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# Inactivation of bacteria in seafood processing water by means of UV treatment

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ABSTRACT

Seafood processing is a large-scale food industrial activity, in the UK and worldwide, which requires substantial quantities of clean water for washing purposes. Therefore, the aim of this study is to assess the feasibility of ultraviolet (UV) treatment to disinfect water coming from shellfish washing process, as to safely recycle it in the process. For this reason, different operating parameters that typically affect UV treatment efficiency, namely the power output of the UV lamp (5 W, 9 W, and 11 W), the turbidity of the washing water (0–52 NTU), and the initial bacterial concentration ($10^4$, $10^5$, $10^6$ CFU $mL^{-1}$) were studied. Water disinfection was monitored by following changes in the concentration of the *Escherichia coli* (*E. coli*) bacteria. Photoreactivation of bacteria after UV disinfection was also investigated. Results showed that the UV treatment can efficiently inactivate bacteria in shellfish processing water, since *E. coli* ($10^6$ CFU $mL^{-1}$) in turbid (i.e. 0.074–35 NTU) seafood processing water were inactivated within the first 15 s of treatment, by means of an 11 W germicidal lamp. Under these conditions, no bacteria photoreactivation was observed after 2 h of exposure to natural light. The disinfection efficiency was decreased when the initial bacterial concentration and water turbidity were increased. In addition, the increase of UV power output resulted in a substantial increase of bacterial inactivation. Furthermore, *E. coli* were reactivated after 2 h of exposure to natural light when the turbidity of the washing water was ≥42 NTU or when the initial bacterial concentration was high (i.e. $10^5$ and $10^6$ CFU $mL^{-1}$).



## 1. Introduction

Shellfish farming and packaging is a large-scale food industrial activity in the UK and worldwide. The UK exports most of the seafood it harvests, thus resulting to high economic gains (e.g. in 2011 just over 435,000 tonnes of seafood, worth £1.46 billion, were exported from the UK (Seafish, 2012a)). High value shellfish, such as langoustine, crab and scallops, are exported to the French, Spanish and Italian markets (Seafish, 2012a). Moreover, Scotland dominates the UK seafood processing industry, while secondary processing units are found in the North England and Wales, thus providing 11,864 full-time jobs in 325 units throughout the UK (data for 2011) (Seafish, 2012a,b). To maintain the high quality and profitability of the UK shellfish species, domestic suppliers have focused on improving the sustainability of their farming, as well as their packaging process.

Shellfish packaging requires vigorous washing and scrubbing with clean water, as to ensure maximum removal of sediments and other debris. Water should be taken from an appropriate source, which is usually sea or tap water (Massachusetts General Laws, 2015). Nonetheless, seawater pumping is an energy intensive process, while also it may be inappropriate due to high pollution levels. It has been extensively reported that seawater in the European continent and worldwide face great challenges due to heavy metal (Besada et al., 2011; Kallithrakas-Kontos and Foteinis, 2016; Wang et al., 2013) and oil pollution (Cohen, 2013). Moreover, many seafood processing industries are sited inland, thus seawater utilization is unpractical. In these cases, tap water is the only solution, but its use can significantly increase operational costs and negatively affect the sustainability of the process. Furthermore, shellfish processing machinery consumes large amounts of water (e.g. for shellfish washing, equipment and floor cleaning), while water reclamation and recycling is not applied. Therefore, water minimization and reuse strategies should be introduced in such industries, as to make seafood washing more efficient and sustainable, thus improving their overall environmental footprint, competitiveness and profitability.

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Ultraviolet (UV) irradiation is a well-established treatment technology for bacterial inactivation in water, air and solid surfaces and is one of the approved technologies used for food processing and preservation (EPA, 2006; Gardner and Shama, 1999; Quek and Hu, 2013; Venieri et al., 2013). The efficiency of UV treatment is attributed to the hazardous effects of UV-C radiation, which can destroy directly the DNA and the outer cell membrane of pathogenic microorganisms (Chatzisymeon et al., 2011; Venieri et al., 2013). UV-C irradiation between 250 nm and 270 nm, where the maximum absorbance of nucleotide bases of the genome occurs, including thymine, cytosine and uracil, can induce damages in DNA and RNA, thus inhibiting cell transcription and replication (Vélez-Colmenares et al., 2012). Specifically, the major DNA lesion, induced by germicidal UV-C irradiation at 254 nm, is the formation of pyrimidine dimers. The presence of these lesions inhibits the normal replication of DNA, and therefore results in inactivation of the microorganisms in short time periods (Nebot Sanz et al., 2007). In addition, UV disinfection does not require chemical reagents, thus another advantage is that there is no formation of hazardous disinfection by-products after treatment (Summerfelt, 2003). However, its main drawback is that many microorganisms, including bacteria, are known to possess the ability to repair their DNA damage in the presence (photoreactivation) or absence (dark repair) of light (EPA, 2006; Nebot Sanz et al., 2007; Quek and Hu, 2013; Sinha and Hader, 2002). This can lead to the reactivation of bacteria, after UV treatment, thus affecting disinfection efficiency and rendering UV treatment unsafe. Till now, few studies have dealt with the use of UV irradiation for food processing, including the inactivation of bacteria on raspberries and strawberries (Bialka et al., 2008), in fruit juices (Gayán et al., 2012; Müller et al., 2011; Santhirasegaram et al., 2015), apple cider (Unluturk et al., 2004), goat milk (Kasahara et al., 2015), and in liquid egg products (Unluturk et al., 2008). However, to the best of the author's knowledge, there is no study dealing with the application of UV for the treatment of seafood processing waters.

