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Solar light and the photo-Fenton process against antibiotic resistant bacteria in wastewater: A kinetic study with a Streptomycin-resistant strain

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ABSTRACT

In this work, we assess the potential of solar light and the solar-assisted photo-Fenton process to inactivate antibiotic resistant bacteria (ARB) in wastewater (WW). A systematic investigation on ARB inactivation and regrowth kinetics after solar-based processes is intended, as well as the modification of their antibiotic resistance (AR). As such, a Streptomycin-resistant (SR) *E. coli* as a model ARB was subjected to solar exposure (with or without UVB light) at various irradiance levels, as well as the solar/H_2O_2 and the photo-Fenton process at neutral pH. We report the good fit of the SR *E.coli* to known kinetic models ($> 96\%$ R^2), and analytically present the necessary treatment timed for total inactivation and halting their post-treatment regrowth capability. For all treatment methods, the AR was found to decrease during treatment, prior to loss of cultivability (5–15% lower time for 4-log inactivation). UVB irradiation was determined as the key factor of loss of cultivability, AR and regrowth. Solar/H_2O_2 and photo-Fenton presented fast inactivation rates, ensured no regrowth of ARB, and indicated moderate effect on modifying the SR of *E. coli*, with similar inactivation times for both strains. The presence of Streptomycin in WW was found to act synergistically on the faster inactivation by all processes tested. Finally, ~1 h of solar-based AOPs was found to ensure 4-log ARB inactivation and no regrowth, even in absence of the residual H_2O_2, indicating their suitability as proper WW disinfection processes.

1. Introduction

The extensive use of antibiotics has gradually become a true bane for Environmental Engineering during the last decades. In 2002, the worldwide antibiotic consumption was between 100.000 and 200.000 tons [1]. 13.288 tons of antibiotics were used in Europe in 1999 according to the European Federation of Animal Health [2], from which 65% were used in human medicine and 29% in veterinary field. Unused drugs are sometimes disposed of in the wastewater system and the non-metabolized drugs are generally ejected in to the wastewater (WW), leading to non-negligible quantities present in it [2]. The points of concentrated points of antibiotic discharge are also tangible threats, namely production sites and healthcare facilities. For instance, ampicillin was found in concentrations of between 20 and 80 μg/L in the effluent of a large German hospital [3] and ciprofloxacin was detected at concentrations between 3 and 89 mg/L effluent of a Swiss hospital [4].

One of the problems with the use of antibiotics is that they may accelerate the development of antibiotic resistance genes (ARGs) and bacteria (ARB), which are health risks to humans and animals. This is becoming a worldwide problem due to the large extent of use of the drugs [5,6] and now is on top of the priority list for the World Health Organization (WHO). A very strong correlation was reported between the quantity of antibiotics produced and the number of resistant bacteria, increasing since 1950's [7].

Urban wastewater treatment plants (WWTPs) are among the main sources of antibiotics' release into the environment [5]. Numerous studies show that ARB highly abundant in secondary effluents and end up in surface waters. In Spain, the population of ARB was monitored upstream and downstream from a WWTP on the Arga River. It was found that for 22 antibiotic resistance strains tested, 21 increased after the WWTP [8] while in the Tamma River in Japan an increase of 6.8% in tetracycline resistant bacteria was found after a WWTP [9]. These increases are due to the fact that processes for treating wastewater provide perfect mixing pots for bacteria carrying genes that confer antibiotic resistance [7], but also due to the inability of WWTPs to handle antibiotics, followed by improper or inexistent tertiary (or quaternary/advanced) treatment.

The Advanced Oxidation Processes (AOPs) have been long proposed as efficient means of degrading antibiotics, but also efficiently

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disinfecting microorganisms when applied after a secondary treatment. Unavoidably, the issue of ARB disinfection has been subjected in investigations, ranging from UV/H_2O_2, TiO_2, solar/H_2O_2 and photo-Fenton mediated solutions, as well as chemical treatments like chlorine and peracetic acid [10–16]. These processes have been found efficient in inactivating ARB in lab and pilot-scale studies, hence their application deserves attention and has place in future upgraded WWTPs, such as the effort performed in Switzerland [17].

In this work, we assess the solar disinfection of WW and the stepwise construction of the photo-Fenton process (solar, solar/H_2O_2, photo-Fenton), as a means of disinfection. The study focuses in the inactivation kinetics, the post-treatment regrowth, and as a possible issue, the antibiotic resistance modification in the treated and stored samples. As a model, a Streptomycin resistant *E. coli* is used, in order to extrapolate the know-how from the antibiotic susceptible to the Streptomycin-resistant strain, while adding to the lack of experimental evidence for this antibiotic and its high-resistant strain. Finally, the effect of the presence of antibiotics during or past the applied treatment methods was assessed.

2. Materials and methods

2.1. Microbial methods

2.1.1. Escherichia coli Streptomycin resistant strain preparation

The streptomycin resistant *E. coli* strain (Lederberg W1485, W1485) employed was provided by the "Deutsche Sammlung von Mikroorganismen und Zellkulturen". Two subsequent master plates were produced with the provided pre-culture in order to dilute and pick a single colony to produce the stock solution. 5 mL of Luria–Bertani broth was inoculated with a colony from bacterial *E. coli* pre-cultures, placed in 50 mL plastic falcons for 8 h and then loop inoculated, after 1% dilution (250 μL in 25 mL) overnight (180 rpm and 37 °C for 15 h), to achieve stationary phase cells. Harvested cells were centrifuged and washed three times (5000 rpm, 15 and 5 min for separation and washing, respectively), followed by reservation in saline solution (neutral pH solution with 8 g/L NaCl and 0.8 g/L KCl); a suspension of 10^9 CFU/mL was achieved.

