Available online at www.sciencedirect.com
SCIENCE DIRECT
ELSEVIER
International Journal of Food Microbiology 109 (2006) 187–197
INTERNATIONAL JOURNAL OF Food Microbiology
www.elsevier.com/locate/ijfoodmicro

The efficacy of electrolysed oxidising water for inactivating spoilage microorganisms in process water and on minimally processed vegetables

Duncan Ongeng [a], Frank Devlieghere [b], Johan Debevere [b], Jozef Coosemans [a], Jaak Ryckeboer [a,c,*]

[a] *Faculty of Bioscience engineering, Katholieke Universiteit Leuven, W. de Croylaan 42, B-3001 Leuven, Belgium*
[b] *Laboratory of Food Microbiology and Food Preservation, Department of Food Quality and Food Safety, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, B-9000 Ghent, Belgium*
[c] *Ecodis nv., Research and Development, Microbiology, Brechtsebaan 30, B-2900 Schoten, Belgium*

Received 21 November 2004; received in revised form 9 November 2005; accepted 29 December 2005

Abstract

The efficacy of Electrolysed Oxidising Water (EOW) for inactivating spoilage microorganisms in process water and on minimally processed vegetables was investigated. The direct effect of EOW on three important spoilage bacteria namely; *Pseudomonas fluorescens*, *Pantoea agglomerans* or *Rahnella aquatilis* was determined by inoculating tap water or "artificial process water" with approximately 8 log CFU/ml pure culture and electrolysing the resultant solutions. The three bacteria were each reduced to undetectable levels at low (0.5 A) and relatively higher levels (1.0 A) of current in tap water and "artificial process water", respectively. The residual effect of EOW on *P. fluorescens*, *P. agglomerans* or *R. aquatilis* was determined by incubating at room temperature 1 ml (approximately 9 log CFU/ml) pure culture suspensions in 9 ml of EOW-T (EOW produced from tap water), EOW-A (EOW produced from "artificial process water" supplemented with approximately 60.7 mg Cl^-/l and 39.3 mg Na^+/l) or deionised water (control) for 0, 15, 45 or 90 min. The bactericidal activity of both EOW-T and EOW-A increased with the concentration of free oxidants and incubation period and the three bacteria were completely reduced at free oxidants-incubation period combinations of 3.88 mg/l–45 min and 5.1 mg/l–90 min in EOW-T and EOW-A, respectively. Two types of industrial vegetable process water; salad-mix and soup process water, which had each a total psychrotrophic count of approximately 8 log CFU/ml were then electrolysed. Without any NaCl addition, only 1.2 and 2.1 log reductions of the psychrotrophs in soup and salad-mix process water was attained respectively. Supplementation of the process water with approximately 60.7 mg Cl^-/l and 39.3 mg Na^+/l afterwards resulted in complete reduction of the psychrotrophic count in both process waters, but soup process water required relatively higher levels of current compared to salad-mix water. Finally, fresh-cut lettuce was washed in EOW-T containing 3.62 mg free oxidants/l, EOW-IP (EOW produced from industrial process water) containing 2.8 mg free oxidants/l or tap water (control) for 1 or 5 min. Washing the vegetables for 1 min in EOW-T resulted in 1.9, 1.2, and 1.3 log reductions of psychrotrophs, lactic acid bacteria and *Enterobacteriacae*, respectively, which increased to 3.3, 2.6, and 1.9 log reductions after washing for 5 min instead. EOW-IP tested in this work had no bactericidal effect on the microflora of fresh-cut lettuce. Electrolysis could therefore be used to decontaminate process water for vegetable pre-washing and to sanitise tap water for final rinsing of vegetables, respectively.
© 2006 Elsevier B.V. All rights reserved.

Keywords: Electrolysed oxidising water; Decontamination; *Pseudomonas fluorescens*; *Pantoea agglomerans*; *Rahnella aquatilis*; Process water; Minimally processed vegetables

1. Introduction

Minimally processed refrigerated fruits and vegetables have become a very important area of potential economic growth in the fresh-cut produce industry (Buta et al., 1999). The economic potential is shown by the solid growth of the industry in the recent past as illustrated by increasing consumption and increasing space devoted to fresh-cut vegetable products in supermarkets and on restaurant menus in most parts of the world

* Corresponding author. Faculty of Bioscience engineering, Katholieke Universiteit Leuven, Division Soil and Water Management, Kasteelpark Arenberg 20, 3001 Leuven, Belgium. Tel.: +32 6 6419100; fax: +32 3 6419109.
E-mail addresses: jaak.ryckeboer@ecodis.be, jaak.ryckeboer@biw.kuleuven.be (J. Ryckeboer).

0168-1605/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijfoodmicro.2005.12.013

(Kaufman et al., 2000). The popularity of fresh-cut vegetables is mainly because today's consumer perceives such products as being fresh, healthy, convenient, tasty, and easy to use in addition to retained nutritional qualities (Wiley, 1994; Garret et al., 2003).

Despite the growth of fresh-cut-vegetable produce market (Kaufman et al., 2000), control of microbial spoilage and protection of consumers against microbiological hazard is still a major challenge to the industry (Parish et al., 2003). Such ready-to-use vegetables retain much of their indigenous microflora after minimal processing, and pathogens may form part of this microflora, therefore posing a potential safety problem (Wiley, 1994; Ahevenainen, 1996; Francis et al., 1999). Since minimally processed vegetables belong to the low-acid foods (pH 5.8–6.0), the characteristic high humidity and the large number of cut surfaces can provide ideal conditions for the growth of microorganisms consequently leading to shelf life reduction (Willocx et al., 1993). Amongst the spoilage microflora, *Pseudomonas fluorescens*, *Pantoea agglomerans* and *Rahnella aquatilis* have been frequently isolated from minimally processed lettuce and other ready-to-eat vegetables (Nguyen-the and Carlin, 1994). These microorganisms have also been found to dominate the microflora of minimally processed vegetable products at the end of shelf life under refrigeration conditions (Brocklehurst et al., 1987). Interestingly, as reported in the UK, *P. fluorescens*, *P. agglomerans* and *R. aquatilis* have also shown significant resistance to quite a number of antibiotics undergoing challenge tests, therefore raising more concern about the safety of ready-to-eat fruits and vegetables (Hamilton-Miller and Shah, 2001).

Disinfection/decontamination is inevitably a critical step in ensuring the safety and shelf life of ready-to-eat vegetables. However, experiments have already shown that feasible decontamination techniques available cannot guarantee the microbiological quality of minimally processed vegetables without compromising their sensorial quality (Beuchat and Ryu, 1997; Beuchat, 1998; Seymour, 1999). In addition to compromised quality, there is also growing concern over potential safety hazards associated with chemical disinfection by products (Simons and Sanguansri, 1997; Richardson et al., 1994; Guten, 2003; Richardson, 2003; Jyoti and Pandit, 2004; Lee et al., 2004a,b).

The use of potable water instead of chlorinated water for cleaning fresh-cut vegetables is being advocated in some European countries like Belgium. However, it has already been shown long before that vigorous washing of vegetables in potable water typically reduces the microbial load by only 1–2 logs and is therefore insufficient to guarantee microbial safety and quality (Beuchat, 1998). Besides commercial washing systems are highly variable to the effect that often limited dirt, foreign matter and microbial removal is attained, thus making shelf life extension by washing almost impossible (Seymour, 1999). Alarming high cost of potable water and waste water treatment has also driven the industry to wash large quantities of vegetables several times in a limited volume of water, leading to microbial accumulation in the process water to high numbers. Subsequent use of such process water would then increase the microbial contamination level of the vegetable instead of reducing it. Process water recontamination is not a new phenomenon (Garg et al., 1990) though not much work has been done on it. A recent case showed that microbial load of shredded lettuce increased by 1.5 log CFU/g after rinsing. Microbiological analysis showed in this case that water used for rinsing had a microbial load amounting to 4.09 log CFU/ml (Allende et al., 2004). There is therefore a need for disinfection techniques capable of inactivating microorganisms both in process water and on minimally processed vegetables so as to cut down water cost through process water reuse.

