Chapter 4

Review of general ECA solution applications

4.1 Food applications

The clear benefits of integrating the ECA technology and its derivative solutions into food production and processing industries have produced a vast array of reports where the technology has been assessed for the control of bacterial contaminants, general spoilage and its impact on the shelf life of perishable produce. Owing to its natural ingredients, it has widespread accreditation as a food grade disinfectant and in some instance it is recognised as a core ingredient in the food and beverage manufacture sectors.

Al-Haq *et al*. (2005) have compiled the results of an extensive survey of the available literature where the ECA technology has been employed in the food industry, and detail the diverse range of electrolytic solution types assessed as well as the plethora of descriptive acronyms under which the technology has been described to date. This survey also details an equally significant number of different types of ECA devices, each with variable electrode design reactor configuration, and consequently a distinctive type of end product solution.

The majority of reports describe studies using electrolysed solutions generated from a plate or non-membrane based reactor system which lacks the capacity for pH modulation (Fig 1). In all cases, the various authors report on applications of acidic electrolyzed solutions generated at a pH < 3, which in accordance with conventional electrochemistry will primarily comprise of volatile dissolved chlorine gas in solution (White, 1992).

It is not surprising that the primary mechanism of action of these solutions has been ascribed to being a function of a combination of low pH and high available chlorine concentration (AAC), and that some Japanese authors reporting on the use of local technology, refute the option that ORP may play a role in microbial inactivation (Al-Haq *et al*., 2005).

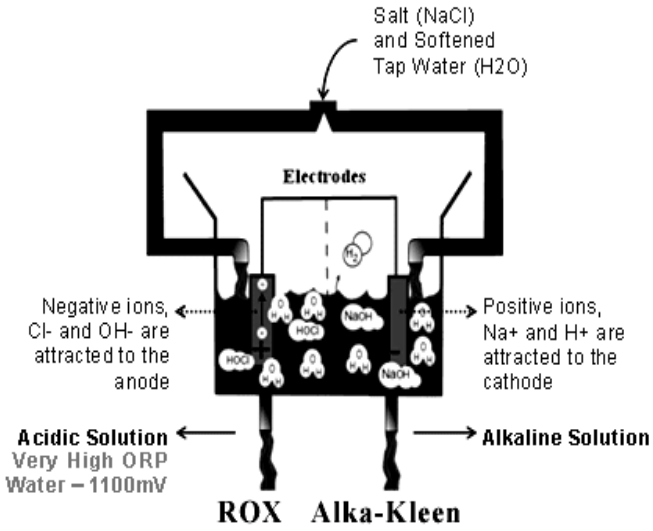

Figure 1. Schematic of the Japanese electrolytic system (Hoshizaki Electric Inc, Japan) used for the production of Acidic electrolyzed water (AEW). (www.hoshizaki.com)

The ROX electrolyser systems produced by Hoshizaki Electric are reported to activate dilute brine solutions (0.05-0.1% NaCl) to produce oxidant solutions with a pH of 2.5-2.8, a Free Available Chlorine (FAC) concentration of 32-35 mg/liter and a REDOX potential of +1100mV (Al-Haq *et al*., 2005).

While effective in the control of superficial spoilage and pathogenic bacteria, a number of reports confirm the adverse consequences of the low pH which includes equipment corrosion, operator discomfort due to chlorine vapours, solution instability, phytoxicity, taint and bleaching of the colour of treated perishable vegetables (Al-Haq *et al*., 2005; Guentzel *et al*., 2007; Fujiwara *et al*., 2009). Despite these limitations, the AEW solutions have been assessed for the control of spoilage and pathogenic bacteria and for shelf life extension in a diverse array of food products ranging from whole and processed fruit and vegetables, grains and allied products, poultry, sea food, and red meats (Park *et al*., 2001; Al-Haq *et al*., 2005; Fabrizio and Cutter, 2005; Kim *et al*., 2005; Huang *et al*., 2006). The substantial effect of ECA solutions against biofilms has previously been described by Thantsha and Cloete (2006).

In converse, relatively few authors have conducted studies using the neutral anolyte produced with the proprietary coaxial Flow Electrochemcial Module (FEM) reactor system as patented and commercialised by Bakhir *et al*., (1995, 1996, 1998, 1999).

Guentzel *et al*. (2008), reported on the use of the neutral anolyte generated from a FEM 3 based reactor, and confirm that the near neutral (pH = 6.5) electrolysed oxidising (EO) water was comprised of hypochlorous acid (~95%), hypochlorite anion (~5%) with trace amounts of Chlorine. They suggest that the high ORP of the solution is disruptive to the integrity of the outer cell barrier, which results in an increased permeability to the HOCl molecule and the subsequent oxidation of cellular reactions and disruption of respiratory pathways.

A 10 minute dip treatment of harvested spinach with a challenge innoculum of *E.coli, Salmonella typhimurium, Listeria moncytogenes, S. aureus* and *Enterococcus faecalis* using neutral oxidant ECA derived solutions, resulted in 5 $\log_{10}$ reduction of all strains within the same time period (Guentzel *et al*., 2008). A similar study using AEW on lettuce leaves resulted in a 2.6 $\log_{10}$ reduction after 3 minutes exposure, and the authors confirmed that the antimicrobial efficacy of the AEW was insignificantly different from that of an acidified chlorinated dip wash (Park *et al*., 2001).

The use of the neutral electrochemically activated solution for the decontamination of poultry carcases artificially challenged with *Salmonella typhimurium* resulted in a 1.39 $\log_{10}$ reduction when sprayed for 17 seconds. A hypochlorite spray at an equivalent FAC of 50 mg/L resulted in a 0.86 $\log_{10}$ reduction (Yang *et al*., 1999).

When chicken skin challenged with pure cultures of *Campylobacter jejuni* were exposed to a variety of dilutions of EAW solutions, it was reported that solutions of high residual chlorine concentrations i.e. >50mg/L displayed a comparable antimicrobial effect relative to that of a chlorine solution of the same chemical concentration. However when the same challenge dose of bacteria was exposed to an EAW dilution of lesser strength and hence reduced residual chlorine concentration i.e. < 25mg/L, the comparable chlorine solution was unable to effect an equivalent reduction in viable count. This confirms the intrinsic capacity of the EAW solutions to exert a greater antibacterial effect relative to standard chlorine based solutions, and substantiates the assertion that the biocidal attributes of the EAW technology exceeds that of purely chemically based formulations (Shimizu and Hurusawa, 1992, Kim *et al.*, 2000; Park *et al*., 2002).

It has been reported that exposure of poultry carcases to a 28mg/L hypochlorous acid solution derived from a mixture of $Ca(OCl_2)$ and HCl, resulted in the development of tolerance by a *Salmonella* isolate within a poultry abattoir (Mokgatla *et al*., 2002). The study details substantially reduced levels of superoxide dismutase and increased levels of catalase enzymes in the HOCl tolerant microbial strain relative to that of a wholly susceptible *Salmonella* strain, and substantiates the findings of an earlier study suggesting that hypochlorous acid exposure can induce similar adaptive responses to that of hydrogen peroxide stress in *E.coli* (Dukan and Touati, 1996). It would be of critical interest to assess the response of the tolerant strain when exposed to a neutral oxidant solution at an equivalent FAC.

The heightened public awareness of the importance of pathogenic diseases associated with Ready-to-Eat (RTE) food products has resulted in a number of studies on the antibacterial efficacy of Electrolysed oxidising water against amongst others *Escherichia coli* 0157:H7, *Salmonella enteriditis* and especially *Listeria monocytgenes* (Venkitanarayanan *et al*., 1999[a,b]; Park *et al*., 2004; Fabrizio and Cutter, 2005).

While the use of acidic electrolysed water (AEW) was shown to be effective in reducing all of the abovementioned pathogens in suspension (Venkitanarayanan *et al*., 1999), it was acknowledged that the application of the AEW solution to the RTE meat products (i.e frankfurters and ham) was ineffective in meeting the current USDA-FSIS requirements of > 2 $\log_{10}$ reduction in *L. monocytogenes* during further meat product processing and packaging (Fabrizio and Cutter, 2005).

Muriana (2005) studied the antimicrobial effects of neutral electrolyzed water (EW) produced by a FEM based reactor system for the decontamination of the processing equipment used with RTE meats contaminated with strongly adherent strains of *Listeria monocytogenes*. Both clean and soiled delicatessen slicing blades contaminated with *L. monocytogenes* were treated with a fine mist spray of sterile distilled water, and a 1:10 and an undiluted EW solution for 15 seconds.

While the sterile distilled water spray was shown to rinse off some of the loosely adherent innoculum, the spray applications of the 1:10 dilution and undiluted oxidant

solutions on the clean blades resulted in a 3.6 log_{10} and >5.66 log_{10} reduction of *L. monocytogenes* respectively (Fig 2).

