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Power ultrasound decontamination of wastewater from fresh-cut lettuce washing for potential water recycling

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ABSTRACT

The decontamination effect of pulsed and continuous power ultrasound, provided at either controlled or uncontrolled temperature regimes, was studied with reference to native microflora and inoculated pathogenic bacteria in wastewater obtained by fresh-cut lamb's lettuce washing. Results showed that decontamination efficacy increased with increasing specific energy and was higher when ultrasound treatment was provided under uncontrolled temperature regime. Continuous ultrasound supplied without temperature control allowed to achieve 3.2 Log reductions of native microflora during 20 min treatment, while 5 Log reductions of inoculated *Listeria monocytogenes*, *Escherichia coli* and *Salmonella enterica* were attained within 5 min of ultrasonication. The heat generated during continuous ultrasound accounted for approximately 58% of the total decontamination effect against *L. monocytogenes*, while it contributed for 100% to *E. coli* and *S. enterica* inactivation.
Industrial relevance: The application of power ultrasound combined with *in situ* generated heat could represent an effective tool for water decontamination and recycling in the fresh-cut industry. In addition, besides safety requirements, this technology would also meet cost-effectiveness criteria and existing standards.

1. Introduction

Nowadays, water scarcity is a major issue at global level. It has been estimated that in the next 15–20 years, the water supply-to-demand gap will be approximately 40%. Tackling the water gap is a challenge for (Horizon 2020) EU research programme. The food sector greatly contributes to water scarcity. It has been estimated that about 20–50% reduction in water consumption in the food sector can be achieved by recycling and reuse of water (Hiddink, Schenkel, Buitelaar, & Rekswinkel, 1999).

The fresh-cut vegetables market has grown considerably in the last few decades in response to an increased demand for fresh-like, healthy and convenient foods. Fresh-cut vegetables production requires intensive use of water to both wash and move vegetables along the production line. In order to secure water supply and protect the environment from the adverse effects of the wastewater discharges (EEC, 1991), water recycling in the fresh-cut industry has to be improved. Recycling of water that is intended to re-enter the washing step, implies wastewater disinfection. As well known, a 5 Log reduction of pathogenic bacteria is the generally accepted requirement for safe water disinfection. Wastewater decontamination may be accomplished by means of chemical and physical interventions (Casani, Rouhany, & Knøchel, 2005; Olmez & Kretzschmar, 2009). Among these, sodium hypochlorite is the most used due to its low cost and easy use (Gil, Selma, López-Gálvez, & Allende, 2009; Olmez & Kretzschmar, 2009). However, not only wastewater containing chlorine has a great environmental impact, but also chlorination disinfection by-products are known to represent a potential risk for human health (Itoh, Gordon, Callan, & Bartram, 2011). Consequently, there is great effort to find suitable technologies to allow wastewater recycling (Artés, Gómez, Aguayo, Escalona, & Artés-Hernández, 2009; Casani et al., 2005; Olmez & Kretzschmar, 2009). Power ultrasound has been suggested as a technology alternative to chlorination for wastewater decontamination (Neis & Blume, 2002; Piyasena, Mohareb, & McKellar, 2003). Ultrasound frequencies higher than 20 kHz are actually considered safe, non-toxic and environmentally friendly (Kentish & Ashokkumar, 2011). During ultrasound treatment, cavitation phenomena occur into the liquid medium causing a rapidly alternating compression and decompression zones, which are in turn responsible for generating shock waves with associated local very high temperatures and pressures, as well as free radicals and hydrogen peroxide (Leighton, 1994; Mason, Joyce, Phull, & Lorimer, 2003). Ultrasound effectiveness in wastewater decontamination was found to increase with the power input and exposure time, and to depend on microorganism type (Elizaquivel, Sanchez, Selma, & Aznar, 2012; Gao, Lewis, Ashokkumar, & Hemar, 2014; Hulsmans et al., 2010; Joyce, Phull, Lorimer, & Mason, 2003; Scherba, Weigel, & O'Brien, 1991). Improved efficiency of ultrasound technology can be obtained by its combination with other biocidal treatments, such as chlorination (Ayyildiz, Sanik, & Ileri, 2011; Drakopoulou, Terzakis, Fountoulakis, Mantzavinos, & Manios, 2009), organic acids (Gómez-López, Gil, Allende, Vanhee, & Selma, 2015) and ultraviolet irradiation