Therefore, the aim of this study is to investigate the feasibility of the UV method to disinfect shellfish washing water, thus being able to safely recycle treated water in the process. For this purpose, washing water from a shellfish processing industry was used and various operating parameters that typically affect UV efficiency were studied. These were the lamp power output, the initial bacterial concentration, water turbidity and treatment time. The effect of bacterial photoreactivation on treatment durability was also examined, as to ensure the feasibility of the process.

## 2. Materials and methods

### 2.1. Shellfish processing water

Shellfish processing water was collected from an industry that uses tap water for shellfish washing, located in the UK. The processing water originates from the industry's shellfish washing line, where tap water is initially used and then it is collected in tanks (about 250 L) and reused, if appropriate, in the washing process. However, shellfish-associated bacteria, including potential pathogens and spoilage organisms, build up in the tanks, thus rendering the used water inadequate for recycling purposes after a short period of time. Therefore, this water has to be disposed of, every about 10 min, when the bacterial concentration becomes too high, thus preventing the efficient water recycling in the washing stage, and fresh tap water needs to be introduced in the system. Shellfish-associated bacteria can include *Vibrio* and *Shigella* species, *Salmonella*, or other toxin-forming bacteria (Iwamoto et al., 2010). In this work, water disinfection was monitored by following changes in the *Escherichia coli* bacteria, which is a common and very popular indicator pathogenic microorganism for potable water (Chatzisymeon et al., 2011), since according to current legislation the quality of seafood washing water should follow the standards of drinking water (MassachusettsGeneralLaws, 2015).

In order to measure bacterial contamination in the used washing water and assess the feasibility of the UV treatment, tap water was continuously (i.e. every 10 min) recycled in the shellfish washing line for up to 40 min. Washing water samples were withdrawn after 10, 20, 30, and 40 min of washing, as to measure their physicochemical and microbiological characteristics. The water samples were collected in sterilized sampling bottles of 1 L, kept at 4 °C and immediately dispatched for further analyses. After measuring their characteristics, samples were sterilized at 121 °C for 15 min and kept in the fridge (4–8 °C).

### 2.2. Bacterial strain

The bacterial strain of *E. coli*, which was used in this work as a water quality indicator, was isolated from the shellfish washing waters by membrane filtration. From the collected samples, 200 μL were passed through a 0.45 μm pore-sized filter (cellulose acetate/nitrate membranes by Sigma–Aldrich) using a vacuum pump VP series (KNF Lab). These membranes were aseptically placed up on plates with Brilliance *E. coli*/Coliform Agar (Oxoid) selective media, thus ensuring that no air bubbles were trapped. The plates were incubated at 37 °C for 20–24 h and *E. coli* colonies with purple-blue colour were picked for further use. Specifically, the isolated *E. coli* were spiked into the sterile industrial washing water to achieve the desired initial bacterial loading for each experimental run. The standard *E. coli* ATCC 23716 (American Type Culture Collection, Rockville, MD, USA) strain was also used. The freeze-dried cultures were rehydrated and reactivated according to the manufacturer's instructions. The concentration of bacterial cells in the shellfish processing water ranged from $10^4$–$10^6$ CFU $mL^{-1}$, as estimated by measuring its optical density at 600 nm on a Cary100 UV–Vis double-beam (Varian, Inc.) spectrophotometer.

### 2.3. UV experiments

Experiments were conducted in an immersion well, batch type, laboratory scale photoreactor shown in Schematic 1. This is a two compartment apparatus and consists of an inner, quartz glass housing the lamp and an exterior cylindrical reaction vessel made of borosilicate glass. The reaction mixture was placed in the exterior cylindrical reaction vessel (compartment 1) and the inner quartz glass was immersed inside the reaction mixture. The UV lamp was placed inside the inner glass tube (compartment 2). It should be noted that this apparatus was constructed and assembled in the workshop of the University of Edinburgh, UK. In a typical experimental run, 300 mL of the shellfish processing water were introduced in the reaction vessel. The bacterial suspension was magnetically stirred, to ensure complete mixing of *E. coli* with the processing water, and then the UV lamp was turned on. UV-C irradiation, with emission wavelength at 254 nm, was provided by an 11 W (11TUV, PL-S, Philips) or a 9 W (PL, 2 PIN, Philips) or a 5 W (5TUV, PL-S, 2G7 base, Philips) germicidal lamp. The temperature was constant at 18 ± 1 °C (i.e. ambient temperature), during each experimental run, since in the shellfish processing industry the washing process takes place at ambient conditions. The exterior reaction vessel was covered with aluminium foil to reflect back UV irradiation. Representative experiments were carried out in triplicates to check the reproducibility of the process. At specific time intervals, 2 mL of the reaction solution were withdrawn and immediately analysed with respect to viable *E. coli* cells, by the serial dilution culture method.