Electrolysed oxidising water (EOW) is a relatively new concept, which has been utilised experimentally in agriculture (Al-Haq et al., 2002; Buck et al., 2003), livestock management (Stevenson et al., 2004), medical sterilisation (Lee et al., 2004a, b; Vorobjeva et al., 2004), food sanitation (Park et al., 2002; Bari et al., 2003; Sharma and Demirci, 2003; Okull and Laborde, 2004) and also in areas that rely on antimicrobial methodologies (Venkitanarayanan et al., 1999a,b; Fabrizio and Cutter, 2003). EOW is conventionally generated by electrolysis of aqueous sodium chloride to produce an electrolysed basic aqueous solution containing dilute sodium hydroxide at the cathode and an electrolysed acidic solution at the anode (Kim et al., 2000). EOW used in this study was generated using the *ecodis*®; a product developed by the company Ecodis nv. (Schoten, Belgium). Basically, the *ecodis*® consists of a disinfection cell (electrolysis cell) and a power supply. The electrolysis cell is made of specially coated, permanent electrodes. Direct current in the (safe) low voltage across the electrodes causes the formation of oxidising agents principally derived from oxygen, as well as free chlorine when chloride ions are present in the solution, both resulting in immediate disinfection. Through transmission of the reactive energy of the oxidants, the disinfection effect is upheld in the water causing a residual activity as well. These free oxidants are formed from the water itself, thus without adding chemicals, and eradicate bacteria, viruses, algae and other microorganisms (Ryckeboer, 2005).

Previous work has shown that the *ecodis*® inactivates *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus* sp., *Aeromonas* sp. (Polanska, 2001), *Escherichia coli* and *Legionella pneumophila* (Daneels, 2004; Vranckx, 2005), but also microorganisms of agricultural importance such as *Bacillus subtilis*, *Ralstonia solanacearum*, *Rhizoctonia solani*, *Pythium ultimum*, *Fusarium oxysporum* f.sp. *lycopersici* and *Trichoderma hamatum* (Vanhoutte, 2002). Furthermore, the *ecodis*® neutralises harmful substances such as cyanides, ammonium, etc. In this way, water reservoirs or the water supply networks downstream of the *ecodis*® are also disinfected and protected against re-infection. A study performed by Corrosion Protection Consultants (CPC, Schoten, Belgium) at 3 mg free oxidants/l showed that the *ecodis*® does not cause any corrosion (Ecodis nv., 2004). Considering the success of the *ecodis*® in the areas mentioned, the system could be a valuable disinfection tool for the minimally processed vegetable industry.

The objective of this study was therefore to investigate the potential of EOW produced by the *ecodis*® for inactivating

spoilage microorganisms in process water and on minimally processed vegetables.

2. Materials and methods

2.1. Bacterial culture and inoculum preparation

In this study, collection strains of *P. fluorescens* (PR3), *P. agglomerans* (PR1) and *R. aquatilis* (PR2), which were isolated at the Laboratory of Food Microbiology and Food Preservation, Ghent University from equilibrium modified packaged mix lettuce stored at 7 °C, were used. The cultures were separately grown aerobically on nutrient agar (Oxoid Ltd., Hampshire, UK) at 30 °C for 24 h. One loop of each bacterium was then cultured separately in 100 ml of sterile nutrient broth in 250 ml Erlenmeyer flasks at 30 °C for 24 h with agitation (150 rpm). Following incubation, the cultures were sedimented by centrifugation (4000×*g* for 20 min) in 50 ml Falcon tubes, washed, and re-suspended in 50 ml of 0.1% peptone water (pH 7.1). A preliminary trial was done (by plating 0.1 ml of the appropriate dilution of the solution on nutrient agar followed by 48 h of aerobic incubation at 30 °C) to determine the number of Falcon tubes needed to produce approximately 8 log CFU/ml in a 10-l container and 8 Falcon tubes were found adequate. For residual effect determination, 10 ml of each culture was also centrifuged, washed and re-suspended in 10 ml of 0.1% Peptone water (pH 7.1) to give approximately 8 log CFU/ml and was confirmed by plating 0.1 ml of the solution of appropriate dilution on nutrient agar followed by aerobic incubation for 48 h at 30 °C.

2.2. Industrial vegetable process water and "artificial vegetable process water" formulation

Two types of industrial vegetable process water (soup water and salad-mix water), collected at the end of a processing shift, were provided by ALGRO nv. (Ghent, Belgium). The total psychotropic count on the first day was determined by plating 0.1 ml of appropriate dilutions on nutrient agar after which the plates were aerobically incubated at 22 °C for 5 days. "Artificial process water" was formulated to simulate industrial process water. It was made from fresh iceberg lettuce bought from a local supermarket. The lettuce was washed with cold tap water and dried for 10 min under a laminar flow. Lettuce juice was extracted by means of a manual kitchen blender followed by filtration through a 40-μm sieve. The lettuce juice was autoclaved at 121 °C for 15 min. 100 ml of the juice was then added to 9.9 l of tap water to constitute 1% of the total solution. In order to increase the concentration of Cl^- ions and to improve the electrolytic conductivity so that sufficient amount of free oxidants could be generated, "artificial process water" was supplemented with approximately 60.7 mg Cl^-/l and 39.3 mg Na^+/l (5 ml of 20% NaCl solution in 10 l of "artificial process water"). Chemical analyses of the industrial and artificially formulated vegetable process waters were then done at the Soil Service of Belgium (Heverlee, Belgium). COD was determined using the potassium dichromate method (ISO 6060), while BOD was determined after 5 days of incubation using a dilution and seeding method (ISO 5815).

2.3. EOW generation and free oxidants concentration measurements

EOW was generated using an *ecodis*® 0.02 (Ecodis nv., Schoten, Belgium). The current passing through the EOW generator was varied from 0.1 to 1.3 A (depending on the experimental run), and the voltage between the electrodes was fixed at 15 V. The flow rate through the machine was fixed at 20 l/h. The *ecodis*® 0.02 was allowed to run for 30 min in order to stabilise before measurements and 15 min in between measurements. The polarities of the electrodes were frequently reversed to prevent deposition of calcium carbonate on the electrodes. Free oxidants concentration measurements were performed using the DPD method (HANNA instruments HI9311; Hungary).

2.4. Direct antimicrobial activity of electrolysis on P. fluorescens, P. agglomerans and R. aquatilis in tap water or "artificial process water"

Pure culture suspensions (approximately 8 log CFU/ml) of *P. fluorescens*, *P. agglomerans* or *R. aquatilis* in tap water or "artificial process water" were separately pumped through the electrolysed oxidising water cell. The current through the cell was varied from 0.1 to 0.7 A during the electrolysis of bacterial suspensions in tap water or 0.1 to 1.3 A for the case of "artificial process water" bacterial suspensions. Oxidised water samples were taken for free oxidants concentration measurements as well as for microbiological analysis. Microbiological analysis was done within 2 min of sample collection in order to determine the population of surviving cells. *P. fluorescens* samples were cultured on Pseudomonas agar (CM 559, CFC supplement SR 103, Oxoid), while those of *P. agglomerans* and *R. aquatilis* were each cultured in Violet Red Bile Glucose Agar (CM 485, Oxoid) using pour plate method with over layer. The plates were then aerobically incubated for 48 h at 30 °C for *P. fluorescens*, or 37 °C for *P. agglomerans* and *R. aquatilis*, respectively.

2.5. The residual antimicrobial activity of EOW-T or EOW-A on P. fluorescens, P. agglomerans and R. aquatilis at room temperature

Electrolysed oxidising water from tap water (EOW-T) containing 0.91, 1.28 and 3.88 mg free oxidants/l were generated at 0.3, 0.5, and 0.7 A, respectively. On the other hand, electrolysed oxidising water from "artificial process" water (EOW-A) containing 0.96, 1.78 and 5.18 mg free oxidants/l were generated at 0.5, 0.8 and 1.3 A, respectively. 9 ml of EOW-T, EOW-A or sterile deionised water (control) was transferred to separate, sterile Falcon tubes, and the caps were tightly closed. 1 ml (equivalent to 9 log CFU/ml) of *P. fluorescens*, *P. agglomerans* or *R. aquatilis* was added to each tube containing 9 ml of EOW-T, EOW-A or deionised water

(control) and the samples were incubated at room temperature for 0, 15, 45 or 90 min. After incubation, surviving bacterial cells in each sample were then determined as mentioned before.

