Biochemical Engineering Journal 104 (2015) 115–122

Contents lists available at ScienceDirect

Biochemical Engineering Journal

journal homepage: www.elsevier.com/locate/bej

Efficacy of antimicrobial combinations to reduce the use of sodium hypochlorite in the control of planktonic and sessile *Escherichia coli*

Ana Meireles, Idalina Machado, Rita Fulgêncio, Filipe Mergulhão, Luís Melo, Manuel Simões*

LEPABE, Department of Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, s/n, 4200-465 Porto, Portugal

ARTICLE INFO

Article history:
Received 18 December 2014
Received in revised form 23 February 2015
Accepted 26 February 2015
Available online 28 February 2015

Keywords:
Biocidal combination
Biofilms
Control
Immobilized cells
Microbial growth
Sodium hypochlorite

ABSTRACT

Foodborne illness outbreaks linked to fresh produce are becoming more frequent and widespread. The types and properties of the chemical agents used for washing, cleaning and disinfection procedures, particularly their toxicity are the key indicators of environmental performance of a minimally processed vegetables (MPV) industry. The main aims of this work were focused on the evaluation of selected disinfectants (chlorine dioxide, peracetic acid, hydrogen peroxide, copper sulphate, vanillin and sodium bicarbonate) alone and combined with sodium hypochlorite on the control of *Escherichia coli* planktonic and sessile cells. The most effective disinfectants tested in planktonic cells were peracetic acid (6 mM) and chlorine dioxide (3 mM) and the best combination with sodium hypochlorite (3 mM) was obtained with peracetic acid (2 mM). In sessile cells, hydrogen peroxide and vanillin had antagonistic effects in combination with sodium hypochlorite whereas sodium bicarbonate efficiency was enhanced when combined with sodium hypochlorite.

1. Introduction

Nowadays, consumers are more conscious of the importance of a healthy life and with what they eat [1]. Since fresh produce is a good and natural source of vitamins and nutrients, its consumption has increased. In parallel, foodborne illness outbreaks are becoming more frequent due to an increasing availability of fresh produce [2–4]. Current decontamination techniques show limited efficiency in reducing pathogen levels [1,5,6]. The chemicals used for washing, cleaning and disinfection procedures, as well as the toxicity of these chemical agents are the key indicators of environmental performance of a minimally processed vegetables (MPV) industry [7]. Sodium hypochlorite (SH) is widely used for MPV, but it produces unhealthy by-products and its efficiency in disinfection is largely reduced by the presence of organic matter [8,9]. Moreover, the possible formation of carcinogenic chlorinated compounds in water raised concerns on the use of SH in food processing [10]. In fact, SH is included in the indicative list of the Directive on Industrial Emissions (IPCC, 2007/0286 (COD)) [11] as a major pollutant for water emissions and on the formation of carcinogenic and mutagenic products in the presence of organic matter [12,13]. Alternative disinfection methods have been recently proposed: physical methods, such as pulsed light and ultrasound [7,14]; and chemical methods, such as ozone [12], phytochemicals [15], hydrogen peroxide (HP) [12], copper sulphate (CS) [16], peracetic acid (PA) [17,18], sodium bicarbonate (SB) [19] and chlorine dioxide (CD) [20]. These methods can help to reduce the use of SH in cleaning and disinfection steps, especially if used in combination. This can result in a synergistic effect, i.e., the combination of disinfectants can lead to a reduction of the disinfectants concentration, compared to when they are applied individually [21].

HP can have a bactericidal (death) or a bacteriostatic (inhibitory) effect on the microorganisms [12,22]. It can be applied on food surface material [23] and its main advantage is the rapid decomposition into water and oxygen by catalase [12]. Despite the fact of having the generally recognized as safe (GRAS) status [24], HP is not allowed by the USA Food and Drug Administration (FDA) [12].

