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Electrolyzed water: Effect on seed decontamination and seed quality

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Abstract

Electrolyzed water (EW) is a new technology that emerged recently with potential application in agriculture, medicine and food industries, mainly in microbiological aspects. This unconventional or 'green' technology has the purpose to decrease the use of natural resources like water with minimal generation of chemical/toxic residues. EW possesses strong bactericidal and virucidal and moderate fungicidal properties. EW treatment may be used as an effective method for reducing microbial contamination on seed. Acidic Electrolyzed Water (AEW) shows wide ranging fungicidal activity, which may facilitate its use as a contact fungicide on aerial plant surfaces. More studies are necessary in relation to this technology and its possible applications in seed technology. The use of EW is an emerging technology and the door is open to further research and development.

Keywords: Electrolyzed water, seed decontamination, seed quality

Introduction

Current environment scenario is directed to rational use of natural resources. Emerging technologies have characteristics of decrease consumption of energy and water. The concept of this technology is to minimize or not to produce chemical residues, and still have potential to many different uses, including in seed technology. Electrolyzed water technology has one or more that premises of green chemistry (Proctor, 2011)^[1]. EW can be a technology with effective application in seed decontamination and seed quality improvement, because adaptations are possible, with easy production and little modifications are necessary to places where water is already used.

Electrolysed water ("Electrolyzed Water") also known as electrolyzed oxidizing water, electroactivated water or electrochemically activated water. It is produced by the electrolysis of ordinary tap water containing dissolved sodium chloride or magnesium chloride or potassium chloride. Typically, tap water has sufficient dissolved salts for the electrolysis of water. The electrolysis of such salt solutions produces a solution of sodium hypochlorite, which is the most common ingredient in household bleach. The resulting water is a known cleanser and disinfectant / sanitizer but is not a surfactant (soap).

Electrolysed water was initially developed in Japan. It has been reported to have strong bactericidal effects on most pathogenic bacteria that are important to food safety. EW is an universal biocide eliminating all kind of virus, bacteria, fungus, spores, algae and mold. It is 100% biodegradable and harmless for Human and Environment. Ecological and cost effective alternative to traditional disinfection methods, 70-80 times more efficient than chlorination/sodium hypochlorite (Sana water solution, 1986)^[2].

This review presents basic aspects like History, composition, advantages and disadvantages, mechanisms and some tendencies of EW on seed quality.

Electrolyzed Water History

Electrolyzed water was first developed in Russia around 1900 for water regeneration, water decontamination and for sanitizing medical devices. However, it was used for the first time for food processing in soda industry in Japan in 1980 (Al-Haq *et al.*, 2005; Hricova *et al.*, 2008; Rahman *et al.*, 2016) ^[3, 4, 5]. Electrolyzed reduced water (ERW) with pH of 8–10 has been developed for health benefit and studied in 1931 in Japan, and its first application in agriculture was initiated in 1954. In 1960, it was applied for medical purposes as health-beneficial water in 1960 and in 1966 the Ministry of Health, Labour and Welfare of Japan confirmed that ERW was effective for chronic diarrhea, indigestion, abnormal gastrointestinal fermentation, antacid, and hyperacidity (Shirahata *et al.*, 2012) ^[6]. With recent development in technology, industries have been attempted to improve the electrolyzed water technology and

Corresponding Author: Sivaji Sivasakthi Ph.D. Scholar, Department of Seed Science and Technology Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India it has become more popular and gained more attraction as a promising non-thermal technology (Yoshida, 2003) ^[7].

Electrolyzed water: production and chemistry

EW is produced by passing of sodium chloride solution (or potassium chloride or magnesium chloride solution) in an electrolysis system with two poles such as anode (+) and cathode (-), with or without membrane or diaphragm (Huang *et al.*, 2008; Cui *et al.*, 2009) ^[8, 9].

During electrolysis, sodium chloride is dissolved in deionized water, which dissociates into Cl^- and Na^+ . Meanwhile, water molecules are electrolyzed and form hydroxide (OH⁻) and hydrogen ions (H⁺).