When the antimicrobial effect on the soiled slicing blades was assessed, the 1:10 dilution and undiluted EW solutions displayed a significantly reduced efficacy i.e. 0.64-log_{10} and 3.34-log_{10} respectively (Fig 2) (Muriana, 2005).

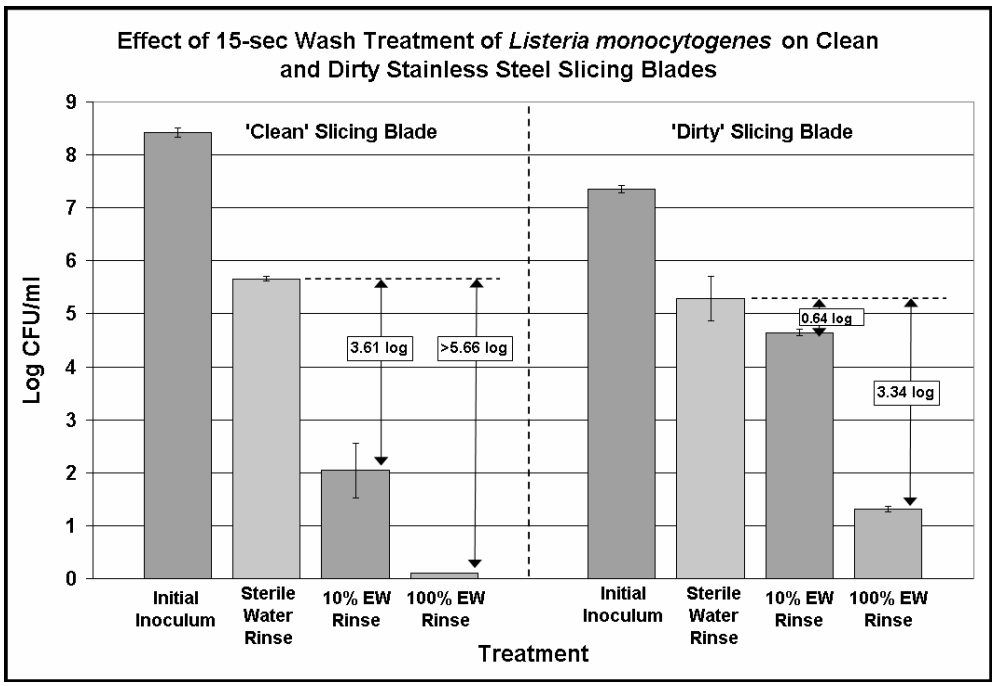

Figure 2. The effect of a 15 second spray treatment of Electrolysed Water (EW) on *Listeria monocytogenes* on clean and dirty stainless steel cutting blades (Muriana, 2005).

An unpublished report complementary to the Muriana study indicated that a 3 minute spray or immersion in an undiluted neutral EAW solution derived from an identical reactor system, resulted in a consistently greater than 2 log_{10} reduction on frankfurter surfaces contaminated with *L. monocytogenes* (Fig 3) (Kirkpatrick and Bagnall – unpublished data, 2005).

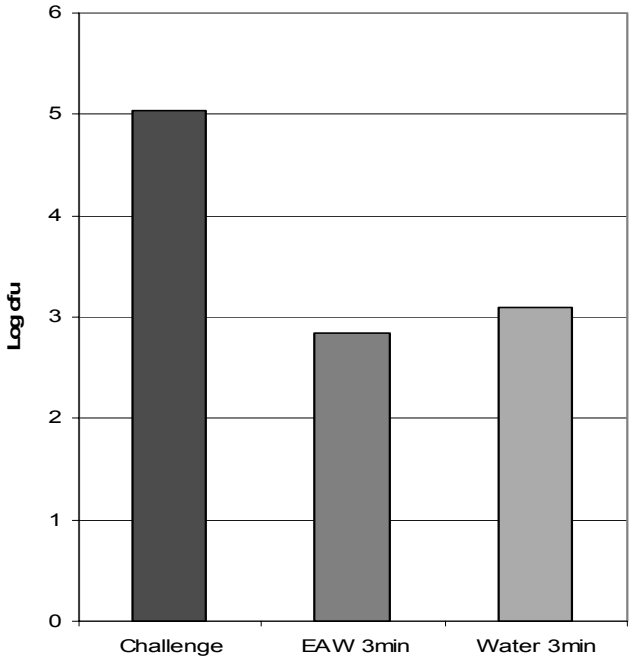

Figure 3. *Listeria monocytogenes* (indicated as log CFU) recovered from frankfurter surfaces after a spray treatment with undiluted EAW and sterile distilled water.

4.2 Oxidation effects

Aside from the widely reported antimicrobial action of the electrochemically activated oxidant solutions, conventional chlorine based formulations have been extensively used for the oxidative manipulation and toxin neutralisation of a variety of chemical compounds. Included here, are the microbially derived toxins which comprise of fungal mycotoxins and bacterial entero- and exotoxins, the inorganic pesticides, herbicides and heavy metals (Prilutsky and Bakhir, 1997).

The use of acidic anolyte (pH 2.5-2.8) to oxidise chemically pure fractions of aflatoxin and Staphylococcal Enterotoxin-A have been reported (Suzuki *et al*., 2002a; Suzuki *et al*., 2002b), and in both instances the inactivation kinetics were suggested to be strongly dependent on the dose of both the hypochlorous acid as well as the hydroxyl radicals present in the acidic oxidant solutions.

In both cases, it was confirmed that the reducing and negatively charged catholyte solution was ineffective in the decomposition of the microbal toxins (Suzuki *et al*., 2002[a]; Suzuki *et al*., 2002[b]). This finding is at odds with the results of the assessments

conducted on the use of the reducing solutions to decontaminate mycotoxins on whole maize kernels, and which will be reported later.

The impact on pesticide and fungicide residues by the oxidant ECA solution has also been evaluated. The oxidant ECA solution was diluted using a 10 fold dilution series and the potential for non-ECA based hydrolysis or unrelated chemical breakdown was excluded by including two untreated control solutions which comprised of both the tap water used as the diluent solution of the oxidant ECA dilution series as well the diluted, non-activated brine solution that was used as the electrolysis feed solution prior to electro-activation. The physicochemical parameters of each solution type were measured before the experimental exposure (Table 1).

Table 1. Physico-chemical properties of the tap water, non-activated salt solution and the diluted oxidant ECA solutions used in the pesticide exposure.

Solution type	ORP (mV)	pH	EC (mS/cm)	FAO (ppm)
Tap water control	280	8.2	0.21	-
2.5gm/lit salt solution	290	7.7	5.22	-
1% Anolyte solution	436	7.5	0.35	≤ 5
10% Anolyte solution	803	7.2	1.34	20-25
100% Anolyte solution	940	6.5	5.45	≤ 200

Legend: ORP – Oxidation-Reduction Potential (mV-milliVolts), EC- Electrical Conductivity (mS/cm – milliSiemens per centimete), FAO – Free Available Oxidant concentration (mg/litre, ppm-parts per million).

The experiment was performed to assess the measure of breakdown of a variety of pesticide and fungicide active ingredients (AI's) after they were exposured to a tap water control, an inactivated brine solution, and a variety of diluted ECA oxidant solutions. A 1 ml aliquot of a cocktail of each of the active ingredients was added to 100ml of the different test and control solution samples. The test samples were agitated with a mechanical stirrer for 5 minutes at ambient temperature and then extracted with an organic solvent and analysed by either gas or liquid chromatography (Table 2).

Table 2. Schedule of pesticide compounds exposed to Anolyte solutions at different dilutions and the percentage breakdown after exposure for 5 minutes.

Active Ingredient	Pesticide Category	1% Anolyte	10% Anolyte	100% Anolyte
		Percentage breakdown		
Malathion	Organophosphorus insecticide	0.0	100	100
Chlorpyrifos	Organophosphorus insecticide	0 0	100	100
Cyprodinil	Anilino-pyrimidine fungicide	0.0	96.3	100
Kresoxim-methyl	Strobilurin fungicide	0.0	0.0	100
Bupirimate	Pyrimidine Fungicide	0 0	52.0	100
Azinphos-methyl	Organophosphorus insecticide	0 0	100	100
Benomyl	Benzimadazole fungicide	8.0	45	100
Aldicarb	Carbamate insecticide	49.5	100	100
Aldicarb sulfoxide	Carbamate insecticide	15 8	100	100
Methomyl	Carbamate insecticide	36.6	100	100

The study showed that the exposure of both pesticide and fungicide compounds to the oxidant ECA solutions resulted in the oxidative breakdown of the active ingredient of all formulations. The organophosphorus and carbamate group of pesticides and the benzimidazole, anilino-pyrimidine, strobilurin, pyrimidine and benzimidazole based fungicides were all oxidised during exposure to the Oxidant ECA solutions (SABS, 2007).