CS is extensively used as a fungicide [25]. The application of copper combined with lactic acid was previously reported [26,27]. The

Abbreviations: CD, chlorine dioxide; CFU, colony forming units; CS, copper sulphate; HP, hydrogen peroxide; LR, log CFU reduction index; LR_c, log CFU reduction of the compound in the combination; LR_i, log CFU reduction of the compound used individually; MBC, minimum bactericidal concentration; MHB, Mueller–Hinton broth; MIC, minimum inhibitory concentration; MIC_c, MIC of the compound in the combination; MIC_i, MIC of the compound used individually; PA, peracetic acid; PCA, plate count agar; PS, polystyrene; SB, sodium bicarbonate; SH, sodium hypochlorite; SS, stainless steel; VN, vanillin.

* Corresponding author. Tel.: +351 225081654.
E-mail address: mvs@fe.up.pt (M. Simões).

http://dx.doi.org/10.1016/j.bej.2015.02.035
1369-703X/

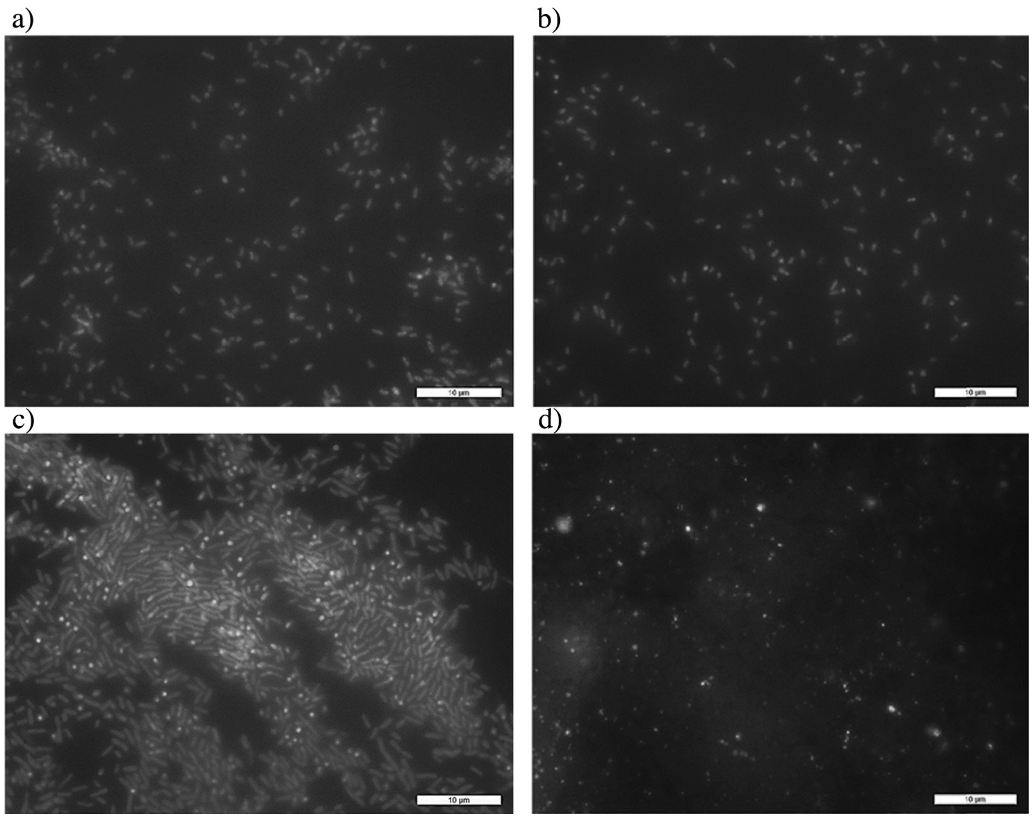

Fig. 1. Microscopy visualization of adhered cells (2 h) on PS (a) and SS (b), and biofilm (24 h) on PS (c) and SS (d). Magnification ×1000 and scale bar 10 μm. Adhered cells and biofilms were stained with 4′,6-diamidino-2-phenylindole (DAPI, Sigma, Portugal). Each slide was stained with 20 μL of DAPI at a concentration of 0.5 μg/mL. After 10 min of incubation in the dark, the slides were mounted with non-fluorescent immersion oil on glass microscope slides. The slides were examined using an epifluorescence microscope (LEICA DMLB2) with a filter with the following characteristics: excitation filter 340–380 nm, dichromatic mirror of 400 nm and suppression filter LP 425. It is possible to observe that biofilms cells are embedded within the extracellular polymeric matrix.

growth of *Salmonella* spp. and *Escherichia coli* O157:H7 were inhibited when both lactic acid and copper were applied. The authors concluded that this combination produced a synergistic inhibition of microbial growth [26]. Gyawali et al. observed a significant growth inhibition of *E. coli* O157:H7, after a 8 h incubation at 37 °C with a combination of copper (40 ppm) and lactic acid (0.2%) [27].

PA is used as a disinfectant in water [28], in the food and biomedical sectors because of its effectiveness against a broad range of microorganisms (bacteria, fungi, and viruses) [29,30]. Due to its high oxidizing potential, this acid is an ideal antimicrobial agent [17,18]. This disinfectant has advantages over SH: (i) it does not react with proteins to produce toxic or carcinogenic compounds; (ii) it has low environmental impact; (iii) and it has been reported to be more active against