150
100
50
0
■ pH= 7.2
■ pH= 8.0
28
28
18
31
282
188
195
226
4
4
125
167
TRI
HYP
HYP + CYA
ELEC
ClO2
BR

Figure 5


E. *coli*
Cost-effectiveness (€ × (mg × min l^{-1}))
4500
4000
3500
3000
2500
2000
1500
1000
500
0
pH= 7.2
pH= 8.0
113
210
59
122
227
224
203
165
965
1309
408
1687
TRI
HYP
HYP + CYA
ELEC
ClO2
BR
Disinfection system
P. *aeruginosa*
Cost-effectiveness (€ × (mg × min l^{-1}))
4500
4000
3500
3000
2500
2000
1500
1000
500
0
pH= 7.2
pH= 8.0
227
210
112
163
2267
1492
1912
1983
1448
1309
2722
3855
TRI
HYP
HYP + CYA
ELEC
ClO2
BR
Disinfection system

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Answers to Reviewers' comments:

Reviewer #2

The literature review presented in the introduction clearly demonstrate the advantages and disadvantages of the various disinfectants. Therefore, to highlight the novelty of this manuscript, authors should clearly indicate the gap in knowledge/research and how this study contributes to filling this gap. Also a clear statement of the research objectives should be given

Answer: The last part of the introduction has been rewritten to highlight the novelty of the manuscript (i.e., the development of a more realistic experimental model), as well as the description of the research objectives. Both are clearly differentiated in two separated paragraphs across the revised version.

Lines 123-137:

"It is important to stress the fact that previous works dealing with the comparative evaluation of biocides in recreational waters only used artificial pool water with test assays in a 0.35-1.0-L beaker format under magnetic agitation (Borgmann-Strahsen 2003; Korich et al. 1990). However, biocide and microorganism dispersion and pool dynamics are more complex than those simulated by bench analysis. Therefore, extrapolation to real situations is debatable. To solve this gap, in the present work we have used a more realistic approach. Our experimental model includes a 250-L indoor pool basin with a pumping system for water recirculation and real-time monitoring of pH, temperature and redox potential. To our knowledge, this is the first time this model has been exploited, and is clearly more representative of real-life scenarios than those carried out in test tubes.

The aim of this work is to present a novel approach for in-depth investigation of the biocidal activity and the cost-effectiveness of a number of unconventional disinfection systems (namely, chlorine dioxide, hydrogen peroxide, BCDMH) in recreational waters without harmful by-product formation against conventional chlorine-laden products (hypochlorite, hypochlorite + isocyanate, tri-chloro and saline electrolysis). "

Reviewer #3

In this study, the biocidal efficacy of 7 different disinfection products in recreational waters, including 3 unconventional and 4 conventional products, were compared based on the CT values and the cost. An experimental 250-L indoor pool basin with a pumping system for water recirculation and real-time monitoring of pH, temperature and redox potential.

Compared with previous work, which used 0.35-1.0 L beaker to evaluate the performance of disinfection products in recreational water. However, disinfection products in this study were analyzed in a much larger and more realistic scale, which is one of the highlights of this study. It made the experimental results closer to the results in the full-scale swimming pool. Also, 7 disinfectants were investigated in sum, which is quite unusual.

The description of the experimental methods and the investigation of the 7 different disinfectants are detailed and clear enough

Answer: We greatly appreciate the comments from Reviewer #3, and the recognition of the advantages from our new experimental model and his/her recommendation for publication of the submission as it is.