2.6. Direct antimicrobial activity of electrolysis on total psychrotrophic counts in industrial salad-mix or soup vegetable process water

Soup or salad-mix industrial process water was transferred to a sterile plastic container with a cover. It was then pumped through the *ecodis*® cell. The current through the cell was varied from 0.1 A to a maximum value of 0.7 A. EOW samples were collected in sterile Falcon tubes for measuring the concentration of free oxidants. Simultaneously, oxidised water samples for microbiological analysis were taken and spread plated within 2 min on nutrient agar. The plates were aerobically incubated at 22 °C for 5 days. Since the first experiment did not yield good results in terms of microbial reduction, another set of experiments was run after supplementing the process waters with 60.7 mg Cl^-/l and 39.3 mg Na^+/l (5 ml of 20% NaCl in 10 l of process water). The current was then varied from 0.5 to 1.3 A.

2.7. Activity of EOW-T or EOW-A on spoilage microflora of fresh-cut lettuce

Fresh heads of iceberg lettuce (*Lactuca sativa* L.) was purchased from a local supermarket. After discarding the wrapper leaves and removing the core, the leaves were cut into small pieces using kitchen knife on a non-sterile working table. The cuts from all the heads were mixed thoroughly and divided into 200 g portions and then grouped into 3 lots. One lot was used for the determination of microbial counts on the fresh produce before treatments. Another lot was washed with EOW-T containing 3.6 mg free oxidants/l generated at 0.7 A. And the third lot was washed with normal tap water, as control. 200 g of the cut vegetable was cleaned in 4 l of the washing water for 1 or 5 min. Another set of experiment was also run with EOW-IP containing 2.8 mg free oxidants/l, generated at 1 A from salad-mix industrial vegetable process water supplemented with 6.07 mg Cl^-/l and 39.3 mg Na^+/l (5 ml of 20% NaCl solution in 10 l of process water). The consumption of free oxidants by organic matter necessitated generation of EOW-IP at higher level of current than EOW-T so as to have sufficient residual antimicrobial activity.

Counts of different groups of microorganisms important in the spoilage of minimally processed vegetables were determined as follows: 30 g of the vegetable was weighed into a sterile stomacher bag containing 270 ml of 0.1% peptone water followed by homogenisation for 60 s using Colworth Stomacher 400 (Steward Laboratory, London, UK). Ten-fold dilution series were then made using 0.1% peptone water. Total aerobic psychotropic count was determined using pour plate method followed by 5 days of aerobic incubation at 22 °C on Nutrient agar. *Enterobacteriacae* were enumerated on Violet Red Bile Glucose Agar (CM 485, Oxoid) using pour plate method with over layer and 48 h of aerobic incubation at 37 °C. The population of lactic acid bacteria was determined using pour plate method with over layer followed by 3 days of aerobic incubation at 30 °C on Lactobacilli Agar (CM 361, Oxoid). Finally, yeasts and molds were enumerated on Yeast Glucose Chloramphenicol (64104, BIO-RAD) using spread plate method followed by 3 days incubation at 30 °C.

2.8. Experimental design and data analysis

Complete randomised block design was used. In order to control the effect of day-to-day variability in the performance of EOW generator from influencing the results, experimental run was used as a blocking variable. Each experiment was run twice, and all the analyses were performed in duplicates. Two-way analysis of variance was then performed on the data collected using S-plus statistical software and the means were separated using Tukey's HSD test at 5% level of significance.

3. Results and discussion

3.1. Direct antimicrobial activity of electrolysis on P. fluorescens, P. agglomerans and R. aquatilis in tap water or "artificial process water"

The predominance of *P. fluorescens*, *P. agglomerans* and *R. aquatilis* on minimally processed vegetables at the end of shelf life under refrigeration conditions reflects their importance at limiting the shelf life of the stored product. Therefore, attempts to eliminate them from the rinse water would be a positive step towards ensuring the microbiological quality and hence shelf life of the processed produce. Generally, in tap water, the bactericidal effect of electrolysis on the three bacteria was very significant ($P \leq 0.05$). The three bacteria showed high sensitivity to the bactericidal activity of the electrolytic process, although *P. fluorescens* was slightly more resistant compared to *P. agglomerans* and *R. aquatilis,* respectively (Fig. 1A;B: only data on *P. fluorescens* and *P. agglomerans* shown for illustration).The bactericidal potency of electrolysis on pure cultures in tap water has little relevance in typical industrial scenario as process water is seldom without organic matter, but nevertheless, the sensitivity of the bacteria observed would be indicative of how water quality might influence the disinfection potential of electrolysis and therefore the salt requirement.

The "artificial process water" used in this study had BOD and COD levels of 2.7 and 13.6 mg O_2/l, respectively. This formulation however, underestimated the organic load of process water used practically in the industry and therefore did not simulate industrial vegetable process water accurately. But nevertheless, inactivation of *P. fluorescens*, *P. agglomerans* or *R. aquatilis* in such a formulation by electrolysis was achieved at relatively higher levels of current compared to the scenario in tap water. The direct effect of electrolysis on the population density of *P. fluorescens*, *P. agglomerans* or *R. aquatilis* in "artificial process water" was also very significant ($P \leq 0.05$). The inactivation profiles of the three bacteria in "artificial process water" were quite similar (Fig. 2; only data on *P. fluorescens* chosen for illustration), but different from the

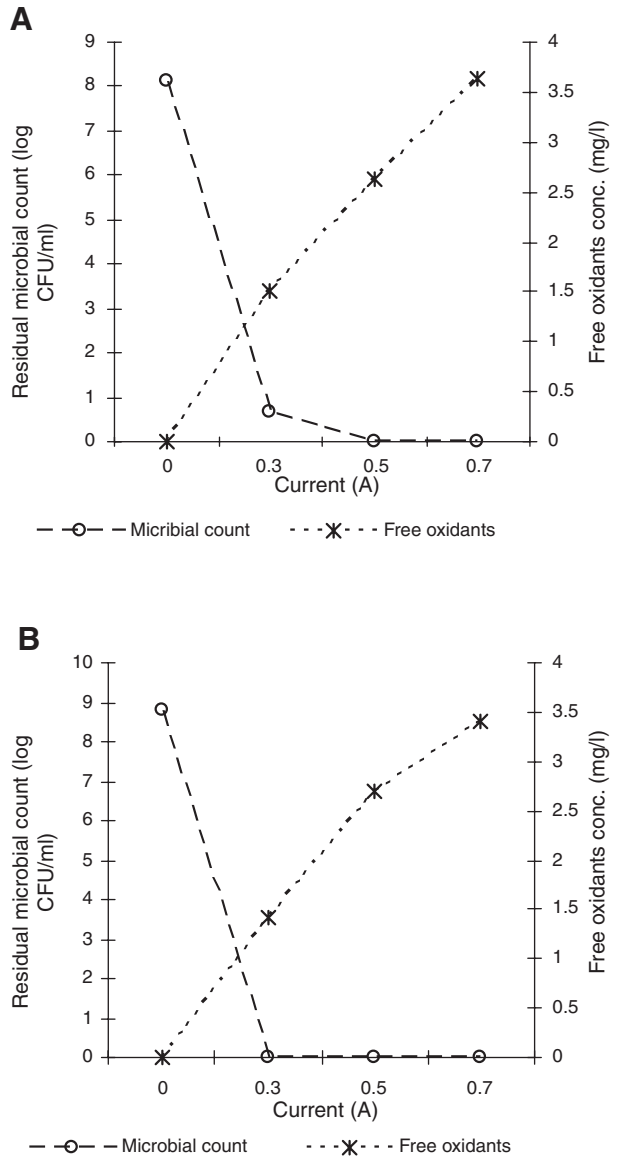

Fig. 1. (A) Inactivation of *P. fluorescens* and evolution of free oxidants as a function of current in tap water by electrolysis. (B) Inactivation of *P. agglomerans* and evolution of free oxidants as a function of current in tap water by electrolysis.

scenario in tap water. Complete inactivation of the three psychrotrophic bacteria in "artificial process water" at higher levels of current compared to inactivation at lower current levels in tap water also explains the influence of organic matter on the efficacy of the decontamination process. These results show that under such conditions, electrolytic process that gives about 2.8 mg residual free oxidants/l would satisfactorily inactivate the three bacteria in process water with similar organic load.