2.1.2. Sampling and plating

1-mL samples were drawn and kept in sterile Eppendorf vials. The disinfection efficiency was measured by viable plate counts on Petri dishes (plastic, 9-cm diameter) containing agar without antibiotics or 4 mg/L of streptomycin. The spread-plating method was used and successive dilutions were made (using a saline solution with 8 g/L NaCl and 0.8 g/L KCl) to ensure countable numbers on the plates, i.e. 20–400 colonies/plate. The plates were prepared with 10 g tryptone, 1 g yeast extract, 8 g NaCl, 15 g agar-agar, 100 mL a 10 g/L dextrose and 3 g/L $CaCl_2$ solution in 900 mL mili-Q water. The plating process took place in 2–3 consequent dilutions and in duplicates on both non-antibiotic hard agar containing plates and on 4 mg/L streptomycin hard agar containing plates. The plates were placed at 37 °C for 18–24 h before counting the colonies. All experiments were performed in duplicates, and in 2–3 consecutive dilutions, with < 15–20% deviation (hence, error bars are not plotted).

2.2. Action of Streptomycin and determination of the antibiotic resistance of the selected strain

Streptomycin is an aminoglucoside antibiotic, which operates by inhibiting protein synthesis by binding to 30S ribosomal subunit of the cell [18]. The resistance of *E. coli* to Streptomycin is a function of its genetic background and specifically, the presence of strA-strB genes and the integron-associated aadA gene cassettes [19]. Hence, a short investigation was launched, comparing the cultivability of the Streptomycin-resistant *E. coli* strain (SR strain) in agar containing 0 through 8 mg/L of Streptomycin, to practically assess the minimum inhibitory concentration of our strain. It was found that 2 and 4 mg/L mildly affect the growth of the strain, whereas 8 mg/L is detrimental for the growth of the strain at 37 °C (Fig. S1 of the Supplementary material). Hence, for the following studies on the disinfection of the SR strain, 4 mg/L were used on the growth media, to follow only the antibiotic resistant fraction of the population.

2.3. Synthetic wastewater preparation

The synthetic wastewater was prepared with the following components following the protocol of Muthukumaran et al. [20], presented in Table S2. Bacterial spiking was performed by adding 100 μL of a 10^9 CFU/mL stock solution to the 100 mL wastewater sample. The bacterial load achieved is of 10^6 CFU/mL. The remaining water characteristics were measured and summarized in Table S3.

2.4. Treatment processes: solar light and AOPs tested in the study

The light source was a bench-scale Suntest solar simulator from Hanau, employing a 1500-W air-cooled Xenon lamp, with an illumination surface of 560 cm^2. 0.5% of the emitted photons fall within the UVB and 5–7% in the UVA region. The solar simulator also contains an uncoated quartz glass light tube and cut-off filters for UVC (290 nm) and IR wavelengths [21]. The five irradiance levels were used in this study (0, 300, 600, 900 and 1200 W/m^2) and irradiance was controlled by a coupled UV radiometer and pyranometer (CUV3 & CM6b, Kipp & Zonen, Delft, Holland). For the experiments without UVB, an identical solar simulator was used, but bearing a different cut-off filter, at 310 nm. Further UVB light was added by changing the reactor to open vessel and recalibrating the irradiance received at the sample. The Fenton and photo-Fenton processes, as well as their blanks were performed in the same conditions (solar simulator, WW, reactors) as the solar experiments. The photo-Fenton experiments took place with either neutralizing H_2O_2 with excess catalase, or not, depending on the focus of the study. Residual H_2O_2 was neutralized by catalase; 1U of enzyme decomposes 1 μmol of H_2O_2 at pH 7. Catalase was used three times more than the stoichiometric amount.

Regrowth of bacteria was measured after the storage of the samples at ambient temperature for 24 and 48 h after the sampling time. The samples were kept in 1.5 mL plastic Eppendorf caps in the dark and the population was measured to assess the post-irradiation events, after their removal from the experimental set-up. According to the study, hydrogen peroxide was neutralized with catalase as before, but in the sample caps. Both inactivation and regrowth kinetics were analyzed and modeled via GInaFiT add-on for Microsoft Excel [22].

A summary of the experiments and the conditions tested is provided below:

- Solar light alone: 300–1200 W/m^2
- Solar/H_2O_2 and Solar/Fe blanks (20 mg/L H_2O_2 or 1 mg/L Fe)
- Solar Photo-Fenton (Fe/H_2O_2 ratio: 1/20), without H_2O_2 removal.

The second part of the investigation involved the addition of 0.1 mg/L Streptomycin and reduction of H_2O_2 to 10 mg/L in the corresponding experiments, the modification of light source described before, as well as neutralization of excess H_2O_2 by catalase.

2.5. Chemicals and reagents

The chemicals information (name, formula, molecular weight, CAS No and supplier) used for the preparation of the synthetic wastewater, the cultivation media (agar plates) and the Fenton reagents are summarized in Table S1.