

BIOCIDAL EFFECT OF TRICLOSAN, GLUTARALDEHYDE AND CTAB ON ACTIVATED SLUDGE MICROBIOTA AND PERFORMANCE

EFEITO DE TRICLOSANO, GLUTARALDEÍDO E CTAB NA COMUNIDADE MICROBIANA E NO DESEMPENHO DE LAMAS ATIVADAS

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ABSTRACT

Mixed liquor and real sewage were collected from three wastewater treatment plants, the biocides effect being initially assessed in respirometric assays. The biocide effect was also assessed in bench-scale trials, but, in this case, with mixed liquor and real sewage from only one WWTP. In the respirometric assays, all biocides showed a dose-dependent effect in the inhibition of microbial biomass respiration. In bench-scale assays, a biocidal effect was perceptible for triclosan since the lowest concentrations, and for glutaraldehyde and cetyltrimethylammonium bromide (CTAB) only at higher concentrations. The Gram negative Type 0092 was more susceptible to triclosan than to CTAB and glutaraldehyde and the Gram positive Type 0041/0675 was significantly affected by the three biocides. In the viability assays, it was shown that biocides addition gave rise to fragmentation of filamentous bacteria but also to loss of floc structure, which has to be taken in consideration when assessing the overall performance of activated sludge systems. Triclosan and CTAB showed a clear fragmentation effect, mainly on the surface of the flocs, since the beginning, and this effect remained until the end of the assay.

Keywords – biocides, activated sludge, filamentous bacteria, performance

RESUMO

O efeito dos biocidas foi inicialmente avaliado em ensaios respirométricos em amostras de licor misto e água residual de três estações de tratamento de águas residuais (ETAR). O efeito biocida também foi avaliado em ensaios laboratoriais, mas neste caso com licor misto e esgoto real de apenas uma das ETAR. Todos os biocidas causaram inibição da respiração da biomassa microbiana. Em ensaios na ETAR laboratorial, o triclosano teve efeito desde as concentrações mais baixas, e o glutaraldeído e o brometo de cetiltrimetilamónia (CTAB) apenas nas concentrações mais elevadas. Quanto ao controlo de bactérias filamentosas, o morfotipo 0092 (Gram -) foi mais suscetível ao triclosano do que à CTAB e ao glutaraldeído e o morfotipo 0041/0675 (Gram +) foi significativamente afetado pelos três biocidas. Nos ensaios de viabilidade, a adição dos três biocidas deu origem à desfilamentação das bactérias filamentosas, mas também à perda de estrutura de flocos, particularmente no caso da CTAB e do triclosano, um aspeto não negligenciável quando se avalia o desempenho global dos sistemas de tratamento de águas residuais por lamas ativadas. O triclosano e o CTAB tiveram este efeito desde o início até ao fim do ensaio e sobretudo à superfície dos flocos.

Palavras Chave – biocidas, lamas ativadas, bactérias filamentosas, desempenho.

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1 HIGHLIGHTS

The performance of activated sludge is constantly threatened by filamentous bulking.

Triclosan, glutaraldehyde and CTAB are biocides used in different contexts.

The biocidal action probably affects the overall performance of activated sludge.

2 OBJECTIVES

The present work aims to demonstrate the potential influence of effective biocidal agents (triclosan, glutaraldehyde and CTAB) in filamentous bacteria, as well as their influence on the overall performance of activated sludge.

3 MATERIALS AND METHODS

3.1 Experiments

Respirometry assays were conducted during this study, using real mixed liquor and real sewage from three different WWTP, all of them located in Braga, Portugal. Bench-scale overall community assays were conducted in Erlenmeyer 500 mL flask using mixed liquor and real sewage only from Braga WWTP.

3.2 Respirometry

The impact of each biocide on sludge activity (toxicity test) was assessed by respirometry, which was based on the experimental protocol described on EPA METHOD 1683 (2001). The protocol was adapted for the experimental conditions. Oxygen uptake rate (OUR) and specific oxygen uptake rate (SOUR) were measured at each biocide concentration in a self-made respirometer (batch respiration meter) with an internal volume of 300 mL in two end-points: 30 minutes and 3 hours.