3.2. The residual antimicrobial activity of EOW-T or EOW-A on P. fluorescens, P. agglomerans and R. aquatilis at room temperature

The effect of incubating pure cultures of *P. fluorescens*, *P. agglomerans* and *R. aquatilis* in solutions of EOW-T at room temperature is shown in Table 1. Irrespective of current or the concentration of free oxidants, at time 0 (simple immediate contact), both treatment and control samples had the same mean log CFU/ml for all the three microorganisms. This observation is quite parallel with results of Venkitanarayanan et al. (1999a) where control and treatment samples (EOW) containing 84.3, 79.8, 73.3 ppm of free chlorine had the same mean log CFU/ml for the case of *E. coli*, *S. enteritis* and *L. monocytogenes*, respectively. This shows that irrespective of the concentration of free oxidants or bacterial species, EOW-T does not have instant bactericidal effect. EOW-T had major antibacterial activity on the three microorganisms starting from 0.5 A (2.28 mg free oxidants/l) onwards except for *P. agglomerans* where 1 log reduction was attained after 1.5 h of incubation in EOW-T containing 0.91 mg free oxidants/l and electrolysed at 0.3 A. Most substantial reduction in the populations of the three microorganisms occurred in EOW-T produced at 0.7 A and containing 3.88 mg free oxidants/l. After 15 min of incubation in this solution, 6.4, 7.6 and 7 log reductions of *P. fluorescens*, *P. agglomerans* and *R. aquatilis* were attained, respectively. And after 45 min, all the three bacteria were not detected anymore. The results clearly show that, to have a substantial antimicrobial effect of EOW-T within a short period of time, a higher level of current is required, which could be achieved through minimal salt supplementation.

The effect of incubating *P. fluorescens*, *P. agglomerans* or *R. aquatilis* in solutions of EOW-A is shown in Table 2. Like it was observed in the case of EOW-T, at whatever level of current, simple immediate contact (time 0) with EOW-A showed completely no effect on microbial population densities. EOW-A generated at 0.5 A (containing 0.96 mg free oxidants/l) also had no effect on the three bacteria irrespective of incubation period ($P>0.05$). However, when current was raised to 0.8 A, the concentration of free oxidants increased to 1.78 mg/l and significant reduction in microbial

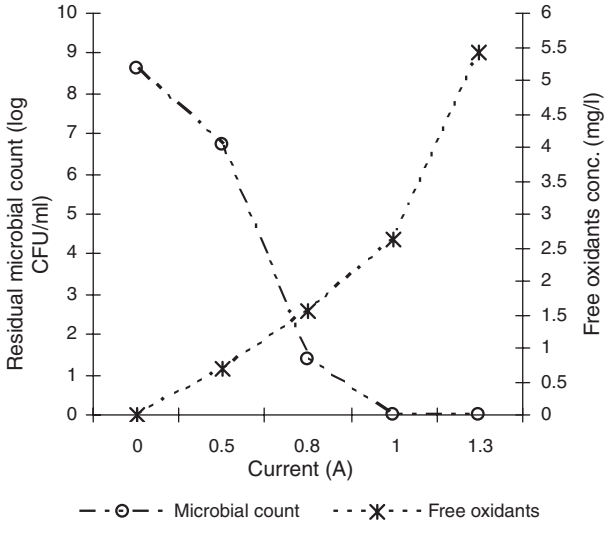

Fig. 2. Inactivation of *P. fluorescens* and evolution of free oxidants as a function of current in "artificial process water" supplemented with approximately 60.7 mg Cl^-/l and 39.3 mg Na^+/l by electrolysis.

Table 1
Inactivation of *P. fluorescens*, *P. agglomerans* and *R. aquatilis* at room temperature by EOW-T

Bacterial species	Surviving bacterial population (mean log CFU/ml) after exposure for:				Current (A)	Free oxidants (mg/l)
	0 min	15 min	45 min	1.5 h		
P. fluorescens	8.70±0.06[a]	8.50±0.06[a]	8.48±0.06[a]	8.10±0.06[a]	0.3	0.91
Control	8.73±0.05[a]	8.71±0.05[a]	8.70±0.05[a]	8.72±0.05[a]		
P. agglomerans	8.80±0.07[a]	8.70±0.07[a]	8.30±0.07[a]	7.80±0.07[b]		
Control	8.83±0.07[a]	8.82±0.07[a]	8.83±0.07[a]	8.79±0.07[a]		
R. aquatilis	8.90±0.05[a]	8.70±0.05[a]	8.50±0.05[a]	8.45±0.05[a]		
Control	8.94±0.05[a]	8.92±0.05[a]	8.93±0.05[a]	8.95±0.05[a]		
P. fluorescens	8.60±0.04[a]	8.41±0.04[a]	6.35±0.04[b]	2.50±0.04[c]	0.5	2.28
Control	8.61±0.04[a]	8.59±0.04[a]	8.60±0.04[a]	8.62±0.04[a]		
P. agglomerans	8.70±0.05[a]	7.17±0.05[b]	5.48±0.05[b]	2.00±0.05[c]		
Control	8.73±0.04[a]	8.71±0.04[a]	8.70±0.04[a]	8.72±0.04[a]		
R. aquatilis	8.80±0.06[a]	8.00±0.06[a]	6.12±0.06[b]	2.43±0.06[c]		
Control	8.83±0.03[a]	8.80±0.03[a]	8.8±0.03[a]	8.82±0.03[a]		
P. fluorescens	8.70±0.05[a]	2.2±30.05[b]	ND	ND	0.7	3.88
Control	8.73±0.04[a]	8.70±0.04[a]	8.69±0.04[a]	8.71±0.04[a]		
P. agglomerans	8.68±0.06[a]	1.10±0.06[b]	ND	ND		
Control	8.69±0.04[a]	8.70±0.04[a]	8.71±0.04[a]	8.73±0.04[a]		
R. aquatilis	8.70±0.06[a]	1.64±0.06[b]	ND	ND		
Control	8.80±0.05[a]	8.79±0.05[a]	8.81±0.05[a]	8.83±0.05[a]		

Means in the same row followed by different superscripts are significantly different ($P \leq 0.05$). ND = not detectable by conventional plating method.

population started to be registered after 45 min of incubation ($P \leq 0.05$). Increasing incubation period to 1.5 h in this solution led to approximately 4 log reductions in the populations of the three bacteria, respectively. It was found out that by increasing current to 1.3 A, the concentration of free oxidants increased to 5.1 mg/l, which lead to 2.95, 2.58 and 3.06 log reductions of *P. fluorescens*, *P. agglomerans* and *R. aquatilis* after 15 min of incubation. However, longer incubation times in this solution resulted in much higher reductions with complete inactivation after 1.5 h incubation for all the three bacteria investigated. This observation shows that organic matter is an important parameter that affects the residual bactericidal effect of EOW.