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(Blume & Neis, 2004; Gómez-López et al., 2015; Mason et al., 2003; Naddeo, Land, Belgiorno, & Napoli, 2009). An increase of microbial sensitivity to ultrasound in combination with temperature increase, experienced with ultrasonic treatment, for wastewater disinfection has been also reported (Gómez-López et al., 2014; Madge & Jensen, 2002; Salleh-Mack & Roberts, 2007). It has been estimated that the heat generated during ultrasound processing accounted for approximately 52% of the resulting disinfection (Madge & Jensen, 2002).

In contrast with the huge number of studies in the literature dealing with ultrasound decontamination of municipal wastewater and effluents as well as model fluids, very few studies investigated ultrasound effectiveness for water decontamination deriving from fresh-cut vegetable production (Elizaquivel et al., 2012; Gómez-López et al., 2014, 2015). It has been demonstrated that power ultrasound was effective in inactivating pathogenic bacteria inoculated in fresh-cut lettuce wash water (Elizaquivel et al., 2012), especially in the presence of the residual peroxyacetic acid concentration that can be found in the wash water (Gómez-López et al., 2015). In these studies, ultrasonic treatments were carried out with temperature control, allowing the inactivation effects of ultrasound only to be evaluated. In another study, Gómez-López et al. (2014) showed that ultrasound disinfection against *Escherichia coli* O157:H7 inoculated in fresh-cut lettuce wash water can be increased by combination with heating. Reductions of 6 Log of this microorganism were actually achieved after 60 and 20 min of ultrasonication with and without temperature control, respectively.

In light of this, there is a lack of knowledge on the efficacy of power ultrasound in combination with *in situ* generated heat against naturally occurring microflora and foodborne pathogens, other than *E. coli*, potentially contaminating fresh-cut vegetable wash water.

In this study, the efficacy of power ultrasound in decontaminating wastewater deriving from fresh-cut vegetable washing was investigated. To this aim, wastewater obtained by washing fresh-cut lamb's lettuce was subjected to power ultrasound, provided in pulsed or continuous modality, with or without temperature control. The decontamination efficacy of the treatments was evaluated on both the native microflora and inoculated pathogenic bacteria, i.e., *Listeria monocytogenes*, *E. coli* and *Salmonella enterica*. These microorganisms were chosen due to their natural occurrence in a water environment and because they are generally considered indicators of fecal contamination (Szewzyk, Szewzyk, Manz, & Schleifer, 2000). The final goal was to find the potentiality of combined ultrasound with *in situ* generated heat in the attempt to implement strategies for efficient management of water resource in the fresh-cut industry. To this regard, the decontamination efficacy was related to the ultrasound cavitation and heat contributions.

2. Materials and methods

2.1. Preparation of fresh-cut vegetable wash water

Lamb's lettuce (*Valerianella locusta* Laterr.) was purchased from a local market. Lettuce leaves were placed into a beaker containing tap water at 18 °C ± 2 °C (the vegetable–water ratio was 1:30 *w*/*v*). After 1 min of washing, water was separated from the leaves by using a domestic salad spinner.

2.2. Bacterial strains and inoculum preparation

The microorganisms used for inoculum were *L. monocytogenes*, *E. coli* and *S. enterica* subsp. *enterica* 9898 DSMZ, obtained from the bacterial culture collection of the Department of Food Science of the University of Udine (Italy). Strains were maintained at −80 °C in Brain Heart Infusion broth (BHI, Oxoid, UK) with 30% sterile glycerol as cryoprotectant until use. Strains were incubated in BHI at 37 °C for 24 h, subsequently cultured in 5 mL of BHI at 37 °C for 24 h, and finally collected by centrifugation at 14,170 *g* for 10 min at 4 °C (Beckman, Avanti TM J-25, Palo Alto, CA, USA) and washed three times with Maximum Recovery Diluent (MRD, Oxoid, UK). The final pellets were suspended in MRD and used as inoculum. A final concentration of approximately 10^6 CFU/mL was obtained for each bacteria suspension.

