Fenton reaction driven by tannic acid and their application in biofilm removal

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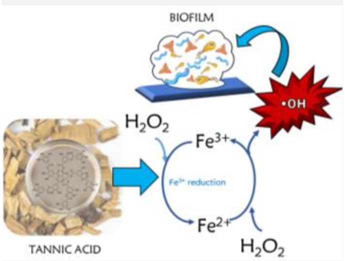

The reactivity of Fenton systems can be driven by iron ligands, their effectiveness for the oxidation of different substrates has been extensively studied. The use of ligands that are non-toxic is the goal to make these systems viable. Depolymerization of CMC was used to optimize conditions by a Fenton system driven by tannic acid (FS-TA). At optimum conditions with 5 ppm Fe, 3.3 mM H_2O_2, 0.11 mM TA and pH 2.36 a comparative depolymerization profile was performed between the Fenton system, conventional Fenton-type and FS-TA and FLS-TA with higher depolymerization observed in the presence of TA.The application of the optimized system on a multispecies biofilm had a removal efficiency of 82.9%.

Introduction

The biofilms (BFs) are bacterial clusters that adhere to surfaces through a matrix of polysaccharide microbial biopolymer matrix, consisting of polysaccharide components, as well as proteins, glycolipids, and extracellular DNA[1].Their matrix and the diverse composition of microorganisms gives them protection against unfavorable external conditions such as the application of biocides[2]. The presence of BPs represents a problem for the food industry, especially in sectors where bacterial counts are constant and can generate large economic losses. Although there are several conventional cleaning agents available on the market, most of them involve the use of substances that can generate toxic products such as organochlorines[3].

Advanced oxidation processes (AOPs) are widely studied methods for degrading persistent organic compounds. These processes involve the use of reagents that generate hydroxyl radical (•OH), which is highly reactive due to this radical has a short half-life of 10^{-9} s. This radical is also biocidal, since it reacts with the cell membrane of microorganisms generating a cascade of reactions called lipoperoxidation that leads to cell death. One of the most studied AOP is the Fenton process which produces •OH and other oxidizing species through the catalytic decomposition of H_2O_2 by iron. The optimum pH for the reaction is 3.0. The pH values above 4.0 cause iron precipitation, which leads to a decrease in its concentration and thus a lower amount of oxidizing species are produced[4]. One way to favor the production of oxidizing species from these processes is the inclusion of iron ligands that increase the availability of iron (in pH ranges above 3.0)[5]. An example of such ligands is tannic acid (TA) which is a type of 1,2-dihydroxybenzene (DHB) widely found in nature[6]. The application of Fenton systems and their variants has been mainly evaluated on E. coli and/or resistance genes and focused on urban water disinfection[7]. At the best of our knowledge, there are no studies on the applicability of these systems on BPs to use in the food industry.

In this work, the ability to remove BPs in vitro by a Fenton and Fenton-like system driven by tannic acid (FS-TA and FLS-TA) was evaluated. Optimal reaction conditions were determined by measuring the oxidation of carboxymethyl cellulose (CMC). In addition, the results were related to OH production.

Material and Methods

FS-TA was optimized by a Central Composite Circumscribed (CCC) (with axial points. To consider the environmental regulation of iron in wastewater, the concentration of this metal was set at 5 ppm. The oxidizing capacity of FS-TA was determined following the viscosity decay of CMC which is a similar substrate to the components of the biofilm matrix. The concentration of CMC used was 3 g/L. The production of •OH was evaluated by EPR using DMPO as spin trapping. measurement was performed at room temperature. Remotion of BFs was tested using BPs formed in a CDC Biofilm Reactor, on stainless steel coupons (type 316, electro-polished, the same used on surfaces in contact with food products). BPs were formed using highly adherent microorganisms extracted from native milking equipment BPs: *Pseudomonas aeruginosa, Klebsiella pneumoniae y Bacillus cereus*. BPs were exposed to a contact time of the FS-TA or FLS-TA of 30 min, after which bacterial counts and qualitative analysis of biofilm integrity by SEM were performed. All the experiments were performed in triplicate.

Results and Discussion

From the experimental design, it was determined that the optimal conditions for depolymerization of CMC were TA, H_2O_2 and pH 2.3 (Figure 1a). Contrary to what was expected for DHB type substances, TA did not promote the oxidation of a substrate at pH above 3. This could be explained by the lower pKa (around 6), compared to other DHBs such as catechol (pKa above 8). This would shift down the pH range in which it behaves as a prooxidant by forming Fe^{3+}-TA monocomplex[8].

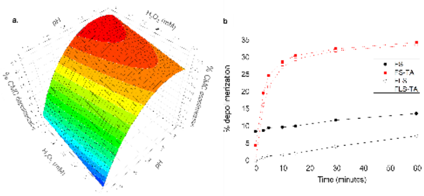

Figure 1. a. optimization of CMC depolymerization. b. Comparative profiles of CMC depolymerization of TA-driven and Fenton and conventional Fenton-type systems.

The depolymerization profiles of CMC under optimal conditions determined were compared with the depolymerization profile of a conventional Fenton (FS) and Fenton-like system (FLS) (Figure 1b). As expected, the FLS showed the lowest depolymerization since the FLS produces radical species of lower reactivity ($^{\bullet}$OOH) and at a lower rate than the FS. On the contrary FS showed the highest depolymerization in the first minutes of the reaction, and then continued with a depolymerization rate like that observed in FLS, this result agrees with the $^{\bullet}$OH production reported for a Fenton reaction[4]. In FS-TA and FLS-TA the depolymerization rate was increases, which means that after 60 minutes of reaction these systems have tripled the percentage of CMC depolymerization. Significant differences were only observed between systems driven by TA during the first 20 minutes of reaction.

A From the EPR measurements (Figure 2) it was observed that the main oxidizing species of the Fenton system when adding TA is still $^{\bullet}$OH although FS-TA presented signals of the DMPO/$^{\bullet}$OH adduct of lower intensity than the conventional Fenton system. This is contrary to what was observed in the depolymerization profile and can be explained considering that in Fenton systems the production of $^{\bullet}$OH is concentrated at the beginning of the reaction, which added to its high reactivity, facilitates radical disproportionation reactions.

Figure 1. DMPO/$^{\bullet}$OH signal. $a_N=a_H=14.9$ G

Multi-species biofilm removal tests showed that FS-TA and FLS-TA achieved 99.99% of efficiency to decrease the BPs microbial load (log/cm^2) of a multi-species BPs supported on a stainless-steel surface at optimal reaction conditions.

Conclusions

The FS-TA and FLS-TA demonstrated effectiveness in eliminating a persistent contaminant such as a multi-species BP. The use of low-cost reagents and environmentally friendly conditions by these systems makes it an interesting option for the removal of BPs present on surfaces exposed to food products.

Acknowledgments

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