3.3. Direct antimicrobial activity of electrolysis on total psychrotrophic counts in industrial salad-mix or soup vegetable process water

Chemical composition of salad-mix and soup process water used in this experiment is presented in Table 3. Although the

Table 2
Inactivation of *P. fluorescens*, *P. agglomerans* and *R. aquatilis* at room temperature by EOW-A

Bacterial species	Surviving bacterial population (mean log CFU/ml) after exposure for:				Current (A)	Free oxidants (mg/l)
	0 min	15 min	45 min	1.5 h		
P. fluorescens	7.84±0.07[a]	7.70±0.07[a]	7.48±0.07[a]	7.30±0.07[a]	0.5	0.96
Control	7.86±0.04[a]	7.84±0.04[a]	7.85±0.04[a]	7.84±0.04[a]		
P. agglomerans	7.88±0.06[a]	7.60±0.06[a]	7.52±0.06[a]	7.34±0.06[a]		
Control	7.98±0.05[a]	8.00±0.05[a]	7.96±0.05[a]	8.00±0.05[a]		
R. aquatilis	8.00±0.06[a]	7.94±0.06[a]	7.63±0.06[a]	7.45±0.06[a]		
Control	8.30±0.05[a]	8.00±0.05[a]	7.96±0.05[a]	8.10±0.05[a]		
P. fluorescens	8.33±0.07[a]	8.00±0.07[a]	7.11±0.07[b]	4.00±0.07[c]	0.8	1.78
Control	8.40±0.07[a]	8.37±0.07[a]	8.40±0.07[a]	8.39±0.07[a]		
P. agglomerans	7.89±0.04[a]	7.41±0.04[a]	6.70±0.04[b]	3. 80±0.04[c]		
Control	8.10±0.05[a]	8.23±0.05[a]	8.00±0.05[a]	8.14±0.05[a]		
R. aquatilis	8.30±0.07[a]	7.80±0.07[a]	7.30±0.07[b]	4.43±0.07[c]		
Control	8.38±0.004[a]	8.40±0.04[a]	8.37±0.04[a]	8.39±0.04[a]		
P. fluorescens	8.26±0.06[a]	5.31±0.06[b]	3.10±0.06[c]	ND	1.3	5.10
Control	8.33±0.04[a]	8.42±0.04[a]	8.35±0.04[a]	8.36±0.04[a]		
P. agglomerans	8.88±0.07[a]	6.30±0.07[b]	2.38±0.07[c]	ND		
Control	8.91±0.06[a]	8.80±0.06[a]	8.90±0.06[a]	8.73±0.06[a]		
R. aquatilis	8.19±0.05[a]	5.13±0.05[b]	0.80±0.05[c]	ND		
Control	8.30±0.03[a]	8.41±0.03[a]	8.10±0.03[a]	8.23±0.03[a]		

Means in the same row followed by different superscripts are significantly different ($P \leq 0.05$). ND = not detectable by conventional plating method.

Table 3
Chemical composition of industrial process waters used in the study

Parameter	Process water		
	Salad-mix[1]	Salad-mix[2]	Soup
Suspended solids (mg/l)	40.25	42.0	35.0
Settable solids (ml/l)	0.1	<0.1	<0.1
BOD (mg O_2/l)	36.0	74.0	41.0
COD (mg O_2/l)	92.1	116.35	41.5
Total hardness (FH)	25.7	33.1	37.5
Chloride (mg/l)	33.39	34.32	157.32
pH	7.3	7.0	7.4
Electric conductivity (mS/cm at 25 °C)	0.741	0.757	1.082
PO_4 (mg/l)	0.560	1.152	0.367
Fe (mg/l)	0.427	0.628	0.357
Ca (mg/l)	69.439	98.302	122.5
Mg (mg/l)	20.017	20.382	16.572
Orthophosphates (mg/l)	0.26	0.25	0.062
Organic nitrogen (mg/l)	1.43	8.63	<0.1
Ammonium nitrogen (mg N/l)	<0.4	<0.52	<0.4
Nitrite (mg N/l)	<0.022	<0.022	<0.022
Nitrate (mg N/l)	<0.76	<0.76	<0.76
Total nitrogen (mg N/l)	<1.76	8.63	<1.76
Carbonate (mg/l)	<1.2	<1.2	<1.2
Sulphide (mg/l)	<0.25	0.31	<0.25
Sulphate (mg/l)	33.33	60.12	43.68
Sulphite (mg/l)	<2	1.6	<2
Dissolved fluoride (mg/l)	0.216	0.283	0.216
Total organic carbon (mg/l)	15.49	16.70	11.01

Salad-mix[1], Soup: industrial process waters used to study direct antimicrobial effect of electrolysis on total psychrotrophic counts. Salad-mix[2]: industrial process water use to generate EOW-IP.

Biological Oxygen Demands (BOD) of the salad-mix (36 mg O_2/l) and soup process water (132 mg O_2/l) were different, their microbial loads were comparable (approximately 8 log CFU/ml). Attempts to perform direct electrolysis without salt addition resulted in very limited effect on microbial populations in both types of process water. As illustrated in Fig. 3A, despite the fact that salad-mix water had no salt supplementation, concentration of free oxidants tended to increase with current, and the maximum current attainable was 0.7 A. At 0.3 A, the concentration of free oxidants was 0.63 mg/l but antimicrobial activity of electrolysis on the psychrotrophs in salad-mix process water was not significant ($P>0.05$). Antimicrobial effect of electrolysis on the psychrotrophs in salad-mix process water started to be realized from 0.5 A, where 1.5 log reduction of the psychrotrophs was attained, corresponding with 0.73 mg free oxidants/l. At 0.7 A, 1.06 mg free oxidants/l was generated and maximal microbial load reduction attained in salad-mix process amounted to 2.1 log CFU/ml.

For the case of soup process water (Fig. 3B), without any salt supplementation, very limited increase in free oxidants with current was observed. The maximum current attainable was also 0.7 A. Electrolysis of soup process water at 0.3 A generated 0.23 mg free oxidants/l but psychrotrophs were not significantly affected ($P>0.05$). Significant reduction of the psychrotrophs in soup process water also started at 0.5 A, and finally, maximal microbial load reduction of 1.2 log CFU/ml was attained in soup process water after electrolysis at 0.7 A corresponding with 0.29 mg free oxidants/l. Decontamination of process water to such residual microbial loads as observed here would not be practically useful, as use of process water with such residual microbial loads to wash vegetables could lead to recontamination. The need to have completely decontaminated water is also exemplified by Garg et al. (1990) who reported an increase in microbial load in processed vegetable due to recontamination from the rinsing water. The difficulty in decontamination of industrial vegetable process water experienced here could have been due to the negative effect of organic load. Soup water seems more difficult to decontaminate compared to salad-mix water, which is not surprising as soup water has higher organic load compared to salad-mix water. As reported by Kiura et al. (2002), the problem with EOW however, is its inactivation following contact with contaminants such as proteins.

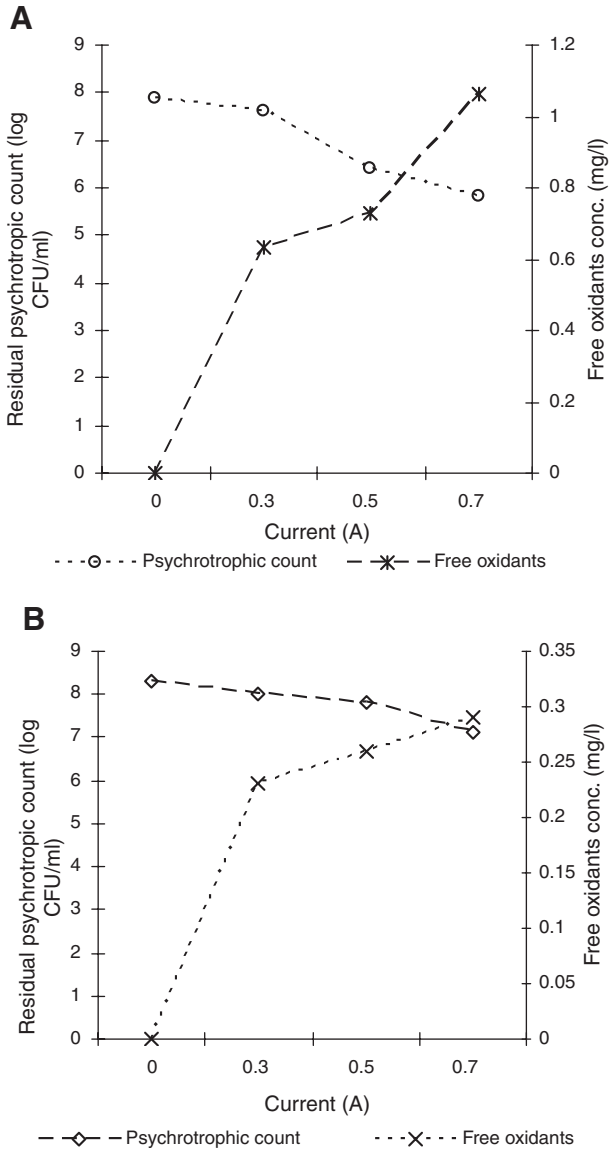

Fig. 3. (A) Inactivation of psychrotrophs and evolution of free oxidants as a function of current in salad-mix process water by electrolysis without any salt supplementation. (B) Inactivation of psychrotrophs and evolution of free oxidants as a function of current in soup process water by electrolysis without any salt supplementation.

