

# 1. *Bacillus, Paenibacillus, Trichoderma, Glomus, Rhizopogon,* and *Pseudomonas* pathogens inactivation by electric field

The effect of *Onewater*<sup>®</sup> technology on different pathogens has been analysed in this paper. In the next parts there will be the generalities of the inactivation and the specific effect on *Bacillus*, *Paenibacillus*, *Trichoderma*, *Glomus*, *Rhizopogon*, and *Pseudomonas* species.

#### 1.1. Generalities

Using *Onewater*<sup>®</sup> microbial death occurs due to either the formation of permanent pores and subsequent destabilization of the cell membrane, or loss of important cell components and destruction of chemical gradients via transport through transient pores. Furthermore, the pores may allow the oxidant species in addition to other toxic species, produced by the electrochemical reactions in water, free access to the interior of the cell, aiding the inactivation process.

Therefore, it is a kind of irreversible electroporation or electropermeabilization. Reversibility is influenced by the magnitude of the current field and the duration of the exposure. For cells, transmembrane potentials above 1V and longer pulse times lead to irreversible permeabilization and cell death [1].

In general terms, the magnitude of the electric field needed to inactivate yeast cells is lower than to inactivate bacteria cells [1]. Hence, yeast is more affected than bacteria when the same electric current is applied to the process. This may be explained by the fact that the transmembrane potential induced by an external electric field depends upon the radius of the cell membrane, with larger cells suffering a greater transmembrane potential from a given electric field [1]. Therefore, it seems logical that fungi should be more affected than bacteria by the same electric field.

Moreover, Gram-negative bacteria have higher sensitivity towards electric current than Gram-positive bacteria [2].





In addition to the electric field, the effect of temperature and pH has to be taken into account for the inhibition of microorganisms. The electrochemical process increases the water temperature above 40°C as minimum, usually reaching 50-60°C; while pH is raised up to 12. A combined effect between electric field and temperature was observed for the inactivation of microorganisms [3].

In conclusion, it would be very difficult for a microorganism to survive under *Onewater*<sup>®</sup> treatment extreme conditions: electric field, high temperature, high pH and oxidative stress.

#### **1.2.** Effect on different bacterial and fungal species

#### Bacillus and Paenibacillus: Gram-positive bacteria

Its inactivation may be achieved by the application of electric fields. Its efficiency has been demonstrated for different *Bacillus* species that the application of electric pulses may reduce microorganisms growth [4],[5]. Taking into account that *Onewater*<sup>®</sup> uses a continuous electric field instead of electric pulses, the effect on microorganisms viability may be increased.

The inactivation of *Bacillus* spores from *B. subtilis* and *B. cereus* has been demonstrated in other papers giving more number of pulses to the treatment [6].

However, the germination of these spores was not stimulated by the electrochemical process. This could be promoted by other chemical treatments.

#### Pseudomonas: Gram-negative bacteria

As the gram-negative bacteria, its inactivation is easily achieved with electric pulses, even more when high temperatures are involved [7].





## <u>Trichoderma:</u> Fungi

*Trichoderma* inactivation data with electric fields was not found, but other conidia fungi producers were investigated: *Penicillium expansum* and *Byssochlamys fulva* [8], [9]. These fungi were effectively inactivated by electric pulses due to the impediment of spores' germination.

#### <u>Glomus:</u> Fungi

No information about the inactivation of *Glomus* was found. *Glomus* species have mainly asexual reproduction by chlamydospores. Taking into account that fungal spores are more sensitive than bacterial and that sexual spores are more resistant than the asexual ones; we can expect that *Glomus* may be effectively inactivated by electric fields [1], [2].

### <u>Rhizopoqon:</u> Fungi

No information was found for *Rhizopogon* inactivation. It has sexual reproduction, by producing basidiospores. Despite the fact that sexual spores are more resistant than asexual spores, inactivation of basidiospores by electric pulses has been demonstrated [1], [2].

# 2. References

- "Comparative electrochemical inactivation of bacteria and bacteriophage" Drees, Abbaszadegan, Maier. Water Research (Elsevier) 37 (2003) 2291-2300.
- [2] "Effects of low electric current (LEC) treatment on pure bacterial cultures" Valle, Zanardini, Abbruscato, Argenzio, Lustrato, Ranalli, Sorlini. Journal of Applied Microbiology (2006).





- [3] "Alkaline phosphatase and microbial inactivation by pulsed electric field in bovine milk" Shamsi, Versteeg, Sherkat, Wan. Innovative Food Science and Emerging Technologies, 9 (2008) 217-223.
- [4] "Pulsed electric field inactivation of diarrhoeagenic Bacillus cereus through irreversible electroporation" Rowan, MacGregor, Anderson, Fouracre, Farish. Letters in Applied Microbiology, 31 (2000) 110-114.
- [5] "Effect of pH, ethanol addition and high hydrostatic pressure on the inactivation of *Bacillus subtilis* by pulsed electric fields" Heinz, Knoor. *Innovative Food Science & Emerging Technologies*, 1 (2000) 151-159.
- [6] "Destruction and inhibition of bacterial spores by high voltage pulsed electric field" Marquez, Mittal, Griffiths. *Journal of Food Science*, 62 (1997) 399-401.
- [7] "Evaluation of pulsed electric field and minimal heat treatments for inactivation of pseudomonas and enhancement of milk self-life" Craven, Swiergon, Midgely, Versteeg, Coventry, Wan. Innovative Food Science and Emerging Technologies, 9 (2008) 211-216.
- [8] "Inactivation of Penicillium expansum in sour cherry juice, peach and apricot nectars by pulsed electric fields" Akdemir, Tok, Mine, Soylu. Food Microbiology 25 (2008) 662-667.
- [9] "Inactivation of molf ascospores and conidiospores suspended in fruit juices by pulsed electric fields" Raso, Calderón, Góngora, Barbosa-Cánovas and Swanson. Lebensm.-Wiss. U.-Technol., 31 (1998) 668-672.

