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# Acidification of potassium acetate and potassium citrate with/without KCl by electro-activation and impact of the solution on spores of *Clostridium sporogenes* PA 3679 at ambient temperature

Omar El Jaam [a, b], Ismail Fliss [a, b], Wassef Ben-Ounis [c, d, e], Mohammed Aïder [a, c, *]

[a] Institute of Nutrition and Functional Foods (INAF), Université Laval, Quebec, Qc, G1V0A6, Canada
[b] Department of Food Sciences, Université Laval, Quebec, Qc, G1V0A6, Canada
[c] Department of Soil Sciences and Agri-Food Engineering, Université Laval, Quebec, Qc, G1V0A6, Canada
[d] Institute of Food Technologies, ITA La Pocatière, Qc, G0R 1Z0, Canada
[e] Université Libre de Tunis, ULT, Tunis, 1002, Tunisia

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## ABSTRACT

Acidity and ORP of electro-activated potassium acetate and potassium citrate with and without KCl was studied and the effect of these solutions on spores of *C. sporogenes* was evaluated at ambient temperature. The ORP and amount of formed acid were proportional to the intensity of the applied electric current and duration of the electro-activation process. Electro-activation at 400 mA during 60 min yielded solution with ORP values of +417.50 to +1043.33 mV and pH of 3.18–3.47. The four solutions were used to study their effect on spores of *C. sporogenes* PA 3679 at ambient temperature. The application of electro-activated solutions resulted in a reduction level of >7 log CFU/mL of the spores. The sporicidal effect of the electro-activated solutions was demonstrated by transmission electron microscopy analysis. The micrographs showed several damages in the spores components such as core, coat and spore cortex.

## 1. Introduction

Electro-activation is a science of studying the activation energy, thermodynamics and physico-chemical properties of aqueous solutions submitted to the action of an electric field under specific conditions where the mass transfer of anions and cations is modulated by special disposition of ion exchange membranes. Basically, electrochemical activation of any aqueous solutions is based on electrolysis. Oxidation and reduction take place in the electro-activation reactor in anodic and cathodic sections, respectively. Free electrons migrate to the anode and positively charged species migrate to the cathode (Aider, Gnatko, Benali, Plutakhin & Kastyuchik, 2012; Shirahata, Hamasaki & Teruya, 2012; Liato, Labrie, Benali & Aider, 2015a). Catholyte and anolyte solutions generated during electro-activation process different properties. Electro-reduction reactions on the cathode side cause an increase of the solution pH and a decrease of the ORP (Soltoft-Jensen & Hansen, 2005), while the obtained solution in the anodic side can be moderately to highly acid with oxidative oxidation-reduction potential (ORP) (Prilutsky & Bakhir, 1997). By using appropriate configuration of the electro-activation reactor, it is possible to produce anolyte with acidic pH of $2.3 < pH < 6.5$ and high ORP of +1000 to +1200 mV. At the other side, a catholye solution with alkaline pH of $7 < pH < 12$ and negative ORP of $-80 < ORP < -900$ can be generated (Gnatko, Kravets, Leschenko & Omelchenko, 2011; Aider et al.,2012; Liato, Labrie, Benali & Aider, 2015a; Liato, Labrie, Benali, & Aïder, 2016). The processes can be controlled by using appropriate ion exchange membranes and cell configurations of the reactor, the factor which permits obtaining the desired pH and ORP of the solution (Aider and Gimenez-Vidal 2012). Moreover, the effect of electro-activation applies on complex molecules and ions on which it affects their activation energy for specific reactions

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* Corresponding author. Department of Soil Sciences and Agri-Food Engineering, Université Laval, Quebec, Qc, G1V0A6, Canada.
*E-mail address:* mohammed.aider@fsaa.ulaval.ca (M. Aïder).

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and other properties such as the dipole moment and electron distribution on the outer orbital. As consequence, electro-activated solutions are highly active in terms of physico-chemical and biological potency (Kim, Hung & Brackett, 2000b; Shironosov, 2009)). Thus, during the electro-activation process, the solutions are in a metastable state with significantly modified properties regarding their oxidation-reduction potential, pH, amount of reactive and active species, including dissolved gases such as oxygen and hydrogen. Consequently, electro-activation makes solutions convenient for different biotechnological and chemical reactions. Moreover, they have potential to be used as a complex powerful hurdle to ensure the microbial food safety and to reduce the use of conventional disinfecting agents (Izumi, 1999; Aider et al., 2012).

Some electrochemically treated solutions have been used for the inactivation of endospore forming bacteria such as *Bacillus anthracis* spores, pathogenic microorganisms, enterotoxins and yeast. They were also used against *Escherichia coli* O157:H7, *Salmonella Enteritidis*, *Salmonella typhimurium*, and *Listeria monocytogenes* in fresh fruits and vegetables, eggs, poultry carcasses, pork, and seafood. These solutions are mainly obtained by electrolyzing NaCl solutions. They also showed some activities in the treatment and prevention of biofilms formation (Deza.M, Araujo & Garrido, 2003; Nan et al., 2010;Aider et al., 2012; Guillou, Besnard, El Murr & Federighi. 2003; Presman & Levitina, 1962; Ayebah, Hung & Frank, 2005, Ayebah, Hung, Kim & Frank, 2006). However, electrochemically activated NaCl solutions have serious inconvenient in food applications. The NaCl molecule acts as precursor for the formation of free chlorine and different chlorine-based chemicals such as sodium hypochlorite. All these chemicals are hazardous for human health, create strong and undesirable smell of chlorine. They also act as bleaching agents. Their presence negatively affect many different molecules with nutraceuticals effect such as β-carotene and phenolic compounds. Thus, it is necessary to use chlorine-free chemicals to produce electro-activated solutions without the aforementioned inconvenients. In this context, salts of food grade organic acids such as citrates and acetates can be used. Indeed, the use of such chemicals offers a possibility of forming their conjugated acids *in situ* of the electro-activation reactor. The generated electro-activated solutions of salts of organic acids are thus expected to possess the same reactivity of their conjugated acids and the activity/reactivity of activated ionic species. Moreover, considering that activation of such solutions will generated acidic medium with high positive (oxidative) oxidation-reduction potential and saturation by oxygen, one can expect that they will be highly effective against strict anaerobic bacteria and spores such as *Clostridium* spp.