2.3. Power ultrasound treatment

An ultrasonic processor (Hieschler Ultrasonics GmbH, mod. UP400S, Teltow, Germany) with a titanium horn tip diameter of 22 mm was used. The instrument operated at constant ultrasound amplitude and frequency of 100 μm and 24 kHz, respectively. Aliquots of 200 mL of wash water inoculated or not with *L. monocytogenes*, *E. coli* and *S. enterica* were introduced into 250 mL capacity (110 mm height, 60 mm internal diameter) glass vessels. The tip of the sonicator horn was placed in the centre of the solution, with an immersion depth in the fluid of 10 mm. The ultrasound treatments were performed for increasing lengths of time up to 20 min. During the ultrasonication experiment, the temperature was either controlled (<35 °C) using an ice bath, to dissipate the heat generated during treatment, or uncontrolled, leaving the temperature to rise due to heat dissipation. The sonicator operated either in pulsed mode or continuous mode. In the pulsed mode, the pulse duration period of 0.5 s was followed by a pulse interval period of 0.5 s, during which the sonochemical reactor was switched off. Before and after each experiment, the ultrasound probe was disinfected by washing with ethanol followed by through rinsing with sterile water.

2.4. Thermal treatment

The total temperature–time combination received by water during continuous ultrasound under uncontrolled temperature regime was applied to the wastewater in the absence of the ultrasound treatment. To this purpose, aliquots of 200 mL of wash water were introduced into 250 mL capacity glass vessels and heated in a thermostatic water bath (Ika Werke, MST BC, Staufen, Germany) under continuous stirring, by mimicking the same temperature rise produced by the probe during continuous ultrasound treatment under the uncontrolled temperature regime.

2.5. Microbiological analysis

Both naturally present and inoculated microorganisms were quantified at different time intervals during the ultrasound and heat treatments. The wastewater samples were diluted 10 fold with MRD (Oxoid, UK). Total viable count of non inoculated water was enumerated by spreading onto plates with Plate Count Agar (PCA, Oxoid, UK) and incubating at 30 °C for 48 h. *L. monocytogenes* and *S. enterica* concentrations were determined by plating on Palcam Agar (PA, Oxoid, UK) and Xylose Lysine Desoxycholate agar (XLD, Oxoid, UK), respectively, at 37 °C for 48 h, while the Coli ID medium (BioMerieux, Mercy L'Etoile, France) was used for *E. coli* concentration determination, followed by incubation at 37 °C for 24 h.

Preliminary trials were carried out on the non inoculated wastewater to check for *Salmonella* spp. and *L. monocytogenes* presence and enumerate *E. coli*. For *Salmonella* spp., 25 mL of wastewater was diluted with 225 mL of Buffered Peptone Water (BPW, Oxoid, UK), homogenized in a Stomacher Lab-Blender 400 (VWR International PBI srl, Milano, Italy) for 2 min and incubated at 37 °C for 24 h. Aliquots of 0.1 mL of BPW were added with 9.9 mL Rappaport Vassiliadis (RV, Oxoid, UK) and incubated at 42–43 °C for 18–24 h. The presence/absence of *Salmonella* spp. was checked by spreading onto XLD agar plates and incubating at 37 °C for 24 h. For *L. monocytogenes*, 25 mL of wastewater were diluted with 225 mL of Fraser Broth (FB, Oxoid, UK), homogenized in a Stomacher for 2 min and incubated at 30 °C for 36–48 h; 1 mL of FB was added with 9 mL of FB and incubated at 37 °C for 24–48 h. The presence/absence of *L. monocytogenes* was checked by spreading onto