4.3 Reducing effects

The use of the ECA solutions to reduce levels of superificial mycotoxins on grains was evaluated as an adjunct intervention to the microbial decontamination of raw grain products prior to milling. While the oxidant solution was effective in reducing the superficial vegetative fungal contaminants normally associated with suboptimal storage i.e. *Aspergillus* spp. and *Penecillium* spp., it was shown to be ineffective

against the field strains of *Diploidia maydis* and *Fusarium* spp. which specifically colonise the endosperm during cultivation (Marais, 1998).

Maize kernels with known levels of mixed mycotoxin contamination were exposed to both the oxidant and reducing solutions under controlled laboratory conditions. Given the short half-life of the reducing anti-oxidant catholyte solution, the assessments were conducted within 2 hours of solution production. In contrast to an earlier report (Suzuki *et al*., 2002b), it was found that the oxidant ECA solution had no inactivation effect on the levels of either aflatoxin or fumonisins, while a 10 minute exposure to the undiluted reducing ECA solution was able to effect a reduction of up to 99% and 75% in both aflatoxin and fumonisin concentrations respectively (SAGL, 2001; 2004).

4.4 Agricultural Applications

Published as well as commissioned studies to assess the antimicrobial efficacy of the electroactivated oxidant solution against economically significant fungal contaminants present in the irrigation water of high risk greenhouse and hydroponic crops detailed significant inactivation efficacy under *in-vitro* conditions (Buck *et al.*, 2002; Mueller *et al*., 2003).

The halogenated oxidant ECA solution was assessed over a range of concentrations and exposure periods as an antimicrobial agent for the inactivation of the microconidia of *Fusarium oxysporum cubenese* (*Foc*), a fungal pathogen of global significance responsible for Fusarium wilt or Panama disease in banana plantations. As part of the same study, the same oxidant solution was evaluated for its antimicrobial efficacy against *Fusarium circinatum*, an economically important fungal pathogen responsible for pitch canker and root rot in pine seedling nurseries.

It was found that the oxidant ECA solution applied at a 1:100 dilution rate, with a 10 minute exposure was effective in eliminating both *Foc* and *F. circinatum* at initial challenge levels of 10^4 mocroconidial spores per ml (Groenewald *et al*., 2002).

A similar study was conducted to evaluate the antimicrobial efficacy of the oxidant ECA solution against common fungal and bacterial pathogens present in intensive hydroponic systems. The oxidant ECA solution was tested over a range of concentrations and exposure periods against water borne *Pythium* zoospores, *Fusarium* condidia and the gram negative bacterium *Ralstonia*, and in all cases a 100% kill was recorded after a 10 minute exposure at a 1:10 concentration (Bagnall, 2007). However, despite favourable antimicrobial efficacy results, the *in-vivo* exposure of cucumber seedlings to the mixed oxidant solution at concentrations in excess of 1:100 resulted in severe phytotoxicity. A similar adverse physiological effect following a foliar spray of cucumber seedlings with acidic electrolyzed water was reported by Fujiwara et al. (2009). An equivalent adverse phytotoxic effect in butter lettuce seedlings was evidenced in a small-scale field trial, when ECA solutions were applied at inclusion rates in excess of 1:20 (Bagnall, 2007). Notwithstanding, the inclusion of the oxidant ECA solution into the recirculating irrigant water at a rate of 1:50 resulted in a 43% increase in biomass yield relative to the untreated control in a small scale hydroponic study (Labuschagne and Bagnall, 2003).

An assessment of the ability of the halogenated oxidant solution to inactivate Tobacco Mosaic Virus (TMV) indicated that the exposure of the virus suspension to a 1:200 strength oxidant solution for a 10 minute period, resulted in a 99% inactivation of the TMV and the prevention of lesions on the leaves of challenged tobacco seedlings (Fig 2) (Labuschagne, 2003).

Untreated Control ECA Oxidant (1:50) ECA oxidant (1:100)

Figure 2. Tobacco Mosaic Virus (TMV) lesions on leaves of virus-challenged tobacco seedlings.

4.5 Medical Applications

Aside from the various reports describing the use of ECA solutions as surface or equipment disinfectants in health care facilities (Kirko *et al*., 1999; Selkon *et al*., 1999; Shetty *et al*., 1999; Thantsha, 2002) a number of invasive medical interventions using the ECA solutions have also been reported (Devyatov and Pertov, 1997; Devyatov *et al.*, 1999; Inoue *et al*., 1997; Nakae and Inaba, 2000; Landa-Solis *et al.*, 2005).

A variety of commercial preparations are currently available (Oculus Innovative Sciences, California, USA) for the treatment of topical disease conditions and the use of the FEM based ECA solution for the treatment of wounds and chronic ulcers has recently been patented (Selkon, 2007). Based on ECA solutions produced by the second generation FEM 2 technology, Puricore have recently launched the Vashe™ Wound Therapy System. Based on the patent registered by Selkon (2007), Vashe™ is promoted as a safe, non-invasive, easy-to-use, wound management process, and has been reported to promote healing by reducing wound bioburden and promoting tissue repair (Puricore, 2007).

The biocompatibility of the ECA solutions have been assessed under the guiding principles for good laboratory practice (GLP) of the Organisation for Economic Co-operation and Development (OECD) as well as the US Food and Drug administration (US FDA). These assessments comprised evaluations of acute eye irritation, acute dermal irritation and acute oral toxicity (LD_{50}), and no adverse effects or consequences to their exposure were noted (Marais, 2002).

4.6 Veterinary Applications

A number of reports attest to the improved disease control and enhanced productivity of livestock reared under intensive production conditions as a result of treatment with ECA solutions (Spirina *et al*., 1997; Marasinskaya, 1999).

In a commissioned study in an intensive pig rearing farm in Denmark, the addition of Electrochemically Activated Water (EAW) to drinking water had a significantly

beneficial effect on the productivity of weaner piglets. In addition to the elimination of potential pathogenic bacteria from the drinking water, a continuous EAW inclusion rate of 1:10 in the drinking water resulted in a higher average daily weight gain and an improved feed conversion ratio in all treated piglets during the first two weeks post-weaning (Maribo, 2002).

A further study on the clinical safety for the invasive use of the ECA solutions reports on the administration of an electrochemically activated saline solution as a post-breeding intra-uterine instillation for the control of mating-induced endometritis in mares. The oxidant ECA solution was aspetically instilled into the uterus within 12 hours of breeding, and it was found that there was no significant difference in conception rates relative to the untreated controls (Annandale *et al*., 2008).

4.7 Disinfection Bi-Products (DBP's)

Historically, the focus of disinfection has been the optimisiation of sustained antimicrobial efficacy relative to the choice and dose of the disinfecting compound applied. However, despite the substantial benefits to community health, the widespread use of chlorine based compounds for the decontamination of drinking water also results in the accumulation of disinfection bi-products which have recently been somewhat tenuously incriminated in a variety of life threatening conditions.

Upward of 700 DBP's have been reported to arise from the disinfection of potable water using chlorine, chlorine dioxide, chloramines and ozone. While alternative strategies to chlorination have reduced Trihalomethanes (THMs), Haloacetic acids (HAAs) and Total Organic Halogen (TOX) levels, these alternatives have also resulted in several new priority DBP's being generated at higher levels when compared to conventional chlorination methods (Lou and Lin, 2008). In fact pre-ozonation with subsequent chloramination has been found to produce significant increases in trihalonitromethanes [chloropicrin] levels (Krasner *et al*., 2006).

A significant proportion of neonatal, cardiovascular and oncological conditions are now being linked to the chronic exposure to these DBP's, but in many cases the veracity of the causal relationships elaborated by epidemiological studies have been

found to be based on largely spurious and unmerited extrapolations. To date, no conclusive evidence has been advanced to demonstrate an incontrovertible link between chronic exposure to THM's in drinking water and a directly allied detrimental health effect, and most research suggesting the causality of the adverse reactions fail to produce statistically credible and repeatable evidence to justify the purported linkage. The inherent difficulty of establishing a direct relationship between a recognised carcinogen and an adverse reaction over an extended exposure period is the tenuous extrapolation from the effects of acute exposure studies as well as the inability to exclude the impact of uncontrolled variables such as lifestyle and inherent predisposition.

In an extensive review of adult leukaemia in Canada, it was found that while THM's may be 'particularly important' in the etiology of chronic myelocytic leukaemia, paradoxically it was proposed that it appeared as if the bi-products of chlorination were also able to afford a 'protective effect' against the incidence of chronic lymphocytic leukaemia. Additionally, the acknowledged bias in data selection and the inability to control interim lifestyle effects resulted in the paradoxical conclusion that non-smokers were at a much higher risk of developing acute lymphocytic leukaemia as a result of consuming chlorinated water (Kasim *et al.*, 2006).