Thus, the main objective of this study was to investigate the effect of electro-activation on the acidity and redox potential of potassium acetate and potassium citrate with or without KCl, and to evaluate their effect on the destruction of *C. sporogenes* PA 3679 spores at ambient temperature.

## 2. Materials and methods

### 2.1. Chemicals and materials

Potassium acetate was purchased from Amresco (Amresco LLC, Cleveland, OH, USA). Potassium citrate was from Fisher Scientific (Fisher Scientific, NJ, USA), KCl was provided by Anachemia (Anachemia, Montreal, Canada). NaCl was purchased from Caledon Laboratories (Caledon Laboratories LTD, Georgetown, Canada). The initial solutions were prepared in distilled water to final concentration of the salt of the organic acid and $Cl^-$ of 0.0625 M and 4 ppm, respectively.

Anion (AM-40) and cation (CM-40) exchange membranes were purchased from the Schekina-Azot (Shchekina, Russian Federation). The activation of these membranes was described as follow: before use, the membranes were wiped with 96%-ethanol, then soaked for 24 h in series of NaCl solutions with different concentrations (320, 160 and 80 $g.L^{-1}$). Finally, membranes were washed with distilled water and stored in a NaCl solution (40 $g.L^{-1}$) until use.

Resistant to corrosion electrodes made of Ruthenium–Iridium coated titanium (RuO2–IrO2–TiO2) were used as anode and cathode. They were purchased from Qixin Titanium Co (Qixin-Titanium Co., Baoji, Shaanxi, China).

### 2.2. Electro-activation reactor and preparation of electro-activated solutions

The process of electro-activation of the studied aqueous solutions was carried out inside a reactor which was composed of three sections: anodic and cathodic sections which were separated by anion (AM-40) and cation (CM-40) exchange membrane from the central section, respectively (Fig. 1). The cathodic section was filled with 0.25 M NaCl solution and was the same whatever the targeted solution in the anodic side. The central section was filled by the same solution which was electro-activated in the anodic section (potassium acetate or potassium citrate) but at a concentration of 0.25 M. The anodic section was used to produce the targeted electro-activated solution and was filled at time zero by a 0.0625 M of potassium acetate or potassium citrate solution with or without KCl (Table 1). The used reactor was adapted from the study of Liato et al. (2015) (Liato, Labrie, Benali & Aider, 2015b). The reactor was connected to a DC-electric current generator (CircuitSpecialists, Tempe, AZ, USA). The electro-activation process was carried out for 1 h and samples were taken at 10, 20, 30, 40, 50 and 60 min. The electric current intensity was set at 100, 200, 300 and 400 mA.

The pH was measured by using a pH-meter (SympHony, VWR, Singapore) with a pH-electrode (VWR, Singapore). The oxidation-reduction potential (ORP) was measured by using an ORP-meter (EcoSense, VWR, Montreal, Canada). The percentage of formed acid (titratable acidity) in the anodic section was determined by using the acid-base titration method. To achieve this measurement, 10 ml of electro-activated solution was titrated with 0.25 M NaOH (VWR, Canada) by using phenolphthalein (Laboratoire MAT INC, Canada) as pH indicator. The end of titration point was reached when a pink colour persists during 30 s.

### 2.3. Evaluation of the sporicidal effect of the electro-activated solutions

Once prepared, the electro-activated solutions of potassium acetate and potassium citrate, with or without KCl, were inoculated with *C. sporogenes* spores suspension to a final concentration of $10^7$ CFU/mL. Then, they were incubated anaerobically at 37 °C for 48 h. The samples were then 10-fold serially diluted in sterile 0.1% peptone water and enumerated on brain heart infusion (BHI) (Becton, Dickinson & Co., Sparks, MD, USA) agar plates.

After treatment, spores were immediately pelleted and fixed by adding 2.5% glutaraldehyde in cacodylate buffer (0.1 M, pH 7.3) for 24 h at ambient temperature (22 °C ± 1 °C). Spores were washed and postfixed with 1% $OsO_4$ in the cacodylate buffer during 90 min at 4 °C. The samples were then dehydrated with serial concentrations of ethanol, embedded in epoxy resin and polymerized at 37 °C and 60 °C overnight for 72 h, respectively. The polymerized samples were sectioned with a diamond knife in an ultra-microtome (60–80 nm), stained with uranyl acetate (3%) and lead citrate (0.1%). They were then observed by using a transmission electron microscopy (JEOL JEM-1230 JEOL Ltd., Tokyo, Japan) at 80 kV with a