biofilms [31,32]. Furthermore, the by-products originated by this acid (water, acetic acid and oxygen) are environmentally friendly [33]. The main drawback associated with PA disinfection is the increase of organic content in the effluent due to acetic acid [34]. CD has attracted interest for the fresh cut industry [20]. It has a higher oxidation capacity than SH and does not react with nitrogen or ammonia to form dangerous products [23], as the major compound formed (chlorite) is classified as non-carcinogenic [20]. This disinfectant is accepted by the FDA for washing vegetables [35,36], but it is not allowed by the EU Food legislation [23]. Furthermore, it has lower reactivity with organic matter and is less corrosive than SH and ozone [12]. The main drawbacks are: (i) its maximum allowed concentration (3 ppm) [35] which is a relatively low value since studies demonstrated that higher concentrations are required to promote a reduction in microbiological content; (ii) its instability, since it is explosive (it has to be generated on site); (iii) its efficiency is pH dependent and the pH values have to be between 6.5 and 7.5 [12]; (iv) and its decomposition when exposed to sunlight [20]. Vanillin (VN) is a phytochemical that is used as flavoring agent in food. This phytochemical has GRAS status and antioxidant properties and is also used as food preservative due to its antimicrobial activity against gram-positive and gram-negative bacteria [37–39]. The main drawbacks are the unawareness of the VN mode of action and also its low solubility in water (1% w/w) [38,40].

Table 1
Calculation and significance of the MIC ratio.

MIC ratio	Value	Result
$\frac{MIC_c}{MIC_i}$	$0 < \frac{MIC_c}{MIC_i} < 0.5$	Potentiation
	$0.5 < \frac{MIC_c}{MIC_i} < 1$	Modest enhancement
	$\frac{MIC_c}{MIC_i} < 1$	Antagonism

MIC_c is the MIC of the compound in the combination and MIC_i is the MIC of the compound when used individually.

Sodium bicarbonate (SB) is usually used as a food additive and has GRAS status. It has been used to control green and blue molds in citrus [41,42]. It is commonly applied due to its wide acceptance in the food industry, low cost, non-toxic properties and because it does not damage the fruits [42]. It can also be applied to eliminate microorganisms from food contact surfaces, such as stainless steel (SS) [19].

The aim of the present study was to test alternative chemical compounds and to explore the combination with sodium hypochlorite, for water and surfaces' disinfection. Therefore, the main objectives were to determine the antimicrobial activity of