3.3 Bench-scale assays

The evaluation of biocides action on the microbial community and on the performance of activated-sludge treatment process was assessed at bench-scale, and was based on the experimental protocol described by Yuan et al. (2015). The protocol was adapted for the current experimental conditions. The concentrations were chosen according to previous works carried on by the present team (Ferreira, 2022). The assessed parameters were Mixed Liquor Suspended Solids (MLSS), Mixed Liquor Volatile Suspended Solids (MLVSS), pH, the Sludge Biotic Index (Madoni, 1994) and abundance of the dominant filamentous bacteria.

3.4 Microscope analysis of filamentous bacteria

Fresh samples from each activated-sludge unit were prepared for examination of the filamentous bacteria. To determine the morphological characteristics of the various filaments, samples were inspected using an optical microscope CX41 (Olympus, Japan) under bright-field and phase contrast (1000 X magnification). Then, Gram and Neisser stains were also observed to enable relative density assessment of the filamentous populations.

3.5 Live/dead viability test

SYTO-BC and PI double-stained preparations were observed under epifluorescence microscopy. Staining conditions for live/dead viability assay were as following: dyes were added to the bacterial suspension at a final concentration of 0.30 μM of SYTO-BC and 1.5 μM of PI.

4 DISCUSSION AND CONCLUSIONS

The biocide effect was proportional to the reduction of the OUR and SOUR indicating an increase of bacterial inhibition, as expected. Overall, all biocides tested showed a time-dependency for the same concentrations and a dose-dependent effect on the biomass respiration inhibition. Triclosan

was the most toxic (Figure 1), followed by glutaraldehyde and CTAB, when mass concentration is considered. However, with the half maximal inhibitory concentration (IC₅₀) conversion from mg/g MLSS to µmol/g MLSS, it is noticeable that the toxicity trend is altered: glutaraldehyde turns out to be the weaker biocide.

