The electrochemical process inactivates oxytetracycline-resistant bacteria

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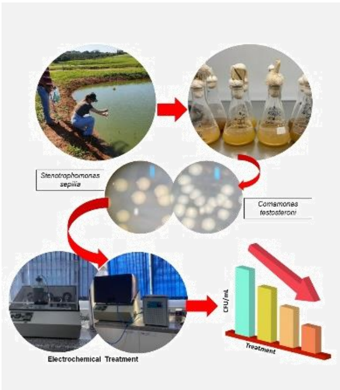

This study explored the use of K_2SO_4 and electrical current to inhibit oxytetracycline-resistant bacteria, a pressing issue in combating antibiotic resistance in aquaculture. The novel finding was that a K_2SO_4 concentration of 4.36 g/L was highly effective in reducing microbial density expressed in CFU/mL, irrespective of the current application. However, a 5A current significantly improved efficacy at lower K_2SO_4 concentrations (1.74 g/L), highlighting the crucial role of electrical current in enhancing treatment effectiveness. Statistical analysis ($p<0.001$) validated these findings, showing significant differences among treatments. K_2SO_4 was identified as a safer alternative to chlorine-based electrolytes, producing fewer toxic byproducts, although monitoring for environmental impact remains necessary.

Introduction

The World Health Organization highlights antibiotic resistance as a major global public health concern [1]. In Brazil, the Brazilian Society for Microbiology estimates around 700,000 deaths annually from multi-resistant bacteria, with projections reaching 10 million by 2050 [2].

Aeromonas spp. has been detected to be resistant to oxytetracycline, and the drug's poor absorption in fish leads to environmental residues [3]. This underscores the need for more effective systems to eliminate antibiotic residues and pathogens, as conventional methods are often insufficient [4].

Therefore, electrochemical treatment emerges as a promising and eco-friendly alternative for inhibiting resistant microorganisms in water. This study explores electrochemical as an advanced oxidation process to inhibit oxytetracycline-resistant bacteria, offering an innovative and hopeful approach to combating antibiotic resistance in aquaculture.

Material and Methods

To capture a diverse range of microorganisms, ten water samples were collected from a tilapia tank at Piscicultura Polletini, Mogi Mirim-SP. At CPQBA - UNICAMP, the samples were enriched in Tryptic Soy Broth - TSB added of oxytetracycline at concentrations of 0, 10, 30, and 100 µg/mL to obtain resistant microorganisms. After determining resistant concentrations, samples were serially diluted and plated on Tryptic Soy Agar - TSA for colony isolation. Following incubation at 28°C for up to 72 hours, Gram staining and 16S rRNA gene amplification via Polymerase Chain Reaction (PCR) and primers 10F and 1525R were used for bacterial identification. The bacterial inoculum was standardized to 1×10^8 cells/mL and diluted to 1×10^4 cells/mL before use.

Electrochemical assays were conducted in batch mode using a 1.2 L glass reactor with a solution of distilled water and K_2SO_4, maintained at 29.5°C. The anode ($70TiO_2/30RuO_2$) was paired with a TiO_2 cathode. A direct current source with power of 30 V/5 A was applied, and the reactor was kept under constant agitation. Electrodes were cleaned before the first use and after each experiment using a 5 g/L K_2SO_4 solution with a constant current of 0.3 mA, followed by washing with distilled water. Samples were collected at 5, 15, 30, 60, and 120 minutes during each treatment and plated on TSA. Each treatment was conducted in nine replicates, using K_2SO_4 concentrations of 1.74 g/L and 4.36 g/L, and electrical currents of 2 A (0.01 A/cm^2) and 5 A (0.025 A/cm^2).

Results and Discussion

The Kruskal-Wallis test showed a significant difference ($p<0.001$) among treatments. The Spearman correlation indicated that the electrical currents (-0.781) combined with the K_2SO_4 concentrations (-0.367) employed effectively reduced CFU/mL counts. However, the highest K_2SO_4 concentration combined with electrical current was most effective in inhibiting oxytetracycline-resistant bacteria.

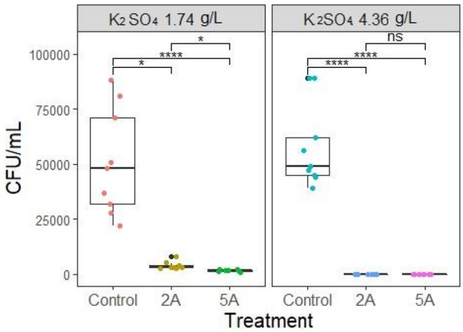

Figure 1. Efficacy of K_2SO_4 concentration and electric current on CFU/mL inhibition during 5 minutes of treatment. Control: No electric current applied; 2A: 2 amps (0.01 A/cm^2); 5A: 5 amps (0.025 A/cm^2); * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, ns: Not significant.

The graph clearly shows that a concentration of 4.36 g/L of K_2SO_4 significantly reduced CFU/mL, regardless of the applied current. Treatments with 2A and 5A currents did not show significant differences when using the highest K_2SO_4 concentration, indicating that K_2SO_4 concentration is the determinant factor for maximum bacterial inhibition. At 1.74 g/L of K_2SO_4, significant CFU/mL reduction occurred with 2A and 5A currents, with 5A being more effective, suggesting that electrical current is more critical at lower K_2SO_4 concentrations.

The significant CFU/mL inhibition can be attributed to both direct and indirect oxidation mechanisms. In direct oxidation, bacteria interact with the anode, forming free radicals that damage cell membranes, intensifying lipid peroxidation and oxidative stress, leading to cell death [5, 6]. During indirect oxidation, the anode reacts with mediating substances in the solution, generating reactive oxygen species [6]. Hydroxyl radicals (·OH) directly attack cellular structures, contributing to microbial death [9].

The type and concentration of the electrolyte influence the oxidants generated during electrolysis, enhancing the electrode's sterilization efficiency [10]. Compared to chlorine-based electrolytes like NaCl or KCl, K_2SO_4 produces fewer toxic byproducts, such as sulfuric acid, sulfite, and sulfate. Chlorine and its byproducts, such as chlorine gas, chlorite, chlorate, and trihalomethanes, can be highly harmful to the environment and to human and animal health [6]. The effectiveness of our treatment is due to electrochemical mechanisms rather than chlorine oxidation. Nonetheless, monitoring byproducts generated from K_2SO_4 is essential to prevent adverse environmental impacts.

Conclusions

The results demonstrate that the electrolyte K_2SO_4 combined with electric current was efficient in inhibiting CFU/mL, regardless of the concentration used. The concentration of 4.36 g/L of K_2SO_4 proved to be the most effective, eliminating all CFU/mL in just 5 minutes, regardless of the applied current. This indicates that a higher concentration of K_2SO_4 is sufficient for maximum bacterial inhibition without the need to increase the electrical current.

Acknowledgments

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