CWPO for the simultaneous degradation of cyanobacteria and cyanotoxins. Impact of cyanobacteria growth stage

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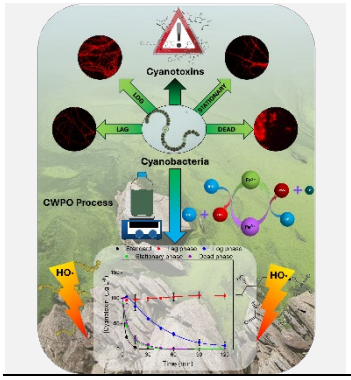

In this work, the impact of cyanobacteria presence throughout their life cycle on the concurrent degradation of cyanobacteria and cyanotoxins by catalytic wet peroxide oxidation (CWPO) was studied. Toxin degradation was impeded by cyanobacteria due to consumption of hydroxyl radicals by the organic matter that competes with the cyanotoxin. The growth stage emerges as a key factor for the CWPO process, particularly during the early life cycle stages, characterized by a higher prevalence of resistant forms (akinetes) and increased cellular metabolism. A lesser effect was exerted by the later stages of the life cycle due to reduced cellular viability resulting from cell autolysis. Nonetheless, complete elimination of cyanotoxins was achieved whenever complete cell degradation occurred.

Introduction

The global proliferation of cyanobacteria in source waters for drinking water treatment plants (DWTPs) has emerged a pressing contemporary issue due to the capacity of certain species to produce highly toxic compounds known as cyanotoxins. All cyanobacteria adhere to a typical biological cycle characteristic of prokaryotic organisms. These cycles comprise four distinct phases: lag, characterized by cellular inactivity; log, with a rapid population expansion; stationary, marked by peak population density; and dead, when the rate of cellular mortality surpasses the rate of growth [1].

The physicochemical processes commonly implemented in DWTPs fail to ensure the complete eradication of cyanobacteria and their toxins. In fact, they can induce cellular lysis, potentially releasing cyanotoxins into drinking water. Advanced oxidation processes (AOPs), in particular the catalytic wet peroxide oxidation (CWPO), characterised by the *in-situ* generation of highly oxidising and non-selective radicals like hydroxyl radicals (HO·), have exhibited high efficacy in degrading cyanotoxins, but the presence of cyanobacteria has been poorly investigated. To our knowledge, previous studies have evaluated only the presence of at most two species [3] on AOPs treatments, overlooking the potential diversity in cyanobacterial populations. Furthermore, while the life cycle of cyanobacteria may significantly affect the efficacy of oxidative processes, its specific influence on CWPO remains unexplored. This study aims to address these gaps by evaluating the degradation of Microcystin-LR (MC-LR), anatoxin (ATX), and cylindrospermopsin (CYN), by CWPO in the presence of several toxic species also considering their main life cycle stages.

Material and Methods

MC-LR (≥99%), ATX (≥99%) and CYN (≥99%) were provided by Laboratorio CIFGA S.A. Hydrogen peroxide solution (33%) and nitric acid (65%) were supplied by Panreac and Scharlau, respectively. All the cyanobacteria strains (UAM 254, *Microcystis aeruginosa*; UAM 292 *Chrysosporum ovalisporum*; UAM 565, *Plantothrix agardhii*; SP33, *Cuspidothrix issatschenkoi*) were supplied from the culture collection of the Universidad Autónoma de Madrid. The magnetite mineral was supplied by Marphil S.L. and was reduced at H_2 atmosphere for 3 h at 400 ºC prior to be used as catalyst (Fe_3O_4-R). CWPO experiments were performed under ambient conditions and pH_0 = 5 in a glass batch reactor (20 mL) equipped with a stirrer (750 rpm). The initial concentration of cyanotoxin and cyanobacteria (followed as chlorophill-*a*) were set at 100 µg L^{-1} while the catalyst and H_2O_2 dose were fixed at 0.2 g L^{-1} and 1 mg L^{-1}, respectively. Cyanotoxin concentration was followed by HPLC-UV (Shimazdu), while cyanobacteria concentration was measured by a fluorometer (BBE Moldaenke).

