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Effective solar processes in fresh-cut wastewater disinfection: Inactivation of pathogenic *E. coli* O157:H7 and *Salmonella enteritidis*

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ABSTRACT

The disinfection of synthetic fresh-cut wastewater (SFCWW) by several solar processes has been investigated as an alternative to the commonly used chlorination in this industry. To this end, a SFCWW recipe was developed based on real sample analysis from the fresh-cut industry and literature data. It is characterized mainly by the presence of organic matter in solution (25 mg/L DOC) and turbidity (100 NTU). The inactivation kinetics of human pathogenic bacteria (*E. coli* O157:H7 and *Salmonella enteritidis*) in SFCWW was assessed by solar photo-inactivation, H_2O_2/solar, iron/solar and solar photo-Fenton processes. Excellent inactivation performance was achieved in all cases demonstrating the capability of solar processes to disinfect water even at high levels of turbidity. The best bacterial inactivation rates were obtained with the H_2O_2/solar process (20 mg/L) in less than 60 min for both *E. coli* and *S. enteriditis*. Moreover, the influence of UVA irradiance (10–50 W/m^2) on the inactivation of both pathogens by the H_2O_2/solar process at several H_2O_2 concentrations was investigated. These results showed a slightly different response for both pathogens against UVA irradiance and H_2O_2 concentration: photo-limitation for both types of bacteria; while only *E. coli* was limited by the H_2O_2 concentrations investigated in this work.

1. Introduction

Over recent decades, in developed countries, the demand for healthy, nutritious and fresh foods marketed as "ready-to-eat" has increased, encouraging rapid development of the so-called fresh-cut produce industry. Fresh-cut products are defined according to theInternational Fresh-Cut Produce Association as '*any fresh fruit or vegetable or combination thereof physically altered from its original form, but remaining in a fresh state*' [1].

This industry is one of the major industrial water consumers (ca. 2–11 m^3/ton of product); as water is used in several steps of the process including cooling, cleaning as well as the product washing step which is the major water consumption step in this industry [2]. The latter is the critical step in ensuring the microbiological safety of the product and to control the risk of cross contamination [3]. In the last few decades, outbreaks of infections linked to the consumption of fresh-cut products have increased in Europe and they have been mainly associated with the faecal pathogens *E. coli* O157:H7 and *Salmonella* spp.

Chlorine is the most widely water disinfectant used in this industry due to its low price and ease of procedure. Nevertheless, it presents some disadvantages, like the formation of toxic vapors and unhealthy disinfection by-products (DBPs) by reactions with the organic matter present in the water that persist in the final product [4]. For these reasons, certain European countries like Germany or Belgium have banned the use of chlorination in this type of industrial process for food [5], requiring the use of alternative procedures.

Different water treatments including electrolyzed oxidizing water, chlorine dioxide, organic acids and filtration, among others, have been studied with the aim of replacing the use of chlorine in the fresh cut industry. The implementation of these technologies has shown good disinfection efficiencies but the altering of organoleptic properties of the fresh product and high-cost in some cases has made them inefficient individually [6]. In this regard, Advanced Oxidation Processes (AOPs) have proven to be effective for decontamination and disinfection of different types of water matrixes. These processes are based on the generation of hydroxyl radicals (HO•), the strongest Reactive Oxygen Species (ROS) after fluoride. It is a non-selective oxidizing agent meaning it may react with almost any organic chemical compound until complete mineralization and/or eventually killing bacterial cells in water [7]. Among the AOPs, those making use ofa UVC-lamp (low Hg pressure, maximum 254 nm) as a photon source such as UVC/H_2O_2 [8] or UVC/TiO_2 [9,10] and ozone [11] have been employed and

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investigated as a disinfection treatment in this industry. However, AOPs driven by natural sunlight as a source of photons to disinfect fresh-cut wastewater (FCWW) have not been yet investigated. The use of an environmentally friendly source of energy such as natural solar radiation may drastically reduce the cost of wastewater treatment.

One of the most common AOPs investigated for solar water disinfection and decontamination is the so-called photo-Fenton process. The main reactions involved in this catalytic cycle are:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + HO^{\cdot} \quad k = 70 M/s \tag{1}$$

$$Fe(OH)^{2+} + h\nu \rightarrow Fe^{2+} + HO^{\cdot} \tag{2}$$

In last years, some studies have reported on the capability of solar photo-Fenton for wastewater disinfection, including the inactivation of different types of waterborne pathogens groups such as bacteria (*E. coli, E. faecalis, Salmonella*, spores of *Clostridium* sp), fungi (like *Fusarium* sp), virus (MS2 bacteriophage, FRNA) and also demonstrating the efficiency of this process to inactivate pathogens at near neutral pH [12]. Others authors had been also reported on the use of complexing agents such as (S,S)-Ethylenediamine-N,N'-disuccinic acid (EDDS) to keep the iron in solution at neutral pH and increase the photo-Fenton efficiency [13]. Recently, some studies have been also demonstrated the photo-Fenton‘s capability to remove simultaneously antibiotic-resistant bacteria and antibiotics presents in real secondary effluents [14].

Furthermore, another solar disinfection process, namely, the photo-inactivation of bacteria assisted by H_2O_2 (H_2O_2/Solar) has recently shown good inactivation efficiencies using low oxidant concentrations under natural sunlight. It is believed that the inactivation mechanism is a combination of a photo-oxidative process induced by solar photons and the generation of internal ROS by photo-Fenton reactions due to reactions between natural occurring iron and H_2O_2 diffused inside the microbial cell. ROS react with DNA, generating lethal and mutagenic nucleic base modifications and single strand breaks ending in cell death [15,16].