Ions with negative charge (Cl⁻ and OH⁻) move to the anode to give up the electrons and form oxygen gas (O₂), chlorine gas (Cl₂), hydrochloric acid (HCl), hypochlorite ion (OCl⁻), and hypochlorous acid (HOCl). Positively charges ions (H⁺, Na⁺) move to cathode to obtain electrons and become hydrogen gas (H₂) and sodium hydroxide (NaOH).

At the end of the electrolysis process, the systems with membrane division produces two types of electrolyzed water *viz.*, acidic electrolyzed water (AEW) from anode side and basic electrolyzed water (BEW) from cathode side (Huang *et al.*, 2008; Cui *et al.*, 2009) ^[8, 9]. AEW can be used as a sanitizer and BEW can be used as a cleaning agent.

Recently, industries and researchers have reported the generation of neutral electrolyzed water (NEW) (Al-Haq *et al.*, 2005; Hricova *et al.*, 2008) ^[3, 4] and slightly acidic electrolyzed water (SAEW) (Nan *et al.*, 2010) ^[10].

The NEW is produced by mixing the anodic solution with OH⁻ ions or by electrolysis of NaCl in a single-cell unit (Hricova *et al.*, 2008; Rahman *et al.*, 2016)^[4, 5], while SAEW is generated by the electrolysis of HCl alone or in combination with NaCl in a single chambered electrolyzed water generator (Forghani *et al.*, 2015; Rahman *et al.*, 2016)^[11, 5].

Table 1: pH and Oxidation-Reduction Potential (ORP) of AEW,BEW, SAEW and NEW (Al-Haq *et al.*, 2005; Hricova *et al.*, 2008;
Nan *et al.*, 2010) ^[3, 4, 10].

Type of water	рН	ORP (mV)
AEW	2-3	>1000
BEW	10-13	-700 to -800
SAEW	5-6.5	850
NEW	7–8	750-1000

Important parameter that influences the effect of EW together pH and ORP is free chlorine concentration (FCC). When the chlorine content increases, the bactericidal activity is higher (Park *et al.*, 2004) ^[12].

Some other factors can interfere the EW production, like water flow. It can change FCC and ORP in inverse proportion.

Salt concentration of brine affect in direct proportion with FCC and electric conductivity. Temperature has little influence in EW parameters (Hsu, 2005) ^[13]. Total chlorine is the group with all forms like chlorohydrins (fatty acids), chloramines and free chlorine (HOCl, OCl⁻, Cl₂) (White, 2010) ^[14].

High FCC must be used in a caution way, because chlorine is dangerous to health of workers, causing damage to respiratory tract (when gas evaporate), irritation to skin (direct contact) and others (WHO, 2000)^[15].

A range of FCC must be determined together with pH for better action in microorganism as well as application form.



Fig 1: Electrolyzed water production unit separated by diaphragm/membrane.

Anodic and cathodic reactions during electrolysis Anodic reactions

 $\begin{array}{l} 2H_2O=4H^++O_2\uparrow+4e\\ 2NaCl=Cl_2\uparrow+2e^-+2Na^+\\ Cl_2+H_2O=HCl+HOCl \end{array}$

Cathodic reactions

 $2H_2O + 2e = 2OH + H_2 \uparrow \\ NaCl + OH = NaOH + Cl$

The advantages and disadvantages of electrolyzed water Advantages of EW

- It can be generated on-site and is relatively inexpensive.
- It is produced by simple electrolysis using pure water with no added chemicals except for a dilute salt solution (NaCl or KCl or MgCl₂); it therefore has less adverse impact on the environment (Koseki *et al.*, 2002; Al-Haq *et al.*, 2005) ^[16, 3].
- It provides electrolyzed water with consistent quality, which can also be stored and has 1–2 years of shelf life.
- EW can be prepared relatively quickly and easily
- Its use reduces the cost and hazards associated with the handling, transportation and storage of concentrated chlorine solution.
- EW at neutral or basic pH, when HOCl or OCl⁻ is present respectively that have good action in microorganisms and low capacity of evaporation (White, 2010) ^[14].
- In the case of NEW it is safer for operators and employees since it does not generate chlorine gas.
- It is easy to modify the chlorine concentration to achieve desired concentrations (Kim *et al.*, 2000) ^[17].
- It reverts to normal water after use, without releasing large amounts of harmful gases such as chlorine (Bonde *et al.*, 1999)^[18].
- According to some researchers, electrolyzed water does not cause resistance in microorganisms (Al-Haq *et al.*, 2005) ^[3]. It is more effective than chlorine (Koseki *et al.*, 2001; Issa-Zacharia *et al.*, 2011) ^[19, 20]. Consequently, the formation of chloramines and trihalomethanes is less (Al-Haq *et al.*, 2005) ^[3].