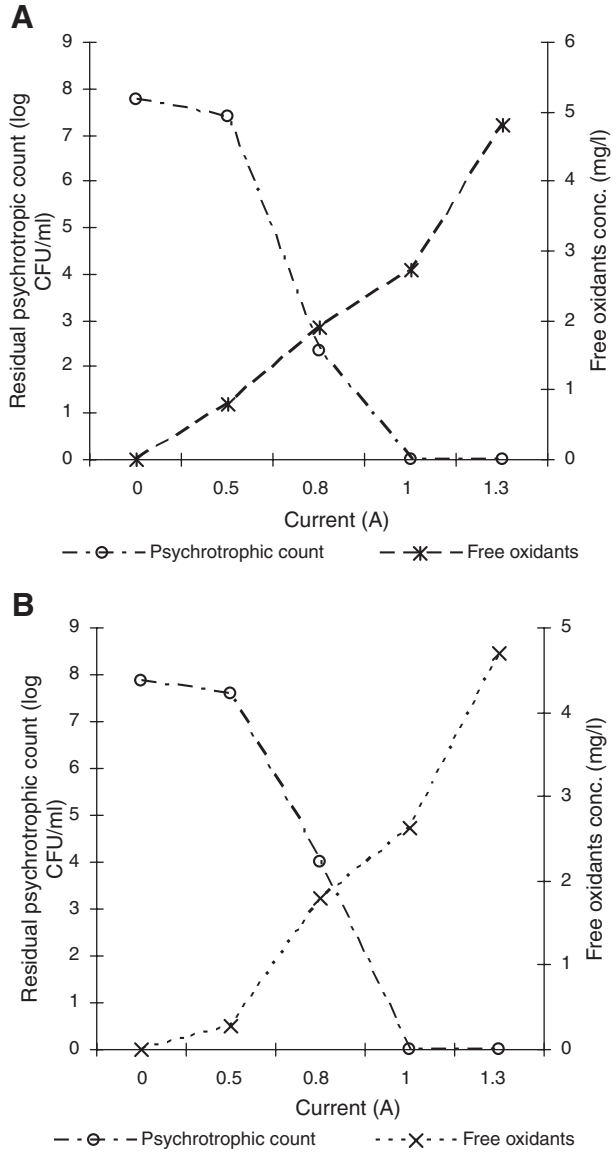

Fig. 4. (A) Inactivation of psychrotrophs and evolution of free oxidants as a function of current in salad-mix process water supplemented with approximately 60.7 mg Cl^-/l and 39.3 mg Na^+/l by electrolysis. (B) Inactivation of psychrotrophs and evolution of free oxidants as a function of current in soup process water supplemented with approximately 60.7 mg Cl^-/l and 39.3 mg Na^+/l by electrolysis.

Supplementation of the raw industrial vegetable process water with 60.7 mg Cl^-/l and 39.3 mg Na^+/l (5 ml of 20% NaCl solution in 10 l of process water) made it possible for current to be varied up to a maximum value of 1.3 A that was concomitant with higher concentrations of free oxidants. Concentrations of free oxidants increased with current and the antimicrobial activity of electrolysis on the psychrotrophs in the two types of industrial vegetable process water followed the same trend as well. In effect, significant reductions of the psychrotrophs in both salad-mix and soup process water then started to be observed from 0.8 A ($P \leq 0.05$). At 0.8 A, electrolysis of supplemented process waters generated 1.9 and 1.8 mg free oxidants/l which resulted in 5.44 and 3.86 log reductions of the psychrotrophs in salad-mix and soup process waters, respectively. From 1.0 A onwards, electrolysis of the salt supplemented process water generated free oxidants well above 2 mg/l and resulted in inactivation of the psychrotrophs in both types of process water to undetectable levels. A critical look at Fig. 4A and B reveals that at most, residual free oxidants concentration of around 3.0 mg/l would be a good indicator of adequate decontamination for both types of process water under the working conditions of this experiment. The addition of salt could have lead to an increase in the electrolytic conductivity of the solution, thus producing sufficient amount of free oxidants to overcome the limiting effect of organic matter. This is in agreement with Kiura et al. (2002) who showed that free chlorine concentration and disinfection potential of EOW increased with NaCl concentration. And that higher free chlorine concentration in EOW produced from solution containing higher NaCl concentration showed better bactericidal effect.

3.4. Activity of EOW-T or EOW-IP on spoilage microflora of fresh-cut lettuce

The effect of EOW-T on the populations of psychrotrophs, lactic acid bacteria, *Enterobacteriacae*, and yeasts and molds on fresh-cut lettuce is shown in Table 4. Total plate count analysis showed that EOW-T containing 3.62 mg free oxidants/l and tap water used as control had no detectable microbial counts. The microbial populations on fresh-cut lettuce before washing were 6.65, 4.8, 3.2, and 0.6 log CFU/g of psychrotrophs, *Enterobacteriacae*, lactic acid bacteria and yeasts, respectively. Molds were not detected. Generally, after washing the vegetables in EOW-T, mean microbiological counts of psychrotrophs, *Enterobacteriacae* and lactic acid bacteria were significantly lower in EOW-T-treated vegetable compared to control samples ($P \leq 0.05$). The population of yeast on untreated vegetable was exceptionally too low to give any conclusions about the antimicrobial effect of EOW-T on yeast. Alternatively, when the vegetables were washed for 5 min instead, EOW-T-treated samples then had 3.3, 2.6, and 1.9 log reductions of psychrotrophs, *Enterobacteriacae* and lactic acid

Table 4
Microbial populations in fresh-cut lettuce before and after washing with EOW-T

Microbial group	Microbial count (mean log CFU/g) before and after washing		
	Before	EOW-T	Tap water (control)
1 min			
PSY	6.65 ± 0.12^a	4.75 ± 0.12^c	5.85 ± 0.12^b
LAB	3.20 ± 0.18^a	1.90 ± 0.18^c	2.60 ± 0.18^b
ETB	4.80 ± 0.09^a	3.60 ± 0.09^c	3.90 ± 0.09^b
YAM	0.60 ± 0.01^a	0.49 ± 0.01^a	0.51 ± 0.01^a
5 min			
PSY	6.65 ± 0.10^a	3.35 ± 0.10^c	5.78 ± 0.10^b
LAB	3.20 ± 0.05^a	1.30 ± 0.05^c	2.50 ± 0.05^b
ETB	4.80 ± 0.34^a	2.20 ± 0.34^c	4.10 ± 0.34^b
YAM	0.60 ± 0.02^a	0.30 ± 0.02^a	0.48 ± 0.02^a

Means in the same column followed by different superscripts are significantly different ($P \leq 0.05$). PSY: Psychrotrophs, LAB: Lactic acid bacteria, ETB: *Enterobacteriacae*, YAM: yeasts and molds.