This work reports on the capability of three solar treatments: photo-Fenton, photo-inactivation and photo-inactivation assisted with H_2O_2 at near-neutral pH as alternative treatments to chlorine compounds used for the inactivation of pathogenic bacteria *E. coli* O157:H7 and *S. enteritidis*. To obtain more realistic results under standardized conditions, this study started with the development, for the first time, of a synthetic fresh-cut wastewater (SFCWW) model which allows an exhaustive and standardized efficiency comparison between different treatments and conditions. The developed SFCWW recipe contains organic matter in solution and turbidity, parameters that may drastically affect the disinfection performance of any solar process. Using this recipe the influence of solar UVA irradiance (ranging from 10 to 50 W/m^2) and reagent concentrations (2.5 mg/L of iron; 2.5–20 mg/L of H_2O_2) was experimentally determined under controlled conditions.

2. Materials and methods

2.1. Bacterial enumeration and quantification

E. coli strain O157:H7 (CECT 4972) and *Salmonella subsp. Enteritidis* (CECT 4155) were obtained from the Spanish Type Culture Collection (CECT). Fresh liquid suspensions were obtained from Nutrient-Broth Agar I and Tryptone Soya Broth for *E.coli* and *S. enteritidis,* respectively and incubated at 37 °C with rotary shaking for 20 h. The initial bacterial concentration in the reactor was 10^6 CFU/mL. All water samples taken during solar experiments were enumerated using the standard plate counting method with ChromoCult®Coliform Agar (Merck KGaA, Darmstadt, Germany) for *E. coli* O157:H7 and Salmonella Shigella Agar (Scharlau®, Spain) for *S.enteritidis* and incubated at 37 °C for 18–48 h, respectively. The detection limit (DL) was 20 CFU/mL.

2.2. Reagents and analytical measurements

Ferrous sulphate heptahydrate ($FeSO_4 \cdot 7H_2O$, Panreac, Spain) and ferric nitrate ($Fe(NO)_3 \cdot 9H_2O$, Panreac, Spain) were used to obtain the desired concentration of Fe^{2+} and Fe^{3+}, respectively. Dissolved iron was measured with 1,10-phenanthroline according to ISO 6332.

Hydrogen peroxide (35% w/v, Merck, Germany) was diluted directly in the solar reactor to obtain the desired concentration. The H_2O_2 concentration was measured using a colorimetric method with Titanium (IV) Oxysulfate (Riedel-de-Haën, Germany) [17].

Catalase (Sigma-Aldrich, USA) was added to samples containing H_2O_2 to eliminate its residual concentration [17].

The following reagents and concentrations were used to formulate SFCWW: Malt extract (55 mg/L); Sodium hydroxide (5 µL of a solution 2 M) (Panreac, Spain); Kaolin (Merck, Germany) (125 mg/L); Sodium chloride (160 mg/L), Ammonium chloride (0.7 mg/L), Magnesium sulphate anhydrous (49 mg/L) (Sigma-Aldrich, USA); Sodium bromide (13 mg/L), Sodium fluoride (0.6 mg/L) (Merck, Germany); Sodium nitrate (65 mg/L), Calcium chloride dehydrate (145 mg/L) (Riedel-de-Haën, Germany); Sodium sulphate anhydrous (10 mg/L), Potassium chloride(205 mg/L) (J.T Baker, USA).

2.3. Experimental procedure

Disinfection assays were carried out in a solar simulator (Atlas Suntest XLS+, USA). Experiments were carried out in an open glass vessel reactor (19 cm diameter) with an illuminated surface of 0.0284 m^2. Assays were done with 700 mL of SFCWW which was completely illuminated and magnetically stirred at 450 rpm. Solar tests were carried out at a controlled temperature below 30 °C to avoid any thermal effect on the bacterial viability.

Prior to solar exposure, reagents and microbial suspension of both pathogens were directly diluted in the reactor to obtain the desired initial concentrations. Both pathogens were inoculated at the same time and treated simultaneously. After 5 min agitation in the dark, the first sample was taken and the reactor was exposed to the artificial irradiation. The inactivation kinetics of each bacteria observed during the solar treatments were calculated using Chick’s law [18].

2.4. Physicochemical FCWW analysis

The following devices were used for FCWW characterization: pH meter (multi720, WTW, Germany), conductivity meter (GLP31, CRISON, Spain), turbidimeter (Model 2100N, Hach, USA). Ion composition of water samples was measured with an ion chromatograph (IC) (Model 850, Metrohm, Switzerland). Dissolved Organic Matter was measured with a TOC analyzer (Model 5050, Shimadzu, Japan).

Absolute transmittance of water with and without turbidity was assessed using a Spectrophotometer (AvacSpec Dual Channel Fiber Optic, Avantes, USA) with an Avantes FC-UV-200-2 probe and a COL-UV/VIS detector (selected spectral range: 300–400 nm).

3. Results and discussion

3.1. Synthetic fresh-cut wastewater (SFCWW) recipe

A SFCWW recipe was developed, based on the wastewater sample characterization obtained from the local fresh-cut industry company:' Verdifresh' (Málaga, Spain). Samples from two different washing-tanks (spinach and lettuce) were collected and analyzed. A total of 8 water samples were collected from 06:20 to 22:00, the typical time that the washed water from each tank is used before being discharged. The averaged chemical characterization is shown in Table 1. Significant differences between spinach and lettuce washing water were observed, and as expected, a significant accumulation of ions throughout the washing time was detected. Several tons of vegetables are commonly