The most common DBP's associated with chlorination comprise Trihalomethanes and haloacetic acids. The four most frequently monitored THM's comprise chloroform, bromochloromethane, dibromochloromethane and bromoform. Chloroform is one of the most widely researched THM's and it has been reported that in order to consume the equivalent of a single dose of an over-the-counter chloroform based cough remedy, an adult would have to consume 8 glasses of chlorinated water every day for an entire year (Freese and Nozaic, 2004). The ongoing assessment of DBP's is far from being exhausted and previously noxious compounds have been relegated down the toxicity scale as a result of the ongoing quantification of the toxicity of previously unknown bi-products. Given that bromine has a significantly greater reactivity relative to that of chlorine (White, 1992), a recent report has confirmed that bromonitromethanes are substantially more cyto- and genotoxic than their chlorinated analogues (Krasner *et al*., 2006).

A further concern relating to the heightened awareness of DBP's, is the reliability and misapplication of the detection methods currently being employed to assess the disinfectant levels in treated water. Aside from the inappropriate choice of reagents (e.g. DPD, Indigo, Acid chrome violet K) to assess specific disinfectant concentrations, the effects of mixed oxidant species, reaction times, masking agents and ionic strength have been shown to significantly impair the integrity of the analytical assay (Gordon *et al*., 2002).

Notwithstanding all of the above, extensive regulations are in place to control the levels of DBP's in drinking water (EPA statute: 832-F-99-062). Irrespective of the disinfectant agent used, adherence to the requirements of consumer health, producer responsibility and the regulatory authorities will remain a trade-off.

The choice of any suitable disinfectant will be dependent upon:

1. Ability to penetrate and destroy infectious agents
2. Safe and easy handling, storage and shipping
3. Absence of toxic residuals or carcinogenic compounds after disinfection
4. Affordability. (EPA: 832-F-99-062).

When the potential for DBP generation using chlorination was contrasted against that of alternative agents (Ozone, UV light, Peracetic acid and mixed oxidant generators), chlorination was found to be most holistic and cost effective strategy to safeguard the quality of drinking water (Freese and Nozaic, 2004)

It has been proposed that the most sustainable solution for controlling DBP formation remains the exclusion of inorganic and organic precursors (Trussell, 1992; White, 1992). Currently flocculation, sedimentation, coagulation and filtration or extraction with activated carbon treatments are the most effective measures that can be employed for the control of THM precursors (Krasner *et al*., 2006; Lou and Lin, 2008). However due to the incremental organic and inorganic burdens in ever diminishing raw water sources, the sustained cost effectiveness of precursor removal will remain under progressively incremental pressure.

It has been claimed that the unique attributes of the ECA technology have the capacity to generate lower levels of DBP's (Bakhir, 2003; Bakhir *et al*., 2003). This is attributed to both the lower dosages of oxidant required to achieve microbial inactivation, as well as the mixed oxidant composition of the disinfectant solution. A study was commissioned to assess DBP production when the oxidant Anolyte solution was used as a disinfectant and biofilm control intervention in dental unit water lines contaminated with mature biofilms. It was reported that the exposure of the well established biofilms to the undiluted ECA solutions resulted in THM levels well below the permissible exposure limit (PEL) of 80 µg/litre as set out by the US-EPA (Puttaiah and Siebert, 2003). However these studies are by no means exhaustive and appropriate investigations will be required to adequately detail the full implications of ECA applications and DBP production (Fenner *at al*., 2006).

As with any raw water source, the levels of Total Organic Carbon (TOC) which includes dissolved humic and fulvic acids, as well as water turbidity, will influence the outcome of any disinfection attempt. In the pursuit of a universal and cost effective biocidal potable water treatment strategy, the prospect of DBP formation will remain a constant reality and compliance with global statutes of permissible exposure limits will always be a challenge.

4.8 Corrosion

Chlorine as well as the other related oxidation products of chlorides are acknowldeged to increase the corrosion potential of susceptible metals and alloys such as stainless steel. The extensive use of the latter material in high care food and beverage processing facilities increases the risk of adverse reactions when the electrolysed oxidant solutions are advocated for cleaning and disinfection purposes. Potentiodynamic studies using the oxidant solutions of a halogenated and a non-halogenated electrolyte were assessed to establish the potential risk of pitting and crevice corrosion in AISI 304 series stainless steel. While the undiluted oxidant solution of the sodium chloride based electrolyte did reveal an increased risk of localised corrosion, no risk of equivalent corrosion was elaborated when a full-strength sodium bicarbonate derived oxidant solution was assessed (Pistorius and Biermann, 2001).

In a survey conducted on the compatibility of a diverse array of metal types exposed to electrolyzed water produced at different pH permutations, it was reported that the corrosion risk of acidic electrolysed water substantially exceeded that of electrolysed solutions produced at neutral pH (Ayebah and Hung, 2005).

It is important to discern between corrosion and rust formation, and the latter arises from the generalised conversion of ferrous to ferric ions which when reduced with oxygen results in the formation of ferric oxides with rust deposits. Localised corrosion describes the focal disruption of the passive diffusion barrier at the metal:oxide interface, the penetration of chloride ions through the impaired interface and the localised dissolution of the underlying metals through direct electron transfer reactions. At a constant chloride concentration, this process in aluminium has been shown to be largely independent of a pH effect (McCafferty, 2003).

As with other disinfectant products, it is fundamentally important to apply the compounds under the appropriate conditions with due recognition of exposure period, ambient conditions e.g. temperature, and the optimum strength of oxidant solution as would be required to achieve the required antimicrobial control. In high risk applications, it may be prudent to consider substantially diluted chloride based or non-halogenated oxidant solutions for antimicrobial and biofilm control interventions (Thantsha and Cloete, 2006).

4.9 Conclusions

The use of the ECA technology as a substitute for conventional chemical based interventions has been reported over an extensive array of applications.

However, the substantial number of different reactor types and the equally diverse array of solutions produced hinders the universal adoption of the ECA technology as being a reliable biocidal intervention with repeatable results.

Disparity of assessment technique as well as that of application protocols similarly distracts from the intrinsic value of the technology, and further assessments should be borne out of consistency of device choice, solution generation specifications as well

as application method. Similarly, laboratory technique also needs to be properly appraised as substantial differences in purported antimicrobial efficacy may be reported which may not necessarily reflect equivalent performance under field conditions.

The performance evaluations over the diverse range of applications have verified the value of the technology in most of the fields assessed. However whilst the ECA technology is described as being essentially benign in comparison to equivalent chemical analogues, the perception that the solutions can be applied without due regard for potentially adverse impacts warrants highlighting. Consideration of issues including material compatibility and effluent management must be revisited prior to the unfettered adoption of the technology.

4.10 References

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Chapter 5

Non-gravimetric measurement of Electro-Chemically Activated water as a biocidal assessment tool

5.1 Abstract

Current methods of evaluating the direct antimicrobial efficacy of biocidal agents have traditionally been dependent upon established bacterial culture techniques. Electro-Chemically Activated (ECA) solutions represent the aqueous products of electrolysis, wherein the gravimetric parameters of dilute salt solutions have been selectively manipulated beyond the range of theoretical physicochemical variances normally associated with conventional electrolysis. These extraordinary variances are best described by the Oxidation Reduction Potential (ORP) or REDOX potential. Serial dilutions of a selection of common gram positive and negative bacteria were exposed to a series of diluted ECA solutions. In order to distinguish between the antimicrobial effects attributed to an elevated REDOX potential and that of established chlorine based compounds, the antimicrobial efficacy of an electro-activated bicarbonate salt solution was evaluated under an equivalent dilution series with the same bacterial bioload titrations.

Both saline and bicarbonate based solutions displayed distinctive biocidal properties which were non-linear in nature. The biocidal efficacy of both highly diluted salt and bicarbonate based anolyte solutions supports the pivotal role that changes to environmental oxidation reduction potential may play in disrupting bacterial homeostasis. By applying an ECA solution of qualified ORP to the micro-environment of the bacterium and by using the measured REDOX potential as a surrogate antibacterial monitor, one is able to afford a reliable real-time measure of biocidal efficacy. It is proposed that the measurement of ORP is an effective and complementary validation tool for conventional antimicrobial assessment procedures.

5.2 Introduction

As the universal solvent, water is near ubiquitously used in the processing and production of perishable foods and beverages. Hence, few other substances have the same capacity to influence the potential risk of transmission of foodborne illnesses and premature product spoilage.

Just as accurate monitoring and recording of disinfection procedures is a critical component of any robust quality-assured safety program, so to, is the need to adequately administer and regulate the hygiene practices associated with process and/or ingredient water (Suslow, 2004).