- It can also prevent enzymatic browning during storage of seeds and foods in modified atmospheric packaging (Koseki *et al.*, 2002; Go'mez-Lo'pez *et al.*, 2007) ^[16, 21].
- As a non-thermal method, the use of AEW does not result in changes in ingredients, texture, scent, flavor, *etc.*, which are brought about by heat-treatment (Yoshida, 2003)^[7].
- In the case of AEW, it is less corrosive and has less impact on quality compared to other acidic solutions.
- NEW gained US Department of Agriculture (DA) certificate for the production of organic produce. In general, NEW has more benefits and less disadvantages compared to AEW due to its pH and available form of chlorine which can make it more effective and less corrosive.

Disadvantages of EW

- AEW is corrosive for some metals and synthetic resin.
- Its effectiveness is reduced by the presence of protein and fat (Iwasawa and Nakamura, 1999) ^[22] because chlorine reacts with protein and fat (Shimada *et al.*, 1997) ^[23].
- Organic matter can cause decrease in EW activity (Cressey *et al.*, 2008) ^[24].
- Among water-electrolyzing machines, some models, if operated at pH<5, produce pungent chlorine gas that causes discomfort for the operator (Al-Haq *et al.*, 2002) ^[25]
- Along electrolysis, Cl₂ and H₂ are produced (Huang *et al.*, 2008) and this can affect worker health, like respiratory tract, besides fact of explosion in higher concentrations.
- The initial purchase of the equipment may be costly.
- With time, the bactericidal activity of AEW is reduced due to chlorine loss (Koseki and Itoh, 2001)^[19].
- AEW contains free chlorine which is phytotoxic to plants and damage plant tissues which make its application in farms impossible (Schubert *et al.*, 1995) ^[26].
- Sub-lethal doses of AEW and NEW can trigger toxin production in mold such as deoxynivalenol (DON) in Fusarium (Audenaert *et al.*, 2012)^[27].

Effect of EW on microorganism

ORP of AEW can cause damage to *Escherichia coli* O157:H7 on bacterial ORP and attack inner and outer membranes, causing necrosis of cells (Liao *et al.*, 2007)^[28] with damage verified with microscopy (Feliciano *et al.*, 2012)^[29]. SAEW have equal or higher activity in bacteria than AEW or sodium hypochlorite (NaOCl) at same concentrations and FCC (Cao *et al.*, 2009)^[30] with advantage of few free chlorine (Rahman *et al.*, 2012)^[31]. HOCl can change bacterial respiration destroying the electron transport chains and affecting adenine nucleotide pool (Albrich *et al.*, 1981)^[32].

Chlorine can affect microorganisms by inhibiting carbohydrates metabolism enzymes that have sulfhydryl groups sensitive to chlorine and this blocked glucose oxidation (Eifert and Sanglay, 2002) ^[33]. One or more mechanisms are responsible by EW activity in microorganisms. Inactivation of key-enzymes, nucleic acid damage, the wall and other vitals can be affected (White, 2010) ^[14]. AEW can decrease dehydrogenase activity of *Escherichia coli* and *Staphylococcus aureus* and change membrane permeability, increasing conductivity, decreasing intracellular ADN and potassium ions (Zeng *et al.*, 2010) ^[34]. The concentration of OH⁻ present in AEW and SAEW can be

The concentration of OH⁻ present in AEW and SAEW can be one point of fungicidal efficiency, because OH⁻ can damage the normal structure of conidia, destabilizing ionic equilibrium (Xiong *et al.*, 2010)^[35]. However, chlorine form is fundamental in disinfection capacity of AEW and SAEW, instead OH⁻ radical (Hao *et al.*, 2012)^[36]. AEW activity is attributed to HOCl indirectly, because after HOCl permeation in bacterial cell, the radical OH⁻ is generated (Mokudai *et al.*, 2012; Mokudai *et al.*, 2015)^[37, 38].