Table 5
Microbial populations in fresh-cut lettuce after washing with EOW-IP

Microbial group	Microbial count (mean log CFU/g) before and after washing		
	Before	EOW-T	Tap water (control)
1 min			
PSY	6.30±0.20[a]	5.40±0.20[b]	5.60±0.20[b]
LAB	3.60±0.11[a]	3.30±0.11[a]	3.40±0.11[a]
ETB	4.50±0.05[a]	3.60±0.05[b]	3.80±0.05[b]
YAM	1.00±0.00[a]	0.57±0.00[a]	0.70±0.00[a]
5 min			
PSY	6.3±0.37[a]	5.10±0.37[b]	5.40±0.37[b]
LAB	3.60±0.08[a]	3.24±0.08[a]	3.30±0.08[a]
ETB	4.50±0.12[a]	3.10±0.12[b]	3.67±0.12[b]
YAM	1.00±0.00[a]	0.52±0.00[a]	0.66±0.00[a]

Means in the same column followed by different superscripts are significantly different ($P \leq 0.05$). PSY: Psychrotrophs, LAB: Lactic acid bacteria, ETB: *Enterobacteriacae*, YAM: yeasts and molds.

bacteria respectively, but still <1 log reductions were observed in the control samples. These results show that antimicrobial activity of EOW-T increased with washing time. Izumi (1999) got similar results when vegetables were treated with EOW containing 20 ppm free chlorine. This observation contrasts sharply with the chlorine disinfection technique; where the effectiveness has been reported not to increase with the washing times (Adams et al., 1989; Beuchat et al., 2001). Secondly, the free oxidants content of the EOW-T is approximately 10–100 times less than 50–200 mg/l free chlorine solutions that have been used by other researchers (Sanz et al., 2002), but still giving promising results. Adams et al. (1989) used a 100 mg/l free chlorine solution for 5 min and reduced the number of bacteria on lettuce leaves by only 2 logs. Although washing vegetables for 5 min in EOW-T substantially reduced the populations of all groups of investigated bacteria, it would still be impractical in an industrial setting as holding the produce in the wash solution for 5 min would not be economical. Line speeds in most vegetable processing plants generally provide contact times of 60 s or less (Delaquis et al., 1999). But nevertheless, EOW-T could still be successfully used to wash less contaminated vegetables at the line speed mentioned. There is also a probable benefit that less disinfection by-products could be formed when EOW-T is used due to low level of free oxidants content. However, this has still to be studied in detail.

The antimicrobial activity of EOW-IP on the populations of psychrotrophs, lactic acid bacteria, *Enterobacteriacae*, and yeasts and molds on fresh-cut lettuce is shown in Table 5. Chemical composition of salad-mix vegetable process water used to generate EOW-IP is shown in Table 3. The EOW-IP as well as tap water used as control had no detectable microbial count, based on total plate count analysis. However, there were no significant differences between EOW-IP-treated and control samples for the populations of psychrotrophs and *Enterobacteriaceae* after 1 min of washing ($P > 0.05$). This shows that EOW-IP had no bactericidal effect on the microflora of fresh-cut lettuce after 1 min of washing. The difference in the populations of psychrotrophs and *Enterobacteriaceae* between EOW-IP-treated and untreated vegetable was just probably due to mere washing-off of the microorganisms that were loosely attached on the surface. A similar situation was also observed after 5 min of washing. This means that increase of washing time did not improve the bactericidal potency of EOW-IP.

The poor antimicrobial activity of EOW-IP in comparison with that of EOW-T was probably due to the negative influence of organic load. Nakajima et al. (2004) have reported the inactivation of free chlorine by bacterial debris during electrolysis of bacterial cells. Those researchers examined bactericidal activity of electrolysed tap water contaminated with bacteria in comparison with that of electrolysed uncontaminated tap water and found that the bactericidal activity of the former was weaker than that of the latter. Additionally, the negative influence of BOD on the bactericidal activity of EOW in solution has already been observed in the early phase of this work. Although EOW-IP had no bactericidal effect on the spoilage microflora of fresh-cut lettuce, microbial recontamination of the washed vegetable did not occur. However, it would still be possible to decontaminate fresh-cut lettuce using EOW-IP produced at higher level of current than the one used in this experiment.

4. Conclusions

Electrolysis as a decontamination technique had strong bactericidal effect on *P. fluorescens*, *P. agglomerans* and *R. aquatilis*. However, residual bactericidal effect of EOW on the three bacteria was dependent on organic load in the solution, concentration of free oxidants and incubation period. In order to overcome the limiting effect of organic load on free oxidants generation so as to give sufficient bactericidal effect, decontamination of industrial vegetable process water using electrolysis required minimal salt supplementation. Although EOW-IP had no bactericidal activity on the spoilage microflora of fresh-cut lettuce compared to EOW-T, EOW-IP showed no evidence of vegetable recontamination. In a typical industrial application, EOW-IP and EOW-T could be used for pre-washing and final rinsing of the vegetables respectively. EOW is therefore a promising alternative decontamination technique for minimally processed vegetable that requires optimisation of the application conditions.

Acknowledgments

This research was sponsored by The Flemish Interuniversity Council in conjunction with Ecodis nv. (Schoten, Belgium).

References

Adams, M.R., Hartley, A.D., Cox, L.J., 1989. Factors affecting the efficacy of washing procedures used in the production of prepared salads. Food Microbiology 6, 69–77.

Ahevenainen, R., 1996. New approaches in improving the shelf life of minimally processed fruits and vegetables. Trends in Food Science and Technology 7, 179–186.

Al-Haq, M.I., Seo, Y., Oshita, S., Kawagoe, Y., 2002. Disinfection effects of electrolyzed oxidizing water on suppressing fruit rot of pear caused by *Botryosphaeria berengeriana*. Food Research International 35, 657–664.

Allende, A., Aguayo, E., Artés, F., 2004. Microbial and sensory quality of commercial fresh processed red lettuce throughout the production chain and shelf life. International Journal of Food Microbiology 91, 109–117.

Bari, M.L., Sabina, Y., Isobe, S., Uemura, T., Isshiki, K., 2003. Effectiveness of electrolyzed acidic water in killing *Escherichia coli* O157:H7, *Salmonella enteritidis*, and *Listeria monocytogenes* on the surfaces of tomatoes. Journal of Food Protection 66, 542–548.

Beuchat, L.R., 1998. Surface Decontamination of Fruits and Vegetables Eaten Raw: A Review. Food Safety Unit, World Health Organisation, Brussels, Belgium. WHO/FSF/FOS/98.2.

Beuchat, L.R., Ryu, J.H., 1997. Produce handling and processing practices. Emerging Infectious Diseases 3, 459–465.

Beuchat, L.R., Ward, T.E., Pettigrew, C.A., 2001. Comparison of chlorine and a prototype produce wash product for effectiveness in killing *Salmonella* and *Escherichia coli* O157:H7 on alfalfa seeds. Journal of Food Protection 64, 152–158.

Brocklehurst, T.F., Zaman-Wong, C.M., Lund, B.M., 1987. A note on the microbiology of retail packs of prepared salad vegetables. Journal of Applied Bacteriology 63, 409–415.

Buck, J.W., Van Iersel, M.W., Oetting, R.D., Hung, Y.C., 2003. Evaluation of acidic electrolyzed water for phytotoxic symptoms on foliage and flowers of bedding plants. Crop Protection 22, 73–77.

Buta, J.G., Moline, H.E., Spaulding, D.W., Wang, C.Y., 1999. Extending storage life of fresh-cut apples using natural products and their derivatives. Journal of Agricultural and Food Chemistry 47, 1–6.

Daneels, A., 2004. Elektrochemische disinfectie van *Legionella pneumophilia* in leidingwater. M.Sc. dissertation. Katholieke Universiteit Leuven, Leuven, Belgium, pp. 1–79.

Delaquis, P.J., Stewart, S., Toivonen, S.P.M.A., Moyls, A.L., 1999. Effect of warm, chlorinated water on the microbial flora of shredded iceberg lettuce. Food Research International 32, 7–14.

Ecodis nv., 2004. De *ecodis®*, revolutionaire technologie voor desinfectie van water: zonder additie van chemicaliën, mét biofilmverwijdering, pp. 1–22.