Current water chlorination protocols are monitored by qualitative assessment of the concentrations of free and total chlorine. Standard colorimetric titration kits afford little more that an estimation of the antimicrobial ranges of active chlorine compounds that are present in the processing and production system. Notwithstanding the notorious unreliability of these qualitative assessments, the capricious antimicrobial efficacy of chlorine compounds outside of their recommended operational pH ranges further constrains the integrity of the quality assured judgement (Park *et al.*, 2004).

The universal validity for the use of free chlorine sensors as surrogate monitors of microbial inactivation has previously been disputed due to a variety of uncontrolled and directly dependent variables which influence the interpretation of the biocidal conditions (Helbling and VanBriesen, 2007).

The “Oxidation-Reduction Potential” (ORP) or REDOX as measured in millivolts, has recently been introduced as an easily standardized approach to maintaining optimal process and ingredient water quality (Suslow, 2004). ORP offers many advantages for “real time” monitoring, and the proposed correlation between REDOX potential and bactericidal effect strongly supports its use as a rapid and reliable, single value, predictive measurement parameter for the degree of microbial inactivation of the biocide treated water (Stevenson, *et al.*, 2004; Suslow, 2004; Kimbrough *et al.*, 2006). In addition, it permits the operator to assess the real-time activity of the

biocidal treatment as opposed to the standard compliance of the applied dosage of biocide (Suslow, 2004).

Historically, interventions that are designed to result in a shift in the ORP of a disinfectant solution have depended upon the calculated addition of oxidant reagents, many of which also have intrinsic biocidal capacity. These studies were thus not able to differentiate between the direct proportion of antimicrobial effect accorded to the oxidant reagent or that due specifically to the elevated ORP. The use of non-halogenated reagents to generate exaggerated shifts in the ORP of ECA oxidant solutions have confirmed the distinctive antimicrobial role that an elevated ORP level will play, independent of the presence of chemical oxidant compounds (Kimbrough *et al*., 2006).

In terms of measuring the ORP of a treated system, reliable probes, with analog and digital recording capabilities have been built into system designs, and these probes have been integrated into audible, visual and remote alarm systems in order to alert an operator of an "out-of-specification" process parameter. The primary advantage for the integration of an ORP measurement tool into the monitoring protocol for any treated water system is that it affords the operator with a rapid and single-value measure of the disinfection potential of the treated water system in any food or beverage processing facility (Suslow, 2004).

Research has shown that exposure to a solution with an ORP value of > 650mV is capable of eliminating spoilage and pathogenic bacteria such as pseudomonad's, *Salmonella spp* and *E.coli* within a few seconds. Liao *et al*. (2007) have demonstrated that progressively higher inactivation efficiencies are directly related to increasing ORP values. Available literature also confirms that progressively more advanced microbial forms such as yeasts, moulds, algae and water borne protozoa require incremental exposure periods to higher milli-volt challenges for inactivation to be complete (http://www.orpmeter.com).

A strong oxidizing agent e.g. hydrogen peroxide or ozone in contact with a viable bacterium effectively disrupts the electronic equilibrium intrinsic to the functional bacterial barrier structure thereby eventuating in a loss of vital function (Zinkevich *et*

al., 2000; Suslow, 2004). As with an oxidative stress scenario, lower ORP values i.e. < +500mV, result in bacterial inactivation or 'stasis' which may be readily reversible when more favourable conditions are restored.

It is readily acknowledged that the water treatment chemicals conventionally used in food and beverage processing systems frequently result in suboptimal disinfectant performance due to the direct shelter afforded to the microbes within the actual food or beverage products, the ubiquitous biofilm growth, as well as the design and construction inadequacies of the process infrastructure. Diligent hygiene monitoring thus remains a Critical Control Point (CCP) that will ensure the maximum disinfection potential of the treated water destined for either sanitation of in-contact process surfaces or where the same is intended for intimate product contact.

5.3 Electrochemical Activation (ECA) of water

The Electrochemical Activation (ECA) of dilute brine solutions has been reported to result in the generation of solutions with anomalous reactional abilities and behavioural characteristics (Prilutsky and Bakhir, 1997; Bakhir, 1999).

Classic electrolysis refers to the decomposition of brine to produce a variety of biocidal chemical compounds. Conversely, ECA refers to the acquisition by the water medium of uniquely distinctive properties which exceed conventional chemical transformations. In the process of the electrolytic decomposition of water, particles or compounds are formed which cannot exist outside of benign non-activated water (Bakhir, 1997; Prilutsky and Bakhir, 1997; Bakhir, 1999; Diao *et al*., 2004).

During electrochemical activation, three categories of products are proposed to be generated within the solution. These comprise:

1. Stable products - these are stable acids (in the Anolyte) and bases (in the catholyte) which influence the pH of the solution,

2. Highly active unstable products – including free radicals and other active ion species with a typical lifetime of less than 48 hours. Included here would be

electrically and chemically active micro-bubbles of electrolytic gases, 0.2-0.5 µm in diameter and with concentrations up to 10^7 ml^{-1} which are uniformly distributed throughout the solution, and

3. Longer lasting, quasi-stable structures, which are formed at or near the electrode surface and which are comprised of free structural complexes of hydrated membranes that form around the ions, molecules, radicals and atoms. (Bakhir, 1997; Prilutsky and Bakhir, 1997).

While these categories are somewhat limited in their description, a more complete analysis of the behaviour of solute molecules at the charged electrode surface is detailed by Stumm and Morgan (1996). While the use of the oxidizing solution as a biocide has been extensively reported, the minimum effective dose of the biocide is typically represented as being a function of the concentration, exposure time and where appropriate, the disinfection hurdle, wherein a minimum point is attained where sufficient free active ingredient is available to neutralize any microbial activity to a prescribed level (Suslow, 2004).

5.4 Objective of the study

The founding hypothesis of this study was to test the contention that non-gravimetric measures of antibacterial efficacy i.e. ORP or REDOX potential may successfully be employed as a surrogate measure for quantifying the biocidal capacity of ECA solutions.

5.5 Materials and Methods

5.5.1 Generation of ECA Biocide

In contrast to the high mineralization oxidant solutions described in other reports (Shetty, *et al.*, 1999, Zinkevich, *et al.*, 2000), the halide-based oxidant biocide (S1) described in this study was generated from a 0,25% saline solution of 99.37% purity. Alternatively, the non-halide based oxidant solution (S2) was generated from a 0.3%

sodium bicarbonate feed solution of 99.7% purity. Both oxidant solution types were prepared using softened water (< 20 mg/L $CaCO_3$) derived from an anionic exchange resin. The solutions were generated by a commercial multi-reactor device installed at the Radical Waters' premises (Kyalami Business Park, South Africa).

5.5.2 Physico-Chemical titrations

After qualified dilution, the measurements of pH, ORP and Electrical conductivity (EC) were conducted for both the saline and bicarbonate derived oxidant solutions using a variety of diluent solutions of different mineralization. The ORP, EC, and pH were all measured before the solutions were used. A Waterproof double junction ORPScan of 1mV resolution was used to measure ORP of the anolyte solutions. Electrical conductivity was measured using a Waterproof ECScan of 0.01mS resolution, and the pH was measured with a waterproof pHScan 2 tester. All probes were supplied by Eutech Instruments (Singapore) and were calibrated with appropriate standards prior to solution measurement. The diluent solutions comprised municipal (tap) water, distilled water (Reitzer Pharmaceuticals, Sunninghill, SA) and de-ionised water (Food Consulting Services, Midrand, SA), and the dilution series ranged from undiluted Anolyte through dilutions of 50, 20, 10, 2 and 1%.

5.5.3 Antibacterial efficacy titration

The modified suspension test as described by Harrigan (1998) describes the addition of a known number of viable microorganisms in suspension to a solution of disinfectant (ECA oxidant) at the required concentration. The antimicrobial efficacy of the exposure is determined by whether a complete microbial inactivation was achieved and is reported as either displaying growth or no growth when plated and incubated.

5.5.4 Preparation of the cell suspension

The test organisms (*Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*) were derived from pure culture strains maintained at the

Department of Microbiology, University of Pretoria, and suspensions were prepared by growing the cells in Nutrient Broth (Biolab) for 24 h at 37°C. Two (2) ml of each cell suspension was centrifuged for 15 min at 2200 rpm. The supernatant was discarded and the pellet was re-suspended in sterile ¼ strength Ringer's solution (Merck). This cell suspension (10^9cfu/ml) was used to prepare the serial dilutions of the microbial suspensions using ¼ strength Ringer's solution (International Dairy Federation, 1962).