Antimicrobial activity of AEW

The antimicrobial mechanism of AEW is not yet fully understood (Suzuki and Watanabe, 2000) ^[39]. AEW may contain chlorine gas (Cl₂), HOCl, and OCl⁻ ions, all of which contribute to FAC (Free available chlorine), *i.e.* uncombined chlorine radicals (FAC is sometimes referred to as available chlorine concentration, ACC). Some researchers believe that the antimicrobial activity of AEW is due to the presence of chlorine species, while others believe that the low pH is responsible. A few studies have suggested that this activity is due to its high ORP. Some scientists say that it is a mixture of all these reasons. The fact remains, however, that AEW possesses strong bactericidal and virucidal and moderate fungicidal properties.

At the low pH of AEW, HOCl is a very weak but effective sanitizer, undergoes virtually no hydrolysis to the much less effective hypochlorite ion (OCI⁻) (Kohno, 1996) ^[40]. Hotta (1995) ^[41] and Kohno (1996) ^[40] suggested that the bactericidal action of AEW is led by non-equilibrium HOCl, existing at low pH, in the electrolysis process. Studies have also suggested that hypochlorous acid (an undissociated form of chlorine) can penetrate microbial cell membranes and subsequently exert its antimicrobial action through the oxidation of key metabolic systems (Hurst et al., 1991)^[42]. Folkes et al. (1995)^[43] suggested that reactive HOCl supplies radical species such as hydroxyl radicals. White (1999) ^[44] suggested that molecular Cl₂ (in equilibrium with HOCl), HOCl and FAC are the major contributors to the sanitizing effect of AEW. Park et al. (2001) [45] suggested that the concentration of chlorine reactants in AEW is influenced by the amperage of the water generator, but other reports contend that the amount of HOCl produced during electrolysis is positively correlated with the amount of NaCl added (Al-Haq et al., 2002)^[25].

The pH value of AEW also plays a role in restricting microbial growth. Iwasawa *et al.* (2002) ^[46] discussed the effect of pH on the bactericidal properties of AEW; Len *et al.* (2000) ^[47] discussed the influence of amperage and pH on these properties. In addition, Len *et al.* (2002) ^[48] discussed the effect of storage conditions and pH on chlorine loss in AEW.

Certain scientists have reported that a high ORP is responsible for the antimicrobial activity of AEW (Venkitanarayan et al., 1999)^[49]. The ORP of a solution is an indicator of its ability to oxidize or reduce, with higher positive ORP values corresponding to greater oxidizing strength (Jay, 1996)^[50]. An ORP of +200 to +800 mV is optimal for growth of aerobic microorganisms, while a range of +200 to +400 mV is favored for growth of anaerobic microorganisms (Jay, 1996) ^[50]. Kim et al. (2000) ^[17] mentioned that the ORP of the treatment solution was the primary factor affecting microbial inactivation. They agreed with reports by McPherson (1993) ^[51] and Carlson (1991) ^[52] concerning water disinfection applications, in which the ORP value of the solution was demonstrated to be a better indicator of disinfection properties than the concentration of residual (free) or total chlorine. Carlson (1991)^[52] and Robbs et al. (1995)^[53] also noted that the killing of bacteria was not based on a defined chlorine reaction and that higher ORP values were required to kill all *E. coli* in a sample. Hence, a certain chlorine measurement alone cannot guarantee disinfection. However, the ORP provides a single measurement of total oxidation capability, regardless of the pH and the concentration of chlorine (Kim *et al.*, 2000) ^[17]. Al-Haq *et al.* (2002) ^[25] suggested that ORP probably plays an influential role, in combination with low pH and FAC, in the disinfection of *B. berengeriana* on European pear.