Fabrizio, K.A., Cutter, C.N., 2003. Stability of electrolyzed oxidizing water and its efficacy against cell suspensions of *Salmonella typhimurium* and *Listeria monocytogenes*. Journal of Food Protection 66, 1379–1384.

Francis, G.A., Thomas, C., O'Beirne, D., 1999. The microbiological safety of minimally processed vegetables. International Journal of Food Science & Technology 34, 1–22.

Garg, N., Churey, J.J., Splittstoesser, D.F., 1990. Effect of processing conditions on the microflora of fresh-cut vegetables. Journal of Food Protection 53, 701–703.

Garret, E.H., Gorny, J.R., Beuchat, L.R., Farber, J.N., Harrie, L.J., Parish, M.E., Suslow, T.V., Busta, F.F., 2003. Microbiological safety of fresh and fresh-cut produce: description of the situation and economic impact. Comprehensive Reviews in Food Science and Food safety 2, 13–19.

Guten, U.V., 2003. Ozonation of drinking water: Part II. Disinfection and by-product formation in presence of bromide, iodide or chlorine. Water Research 37, 1469–1487.

Hamilton-Miller, J.M.T, Shah, S., 2001. Identity and antibiotic susceptibility of enterobacterial flora of salad vegetables. International Journal of Antimicrobial Agents 18, 81–83.

Izumi, H., 1999. Electrolysed water as a disinfectant for fresh-cut vegetables. Journal of Food Science 64, 536–539.

Jyoti, K.K., Pandit, A.B., 2004. Ozone and cavitation for water disinfection. Biochemical Engineering Journal 18, 2–9.

Kaufman, P.R., Handy, C.R., Mclaughlin, E.W., Park, K., Green, G.M., 2000. Understanding the dynamics of produce markets/consumption and consolidation. USDA Economic Research ServiceAgriculture Information Bulletin, vol. 758.

Kim, C., Hung, Y.C., Brackett, R.E., 2000. Roles of oxidation–reduction potential in electrolyzed oxidizing and chemically modified water for the inactivation of food-related pathogens. Journal of Food Protection 63, 19–24.

Kiura, H., Sano, K., Morimatsu, S., Nakano, T., Morita, C., Yamaguchi, M., Maeda, T., Katsuoka, Y., 2002. Bactericidal activity of electrolyzed acid water from solution containing sodium chloride at low concentration, in comparison with that at high concentration. Journal of Microbiological Methods 49, 285–293.

Lee, S.C., Guo, H., Lam, S.M.J., Lau, S.L.A., 2004a. Multi-pathway risk assessment on disinfection by-products of drinking water in Hong Kong. Environmental Research 94, 47–56.

Lee, J.H., Rhee, P., Kim, J.H., Kim, J.J., Paik, S.W., Rhee, J.C, Song, J.H., yeom, J.S., Lee, N.Y., 2004b. Efficacy of electrolyzed acid water in reprocessing patient-used flexible upper endoscopes: comparison with 2% alkaline glutaraldehyde. Journal of Gastroenterology and Hepatology 19, 897–903.

Nakajima, N., Nakanoa, T., Haradaa, F., Taniguchia, H., Yokoyamaa, I., Hirosea, J., Daikokua, E., Sanoa, K., 2004. Evaluation of disinfective potential of reactivated free chlorine in pooled tap water by electrolysis. Journal of Microbiological Methods 57, 163–173.

Nguyen-the, C., Carlin, F., 1994. The microbiology of minimally processed fresh fruits and vegetables. Critical Reviews in Food Science and Nutrition 34, 371–401.

Okull, D.O., Laborde, L.F., 2004. Activity of electrolysed oxidising water against *Penicillium expansium* in suspension and on wounded apples. Journal of Food Science 69, 23–27.

Parish, M.E., Beuchat, L.R.L., Sauslow, T.V., Harris, L.J., Garret, E.H., Farber, J.N., Busta, F.F., 2003. Methods to reduce/eliminate pathogens from fresh and fresh-cut produce. Comprehensive Reviews in Food Science and Food safety 2, 161–173.

Park, H., Hung, Y., Brackett, R.E., 2002. Antimicrobial effect of electrolyzed water for inactivating *Campylobacter jejuni* during poultry washing. International Journal of Food Microbiology 72, 77–83.

Polanska, M., 2001. Development of a new method for disinfection of drinking water. M.Sc. dissertation. Katholieke Hogeschool Sint-Lieven, Gent, Belgium and Wroclaw University of Technology, Wroclaw, Poland, pp. 1–156.

Richardson, S.D., 2003. Disinfection by-products and other emerging contaminants in drinking water. Trends in Analytical Chemistry 22, 666–684.

Richardson, S.D., Thruston, A.D., Collette, T.W., Patterson, K.S., Lykins, B.W., Majetich, G., Zhang, Y., 1994. Multispectral identification of chlorine dioxide disinfection by-products in drinking water. Environmental Science and Technology 28, 529–599.

Ryckeboer, J., 2005. Bestrijden van microbiële contaminaties in leidingen met elektrolyse. Milieutechnologie 5, 4–7.

Sanz, S., Giménez, M., Olarte, C., Lomas, C., Portu, J., 2002. Effectiveness of chlorine washing disinfection and effects on the appearance of artichoke and borage. Journal of Applied Microbiology 93, 986–993.

Seymour, I.J., 1999. Review of current industry practice on fruit and vegetable decontamination. Review, vol. 14. Campden and Chorleywood Food Research Association Group, Chipping Campden, Gloucestershire, UK, pp. 1–38.

Sharma, R.R., Demirci, A., 2003. Treatment of *Eschericia coli* O157:H7 inoculated alfalfa seeds and sprouts with electrolysed oxidising water. International Journal of Food Microbiology 86, 231–237.

Simons, L.K., Sanguansri, P., 1997. Advances in the washing of minimally processed vegetables. Food Australia 49, 75–80.

Stevenson, S.M.L., Cook, S.R., Bach, S.J., McAllister, T.A., 2004. Effects of water source, dilution, storage, and bacterial and fecal loads on the efficacy of electrolyzed oxidizing water for the control of *Escherichia coli* O157:H7. Journal of Food Protection 67, 1377–1388.

Vanhoutte, S., 2002. Ontsmetting van bevloeiingswater in de tuinbouw met behulp van vrije radicalen. M.Sc. dissertation. Katholieke Universiteit Leuven, Leuven, Belgium, pp. 1–99.

Venkitanarayanan, K.S., Ezieke, G.O., Hung, Y.C., Doyle, M.P., 1999a. Efficacy of electrolyed oxidising water for inactivating *Escherichia coli* O154:H7, *Salmonella enteritidis*, and *Listeria monocytogenes*. Applied and Environmental Microbiology 65, 4276–4279.

Venkitanarayanan, K.S., Ezieke, G.O., Hung, Y.C., Doyle, M.P., 1999b. Inactivation of *Escherichia coli* O154:H7, *Salmonella enteritidis*, and *Listeria monocytogenes* on plastic kitchen cutting boards by electrolysed oxidising water. Journal of Food Protection 62, 857–860.

Vorobjeva, N.V., Vorobjeva, L.I., Khodjaev, E.Y., 2004. The bactericidal effects of electrolyzed oxidizing water on bacterial strains involved in hospital infections. Artificial Organs 28, 590–592.

Vranckx, L., 2005. Elektrochemische desinfectie van *Legionella pneumophila*, impact op amoeben en biofilms. M.Sc. dissertation. Katholieke Universiteit Leuven, Leuven, Belgium, pp. 1–105.

Wiley, R.C., 1994. Introduction to minimally processed fruits vegetables. In: Wiley, R.C. (Ed.), Minimally Processed Refrigerated Fruits and Vegetables. Chapman and Hall, New York, USA, pp. 1–14.

Willocx, F., Mercier, M., Hendrickx, M., Tobback, P., 1993. Modelling the influence of temperature and carbondioxide upon the growth of *Pseudomonas fluorescens*. Food Microbiology 10, 159–173.