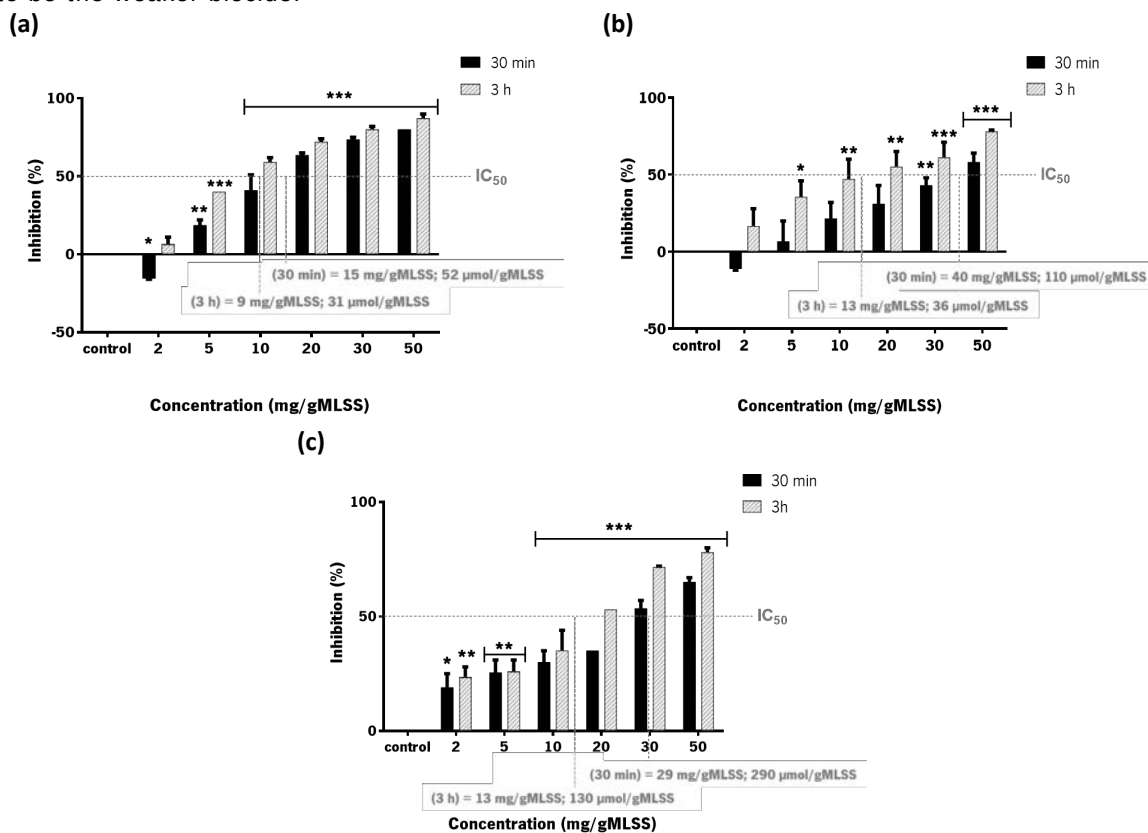


Figure 1. OUR inhibition (%) of activated-sludge when exposed to (a) triclosan, (b) CTAB and (c) glutaraldehyde concentrations (mg/g MLSS) in the two end-point assays (30 minutes and 3 h) in WWTP1. The IC₅₀ value of each biocide after 30 minutes and 3 h is represented by a dashed line. All the columns show the mean $\pm$ SEM (n=2). Statistically significant difference with control (0 mg/g MLSS): * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

Concerning the action of these biocides in filamentous bulking control, in the case of Type 0092 (Gram negative), CTAB addition allowed a positive effect on reducing its abundance only at the highest concentration (50 mg/g MLSS), triclosan caused reduction of these filaments straight after the adding only for concentrations higher than 20 mg/g MLSS and for the concentrations of 5 and 10 mg/g MLSS after a delay of at least 24 h and glutaraldehyde caused a lethal immediate effect since the concentration of 10 mg/g MLSS. Considering Type 0041 (Gram positive), CTAB and glutaraldehyde caused a rapid decrease of one score unit with all concentrations, but triclosan had no effect in the lowest concentration (2 mg/g MLSS), a delayed effect with the intermediate concentrations and only the highest concentrations caused an instantaneous decrease of one score unit (Figure 2). These observations, however, must be taken carefully, as the initial scorings were often different and, for this, the definitive limiting effect cannot be strictly established.

Along with the establishment of the effectiveness of the three biocides - triclosan, CTAB and glutaraldehyde - on the control of filamentous bacteria, it must be ascertained if the biocides do not hamper the performance of the other microorganisms present in the activated-sludge. Overall, the three tested biocides, during the 72 hours of treatment showed a clear biocidal effect at the used concentrations. The toxic effect of triclosan, both in the microbial community and in the performance of activated-sludge units, was noticeable only after 24 hours, except in the case of the highest concentrations. CTAB caused the weakest effects on what performance and health state of the various communities is respected, with the exception of the highest concentration.

Glutaraldehyde led to intermediate responses, with the highest concentrations leading to significant alterations but the lowest ranging from no alterations to slight or delayed effects.

It was possible to conclude that, concerning the dominant filamentous bacteria obtained in activated-sludge units, for Gram negative (Type 0092) only CTAB showed a positive effect by reducing the abundance of filamentous bacteria to acceptable values. For the Gram positive (Type 0041/0675), CTAB and glutaraldehyde exhibited positive effect of biocides addition independent of concentration and incubation time.