A cascade of redox reactions occurs during electrolysis, producing many reactive and toxic compounds, such as ozone, and highly reactive and short-lived radicals such as O⁻, Cl⁻, and OH⁻ in AEW. These compounds contribute to the sanitizing effect of AEW (Shiba and Shiba, 1995)^[54].

A significant number of scientists believe that all three factors (chlorine, pH, and high ORP) contribute towards disinfection by AEW (Al-Haq *et al.*, 2002) ^[25]. However, the presence of chlorine and a high ORP seem to be the main contributors to antimicrobial capacity. Some Japanese scientists believe that ORP is not the reason behind the sterilization effect of AEW and that it should be called AEW rather than EO water.

Antimicrobial activity of BEW

BEW (also known as alkaline electrolyzed water (AlEW) or Electrolyzed reduced water (ER water)) has a pH greater than 11.3 and an ORP of -800 mV or less. Thus, it has strong reducing potential, which leads to reduction of free radicals in biological systems. It may also be useful in the treatment of organ malfunctions (Kim *et al.*, 2000) ^[17]. BEW is recognized to have a surface-active effect due to the presence of dilute NaOH, dissolved hydrogen and active hydrogen (Yamanaka, 1995) ^[55].

Effect of Electrolyzed water on seed decontamination and seed quality

EW can decontaminate the infected seeds and also improves the seed physiological parameters for some extant in many crops. Slightly acidic electrolyzed water (SAEW) has killed the all viable Enterobacteriaceae and improved the seedling quality in alfalfa seeds soaked in SAEW for 6 hours (Zhang *et al.*, 2020) ^[56]. Fuentes *et al.* (2019) ^[57] reported that soaking of tomato seeds in Neutral Electrolyzed Water for 30 min reduced the *Fusarium* and *Aspergillus* sp. and improved the seed germination percentage. Stan and Daeschel, 2003 found that soaking of alfalfa seeds in Acidic Electrolyzed Water reduced the *Salmonella enterica* population and increased the seed germination. Kim *et al.* (2006) ^[58] suggested that AEW seed treatment has reduced the *Escherichia coli* O157:H7 population and maintained the seed germination at higher level in alfalfa and broccoli seeds.

Electrolyzed water soaking reduced the microorganism count and improved the seed germination and γ -aminobutyric acid accumulation in brown rice (Liu *et. al.*, 2013) ^[59]. Kim *et al.* (2003) ^[60] suggested that electrolyzed oxidizing (EO) water reduced initial Salmonella population in alfalfa seeds and sprouts. AEW reduced the total bacterial, coliform, yeast and mold counts on mung bean sprouts (Liu and Yu, 2016) ^[61]. Muller *et al.* (2003) ^[62] reported that EO water is a viable option for controlling powdery mildew on gerbera daisies and provides growers an additional tool to reduce the use of traditional fungicides in greenhouses.

Foliar spray with acidic electrolyzed water on tomato plant reduced the bacterial leaf spot (*Xanthomonas campestris* pv. *vesicatoria*), scab (*Streptomyces scabies*) and wilt diseases (*Fusarium. oxysporum f.sp. lycopersici*) and also reduced the number of diseased fruits/plant, number of spots/fruit; and increased the number of healthy fruits/plant and total fruit yield/plant (Abbasi and Lazarovits, 2006)^[63]. Yu and Liu, (2019)^[64] found that electrolyzed water soaking improved the α -amylase activity, protease activity, phytase activity and lipase activity in triticale during seed germination.

Conclusion

EW treatment may be used as an effective method for reducing microbial contamination on seed. EW shows widerange of fungicidal activity, which may facilitate its use as a contact fungicide on aerial plant surfaces and for general sanitation in greenhouses. It provides growers an additional tool to reduce the use of traditional fungicides in greenhouses. As EW is produced on-site and on demand for direct use, it can also reduce health hazards for workers by eliminating the need to handle concentrated chemicals. It may be useful as a seed disinfecting agent. Both AEW and BEW are useful in seed sanitation. The use of EW is an emerging technology and the door is open to further research and development.

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