5.5.5 Test procedure

The experiment was designed to construct a matrix for the evaluation of the effect of a decreasing Anolyte concentration versus decreasing cell count for each bacterial species. The Anolyte at the required concentration was distributed in 9 ml quantities in sterile borosilicate test tubes. An aliquot of 0.1 ml of the cell suspension (at the predetermined concentration) was added to the disinfectant solution, and 0.1 ml was added to sterile ¼ strength Ringer's solution as a control. After an exposure period of 8 min, 0.1 ml was plated on Nutrient Agar (Biolab). The plates were incubated at 30°C, and were inspected for bacterial growth after 24-48 h. A 100% inactivation rate was recorded when no growth was visible (International Dairy Federation, 1962).

5.6 Results

5.6.1 Halide based anolyte – NaCl

5.6.1.1 Physico-Chemical titrations

Freshly generated anolyte was produced using the proprietary coaxial FEM based electrode, with an influent saline concentration of 2.5g/l, an intra-reactor flow rate of 350ml/min and a power loading of 12V and 5A. The oxidant anolyte solution was quantified in accordance with previously described post-production parameters (Table 1) (Bakhir, 1999).

Table 1. Physicochemical attributes of the undiluted saline anolyte solution.

Solution parameter	Value
Salt concentration	2,5 g/l
ORP	969mV
EC	4.15 mS/cm
pH	7.3

The saline based anolyte (S1) was diluted according to the prescribed dilution series for each different diluent type and the graphic representation of the value variations for each physicochemical parameter for each dilution are detailed in Figures 1, 2 and 3.

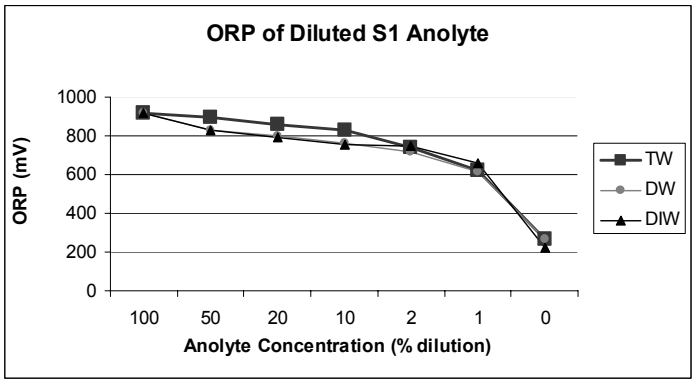

Figure 1. Changes in ORP of saline anolyte solution when diluted at different strengths and with different diluents (Legend: S1- NaCl, TW – Tap Water, DW – Distilled Water, DIW – De-Ionised Water).

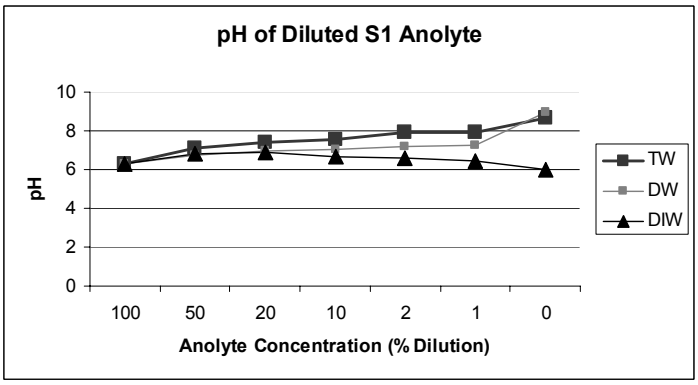

Figure 2. Changes in pH of saline anolyte solution when diluted to different strengths and with different diluents. (Legend: S1- NaCl, TW – Tap Water, DW – Distilled Water, DIW – De-Ionised Water).

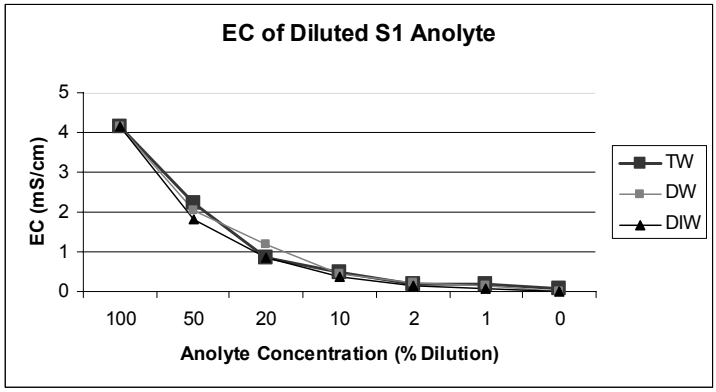

Figure 3. Changes in the Electrical Conductivity (EC) of saline anolyte solution when diluted at different strengths and with different diluents (Legend: S1 - NaCl, TW – Tap Water, DW – Distilled Water, DIW – De-Ionised Water).

As evidenced from the dilution series above, there were insignificant differences between the three diluent solutions and the physicochemical parameters of the anolyte when diluted. The progressive dilution of the saline based anolyte resulted in a non-linear reduction in the REDOX potential, while the pH of the diluted anolyte rapidly assumed the pH value of the diluent solution. The dilution of the anolyte resulted in a near linear reduction trend of the electrical conductivity of the diluted solutions.

5.6.1.2 Antibacterial efficacy titration

A series of logarithmic dilutions of NaCl based anolyte was prepared and the variations in physicochemical parameters for each dilution were recorded (Table 2).

Table 2. Physicochemical parameters of the serial dilution of the NaCl anolyte solutions.

Dilution	pH	EC	ORP
Neat	6.60	5.54	972
1:10	6.13	6.76	836
1:50	6.64	6.81	742
1:100	6.87	6.86	587
1:1000	6.92	6.84	461
1:10 000	7.13	6.92	442
Diluent*	7.08	6.89	451

* ¼ strength Ringer's solution was used as the diluent.

5.6.1.3 Antibacterial efficacy

Suspensions of the different bacterial strains were serially diluted to predetermined cellular concentrations and were exposed to the range of anolyte dilutions as described above. After plating and incubation, the presence of bacterial growth was recorded and the results are tabulated per bacterial species and were detailed as either growth or no growth (100% inactivation).

Table 3. Inactivation of *B. subtilis* over a range of saline anolyte dilutions.

Anolyte concentration	Cell concentration (cfu/ml)				
	10^6	10^5	10^4	10^3	10^2
Control	Growth	Growth	Growth	Growth	Growth
Undiluted	No growth	No growth	No growth	No growth	No growth
1:10	Growth	Growth	No growth	No growth	No growth
1:50	Growth	Growth	Growth	No growth	No growth
1:100	Growth	Growth	Growth	No growth	No growth
1:1000	Growth	Growth	Growth	No growth	No growth
1:10 000	Growth	Growth	Growth	Growth	No growth

While the undiluted anolyte achieved inactivation of all bacterial challenges, it was only when the challenge dose was reduced to a $\log_{10} 4$ count that the 1:10 dilution of saline based anolyte resulted in complete inactivation. Further reductions in the challenge dose resulted in total bacterial inactivation even at higher anolyte dilutions.

Table 4. Inactivation of *S. aureus* over a range of saline anolyte dilutions.

Anolyte concentration	Cell concentration (cfu/ml)				
	10^6	10^5	10^4	10^3	10^2
Control	Growth	Growth	Growth	Growth	Growth
Undiluted	No growth	No growth	No growth	No growth	No growth
1:10	No growth	No growth	No growth	No growth	No growth
1:50	No growth	No growth	No growth	No growth	No growth
1:100	Growth	No growth	No growth	No growth	No growth
1:1000	Growth	Growth	Growth	No growth	No growth
1:10 000	Growth	Growth	Growth	No growth	No growth

Complete inactivation of high dose *S. aureus* challenge was achieved up to a 1:50 anolyte dilution and the microbial inactivation rate described an inverse linear trend with increasing anolyte dilution thereafter. Complete inactivation of all cell suspensions with counts of 3 $\log_{10}$ and below was achieved with anolyte dilutions of up to 1:10 000.

Table 5. Inactivation of *E. coli* over a range of saline anolyte dilutions.

Anolyte concentration	Cell concentration (cfu/ml)				
	10^6	10^5	10^4	10^3	10^2
Control	Growth	Growth	Growth	Growth	Growth
Undiluted	No growth	No growth	No growth	No growth	No growth
1:10	No growth	No growth	No growth	No growth	No growth
1:50	Growth	No growth	No growth	No growth	No growth
1:100	Growth	Growth	Growth	No growth	No growth
1:1000	Growth	Growth	Growth	Growth	Growth
1:10 000	Growth	Growth	Growth	Growth	Growth

Aside from the undiluted anolyte solution, inactivation of the highest concentration of *E.coli* organisms was only achieved up to a 1:10 strength anolyte solution. No inactivation of the bacterial cells in suspension was achieved with anolyte dilutions of 1:1000 or higher.

Table 6. Inactivation of *Ps. aeruginosa* over a range of saline anolyte dilutions.