Results and Discussion

Figure 1 illustrates the influence of the presence of the four strains at different life cycle on the cyanotoxin degradation by CWPO while Figure 2 depicts the Chl-A removal after the process.

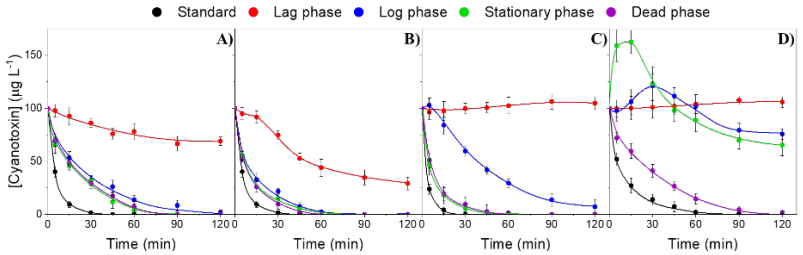

Figure 1. Effect of the different stage of the life cycle over the degradation of A) MC-LR in presence of UAM 254; B) MC-LR in presence of UAM 565; C) CYN in presence of UAM 292 and D) ATX in presence of SP33 upon CWPO.

Analysing the Figure 1, in all scenarios the presence of cyanobacteria impedes cyanotoxin degradation compared to control experiments without microorganisms (Standard experiments). The decrease in the reaction rate observed in the presence of cyanobacteria can be explained by the consumption of radicals by these organic species due to the non-selective nature of HO·. Notably, the specific stage of the cyanobacteria life cycle significantly impacts on the cyanotoxin and cyanobacteria degradation during CWPO. In none of the cases studied, complete cyanotoxin and cyanobacteria elimination is achieved within a two-hour reaction period in the presence of cyanobacteria under the lag phase. These findings imply that cells during this phase exhibit a significant resistance, leading to an intense competition for hydroxyl radicals. Moreover, filamentous cyanobacterial strains with cellular specialization (Figure 1C and D) contain a high proportion of akinetes (spore-like cells), leading to significant interference in the process due their thicker cell walls compared to vegetative cells. A parallel trend is observed when examining the influence of the log phase. Notably, in presence of SP33, an increase in the concentration of dissolved cyanotoxin is observed during the reaction due to cell lysis and the subsequent release of cyanotoxins into the environment. Although this effect is expected for all the studied cases, it is only noticeable for this strain due to the lower cell size of SP33. The presence of smaller cells means that the same Chl-A concentration result in a higher proportion of particulate organic matter, and thus, implies a more pronounced competition for hydroxyl radicals and a lower cyanotoxin degradation rate.

A higher increase in the concentration of dissolved toxin is observed in the stationary phase, reaching the peak concentration at shorter reaction times during this phase. These findings, coupled with a highest Chl-A removal in the latter phase (Figure 2) suggest that cyanobacteria are more efficiently broken down during this phase than in all the previous phases. This results in a faster cellular lysis and subsequent release of cyanotoxins within shorter reaction times prior to its removal.

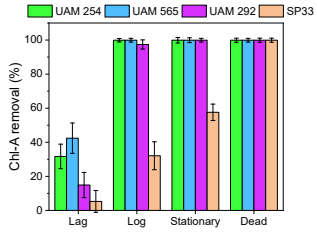

Figure 2. Chl-A removal after 120 min CWPO process for the different toxic cultures and growth phase studied.

Finally, the complete degradation of the toxin and the bacteria is achieved in less than 2 h during the dead stage. This can be attributed to the fact that numerous cells are damaged and undergo partial self-lysis. Although akinetes are re-formed during this phase, they are not yet fully developed, as complete maturation requires additional time [1]. This circumstance streamlines the degradation of dissolved organic matter, as there is less particulate organic matter competing for hydroxyl radicals. Consequently, this phase has the least impact on the CWPO process.

Conclusions

The growth stage emerges as a key factor in understanding the performance of the CWPO process. Cyanobacteria in their early life stages present a more pronounced interference compared later stages due to the heightened presence of resistance forms in early life stages and an accelerated metabolism. All in all, once all cells are fully degraded, complete elimination of the cyanotoxin is also achieved.

Acknowledgments

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