Anolyte concentration	Cell concentration (cfu/ml)				
	10^6	10^5	10^4	10^3	10^2
Control	Growth	Growth	Growth	Growth	Growth
Undiluted	No growth	No growth	No growth	No growth	No growth
1:10	Growth	No growth	No growth	No growth	No growth
1:50	Growth	No growth	No growth	No growth	No growth
1:100	Growth	No growth	No growth	No growth	No growth
1:1000	Growth	Growth	Growth	No growth	No growth
1:10 000	Growth	Growth	Growth	Growth	Growth

While the undiluted anolyte displayed total inactivation of all *Ps. aeruginosa* organisms, no further inactivation of the high bacterial cell count preparations was

achieved with the diluted anolyte solutions until the suspension had been diluted to a 5 $\log_{10}$ cell count. At this cell concentration, the 1:100 anolyte dilution was still able to achieve a complete inactivation of all bacteria. No inactivation was obtained even at low cell suspension counts when the anolyte solution was diluted beyond 1:1000.

5.6.2 Non-halide based anolyte – $NaHCO_3$

5.6.2.1 Physico-Chemical titrations

A 3g/l $NaHCO_3$ solution of 99.7% m/m purity grade was used in the preparation of the non-halide based anolyte. This solution was activated using the customised sodium bicarbonate (S2) ECA device. The intra-reactor flow rate was preset to 350ml/min and the power supply to the reactor was increased to 24V and 3A relative to that of the saline based generation. The characteristics of the solution were measured in accordance with previously described conventions (Table 7).

Table 7. Physicochemical attributes of the undiluted sodium bicarbonate anolyte solution.

Solution parameter	Value
$NaHCO_3$ concentration	3 g/l
ORP	958mV
EC	1.36 mS/cm
pH	6.88

Relative to the saline based anolyte of reduced mineralization i.e. 2.5g/l, there was a slight reduction in REDOX potential and a substantially reduced electrical conductivity of the bicarbonate derived anolyte solution. While the ionic dissolution of the bicarbonate salt has been shown to be substantially more temperature dependent than that of NaCl, both feed salt solutions were prepared with water at ambient temperature i.e ± 20^{o}C, and the reduced EC is a direct reflection of the diminished electrical reactivity of the dissolved bicarbonate salt in solution.The bicarbonate derived anolyte was diluted according to the prescribed dilution series and the graphic representation of the variations in physicochemical parameters for each dilution are presented in figures 4, 5 and 6.

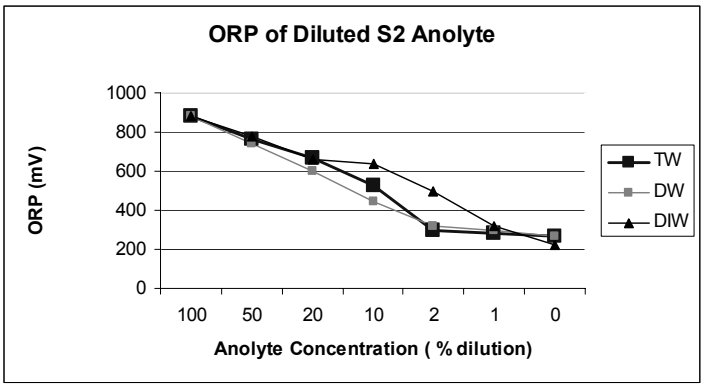

Figure 4. Changes in anolyte ORP when diluted at different strengths and with different diluents (Legend: S2 - $NaHCO_3$, TW – Tap Water, DW – Distilled Water, DIW – De-Ionised Water).

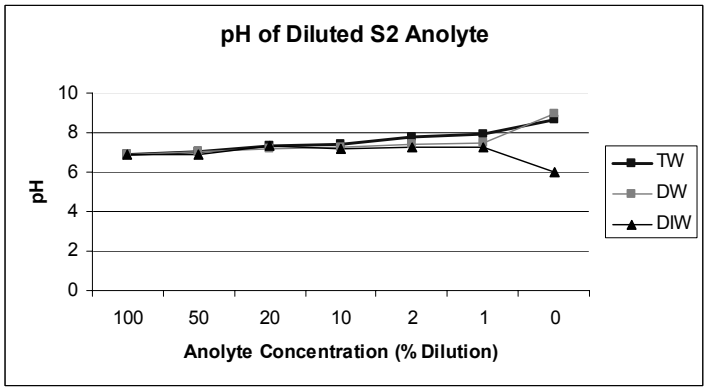

Figure 5. Changes in anolyte pH when diluted to different strengths and with different diluents. (Legend: S2 -$NaHCO_3$, TW – Tap Water, DW – Distilled Water, DIW – De-Ionised Water).

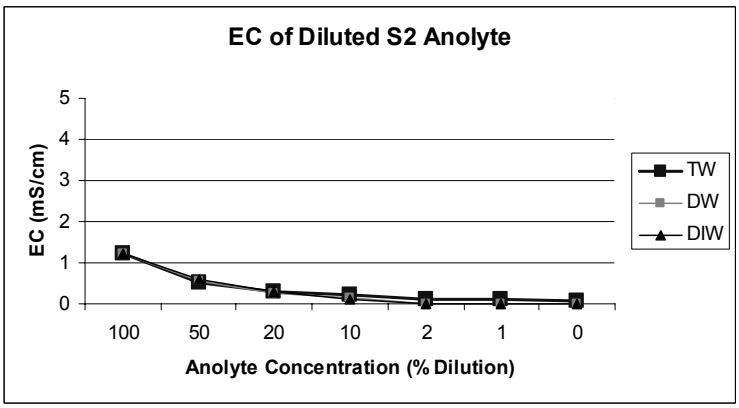

Figure 6. Changes in the Electrical Conductivity (EC) of anolyte solutions when diluted at different strengths and with different diluents (Legend: S2-$NaHCO_3$, TW – Tap Water, DW – Distilled Water, DIW – De-Ionised Water).

The changes in the physicochemical parameters of the sodium bicarbonate anolyte displayed substantial deviations over the dilution series relative to that of the saline based anolyte. The reduction in REDOX potential of the $NaHCO_3$ based anolyte occurred more rapidly and the ORP fell below the proposed antibacterial hurdle value of 600mV at approximately 15% strength versus that of a 1% strength detailed with the NaCl based anolyte. The pH of the diluted anolyte rapidly equilibrated with the pH of the diluent solution, while the EC described a linear reduction albeit from a significantly lower initial conductivity value.

5.6.2.2 Antibacterial efficacy titration

As with the NaCl derived anolyte, an equivalent series of logarithmic dilutions of the $NaHCO_3$ based anolyte were prepared and the variations in physicochemical parameters for each dilution were recorded (Table 8).

Table 8. Changes in physicochemical parameters of the diluted Sodium Bicarbonate anolyte solutions.

Dilution	pH	EC	ORP
Neat	7.63	1.36	958
1:10	8.17	5.45	842
1:50	7.99	5.51	784
1:100	6.73	5.73	468
1:1000	7.23	5.01	386
1:10 000	7.18	5.69	377
Diluent*	7.75	5.77	444

* ¼ strength Ringer's solution was used as the diluent throughout this study.

4.6.2.3 Antibacterial efficacy

The serial dilutions of the bacterial strains were prepared and exposed to the $NaHCO_3$ anolyte in accordance with the previously described protocol, and the same evaluation and recording procedure was followed.

Table 9. Inactivation of *B. subtilis* over a range of sodium bicarbonate anolyte dilutions.

Anolyte concentration	Cell concentration (cfu/ml)				
	10^6	10^5	10^4	10^3	10^2
Control	Growth	Growth	Growth	Growth	Growth
Undiluted	No growth	No growth	No growth	No growth	No growth
1:10	No growth	No growth	No growth	No growth	No growth
1:50	Growth	Growth	No growth	No growth	No growth
1:100	Growth	Growth	No growth	No growth	No growth
1:1000	Growth	Growth	Growth	Growth	Growth
1:10 000	Growth	Growth	Growth	Growth	Growth

Bicarbonate based anolyte was effective in achieving complete inactivation of all *B. subtilis* cell suspensions up to a 1:10 dilution. Solutions diluted below 1:10 strength were only effective in inactivating cell suspensions of $4\log_{10}$ and below and there was incomplete inactivation of all suspensions when the anolyte was diluted to 1:1000 and more.

Table 10. Inactivation of *S. aureus* over a range of sodium bicarbonate anolyte dilutions.

Anolyte concentration	Cell Concentration (cfu/ml)				
	10^6	10^5	10^4	10^3	10^2
Control	Growth	Growth	Growth	Growth	Growth
Undiluted	No growth	No growth	No growth	No growth	No growth
1:10	No growth	No growth	No growth	No growth	No growth
1:50	No growth	No growth	No growth	No growth	No growth
1:100	No growth	No growth	No growth	No growth	No growth
1:1000	Growth	Growth	Growth	No growth	No growth
1:10 000	Growth	Growth	Growth	Growth	Growth

As with the exposure tabulation of the saline based anolyte dilutions, the bicarbonate based anolyte was effective in inactivating all cell suspensions beyond a 1:50 dilution. It would thus appear that *S. aureus* is equivalently sensitive to exposure to both saline and bicarbonate based anolytes even at substantial dilutions. This matched antibacterial efficacy is achieved without the addition of recognized halogen derived

biocidal compounds in the diluted bicarbonate based anolyte solutions. However, given that ¼ strength Ringers was used as the diluent solution, it is feasible that some of the constituent chloride ions (1.358gm/l or 1358ppm) may have undergone transformation into reactive chlorine species following admixture with the bicarbonate based anolyte.

Table 11. Inactivation of *E. coli* over a range of sodium bicarbonate anolyte dilutions.

Anolyte concentration	Cell Concentration (cfu/ml)				
	10^6	10^5	10^4	10^3	10^2
Control	Growth	Growth	Growth	Growth	Growth
Undiluted	No growth	No growth	No growth	No growth	No growth
1:10	No growth	No growth	No growth	No growth	No growth
1:50	No growth	No growth	No growth	No growth	No growth
1:100	No growth	No growth	No growth	No growth	No growth
1:1000	Growth	Growth	No growth	No growth	No growth
1:10 000	Growth	Growth	Growth	Growth	Growth

Aside from the 1:10 000 dilution, the bicarbonate based anolyte was effective in achieving inactivation of all *E.coli* suspensions with bacterial counts of up to 4 $\log_{10}$. The diluted anolyte solutions were effective in the inactivation of bacterial counts of 6 $\log_{10}$ and below using a 1% anolyte concentration, and this corresponded to an ORP of the diluted anolyte of 468mV (Table 8).

Table 12. Inactivation of *P. aeruginosa* over a range of sodium bicarbonate anolyte dilutions.

Anolyte concentration	Cell concentration (cfu/ml)				
	10^6	10^5	10^4	10^3	10^2
Control	Growth	Growth	Growth	Growth	Growth
Undiluted	No growth	No growth	No growth	No growth	No growth
1:10	No growth	No growth	No growth	No growth	No growth
1:50	No growth	No growth	No growth	No growth	No growth
1:100	No growth	No growth	No growth	No growth	No growth
1:1000	Growth	Growth	Growth	Growth	Growth
1:10 000	Growth	Growth	Growth	Growth	Growth

As with table 11 above, the bicarbonate based anolyte achieved complete inactivation of all cells in suspension up to a 1% concentration of bicarbonate derived anolyte. No inactivation was achieved when the suspensions were exposed to anolyte dilutions of 1:1000 and above.

5.7 Discussion

From the coordinate plots detailed in Figures 1 and 4, it is evident that the changes in the ORP of the diluted solutions do not describe a linear trend when contrasted against the incremental dilution percentage. While there was a direct correlation between the Anolyte dilution series and the proportionate reductions in Electrical Conductivity (EC) (Figures 3 and 6) arising from an anticipated linear dilution effect, the same correlation could not be ascribed to the non-linear trend detailed when the REDOX potential was measured over the equivalent dilution series. There appears to be a mismatched, yet directly causal relationship between the dilution effect on the linear parameters (concentration of chemical constituents) and the non-linear effects on the REDOX potential when contrasted against the capacity to predict the irreversible damage to the different bacterial strains.

The data from the two antibacterial titration series confirms that the non-halide based Anolyte was effective as a broad spectrum antibacterial agent against most of the test bacteria, with the qualified exception of *B. subtilis*, which at the highest bacterial cell concentration was not inhibited at an Anolyte dilution which exceeded 1:10. In contrast, all other test microorganisms with titres of up to 6 $\log_{10}$ were inhibited with Sodium bicarbonate based Anolyte when diluted up to a final exposure concentration of 1%.

Contrary to the conventional dogma which draws a correlation between specific bacterial susceptibility and a specific concentration of biocidal compound (i.e. MIC), the antibacterial efficacy of the Anolyte as an biocidal agent appears not to be predicatated upon by the type of cell barrier structure of the test microorganisms (Gram negative vs. Gram positive), but rather the data suggests that the antibacterial response tends to be strain specific. While by no means exhaustive, it is feasible to speculate that bacteria with an intrinsic capacity for endospore formation (*B.subtilis*)

as well as EPS formation (*Ps.aeruginosa*), may well display a non-adaptive and adjunct capacity to withstand the adverse stressors that the exposure to an overtly oxidative milieu may elicit.

It is conventionally recognised that the classical chemical biocide model and its associated gravimetric antibacterial efficacy titrations will elaborate a repeatable 'Minimum-Inhibitory Concentration' (MIC). This MIC measure subscribes to a conventional linear dilution model which predicts the minimum concentration of an active biocidal ingredient that would be required in order to eliminate a prescribed bacterial bioload within a predictable time frame. However, when the Anolyte solutions are subjected to an equivalent dilution series, the progressive dilution of the biocidally active chemical constituents as reflected in the EC dilution series (Figures 3 and 6), do not correspond with the hithertofore acknowledged levels of active chemical ingredient i.e. MIC which are conventionally reported as being a prerequisite for antibacterial compliance.

The distinctly elevated levels of REDOX potential suggests a causal relationship with the degree of bacterial inactivation, and the resultant biocidal effect does not appear to be dependent upon the proportionate concentration of conventionally recognised biocidal agents i.e. halide based reagents. This tentative causal relationship has also been proposed by Kim *et al*., (2000), Park *et al*., (2004) and Kimbrough *et al*., (2006). The measurement of the REDOX potential of the progressively diluted anolyte solutions thus appears to be a reliable non-gravimetric measure for the prediction of its antibacterial efficacy, and it is proposed that the predictability of its composite antibacterial attributes may be substantially augmented were it to be integrated with the roles that pH and electrical conductivity may play in overall biocidal effect.

Traditional antibacterial efficacy assessment protocols call for a procedurally qualified and quantified sampling of biocide treated environments in order to establish the success of the bacterial control strategy. Such prescriptive procedures and their allied protocols are seldom designed to afford blanket representation of highly variable environments which are likely to be contaminated with different bacteria. Similarly, and notwithstanding the vagaries of individual sampling techniques and the spectrum of other environmental features i.e. temperature, time, sampling asepsis etc.,

that may impact upon the relevance of a sampling effort, bacterial cultures also require extended periods of incubation under highly specific culture conditions prior to any definitive indication being afforded as to the levels of residual bacterial contamination that may follow an antibacterial intervention.

While this study readily acknowledges the indispensability of conventional sampling and culture procedures, the time constraints allied to the delays in the availability of results especially with highly specific culture conditions, further reaffirms the limits that conventional sampling and culture protocols impose on optimum productivity goals. In light of the relative novelty of this approach for assessing antibacterial potential, it would be reckless to promote the exclusive adoption of the REDOX monitoring technology without the retention of established and complementary bacterial control and assessment measures as are currently accepted and practiced globally. However, despite this novelty, quality assurance managers now have access to a tool whereby it is possible to control water and product disinfection on a real-time and in-process basis without having to rely exclusively upon a deferred laboratory based result which is constrained by the limitations of conventional microbial sampling and culture protocols.

5.8 Conclusion

Traditional conventions for bacterial control of process and potable water in food or beverage production are dependent upon a basket of features, wherein the goal of minimizing the effective dose of the sanitizer used for bacterial disinfection is balanced against the adverse dose dependent implications on product sensory quality, the environment and crucially, human health.

Aside from confirming the safe and novel biocidal capabilities of the ECA solutions, of greater significance was the quantification of the intrinsic measurable capacity of the ORP of diluted ECA solutions as being a reliable tool to predict the biocidal effect of a given Anolyte dilution when dosed into a system with a projected bacterial bioload. The study provides evidence to support the contention that Electro-Activated water solutions may exert their antibacterial effect in a manner which is distinctive from that reported for conventional chemical based remedies. That bacterial

inactivation could be achieved at significant dilutions of both of the two types of anolyte solutions reaffirms the hypothesis that the antibacterial effect is unlikely to be associated with a purely gravimetric measure of biocidal reagent. Additionally, the exclusion of reactive halogen based species from the antibacterial efficacy appraisal through the use of a Sodium Bicarbonate derived anolyte, substantially confirmed the antibacterial effect of the non-halogen reagent.

In conclusion, the measurement of an array of anomalous physicochemical attributes of the ECA solutions on a real-time and in-process basis may provide a reliable tool to describe optimal antibacterial concentrations in different process water systems, and consequently may permit its users to enhance quality assurance measures and decisions without compromise to product quality or consumer satisfaction.

5.9 Acknowledgements

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