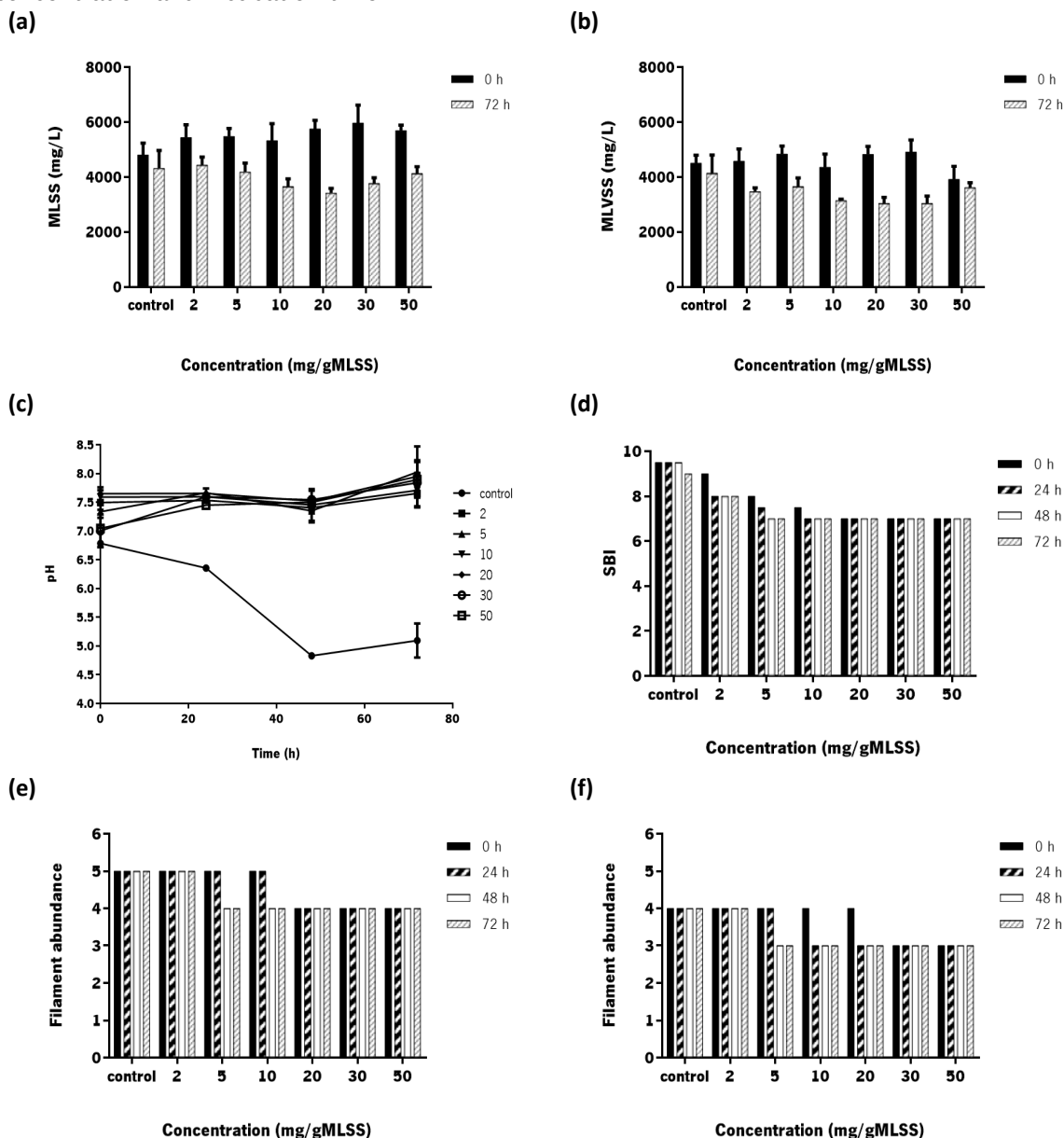


Figure 2. Triclosan assay. Variations of (a) MLSS (mg/L), (b) MLVSS (mg/L), (c) pH, (d) SBI, (e) Type 0092 filament abundance and (f) Type 0041/0675 filament abundance, for the control (0 mg/g MLSS) and 2, 5, 10, 20, 30 e 50 mg/g MLSS of triclosan. Plots from (a) to (c) show the mean $\pm$ SEM (n=2).

Staining the microbial community with SYTO-BC and PI allowed to follow the evolution in the proportions of live populations vs death populations (Figure 3). Loss of floc structure (smaller and lower density), dead cells located essentially inside the flocs, fragmentation of filamentous bacteria to single cells and dead protozoa were observed as a result of biocide addition in the activated-sludge unit. Among the tested biocides, triclosan and CTAB showed a clear fragmentation effect,

mostly in the outer layer of the flocs. The same effect was observed after 48 h and 72 h of treatment.

It is clear that the effectiveness of biocides, particularly in controlling the growth of filamentous bacteria, must be verified by other monitoring, regular analysis of the effluent quality and microscopic observation - as long as that the other components and the general performance are monitored, including the possible effects on the microfauna, because they have different effects on the various components of the communities and on the general performance of the WWTP.

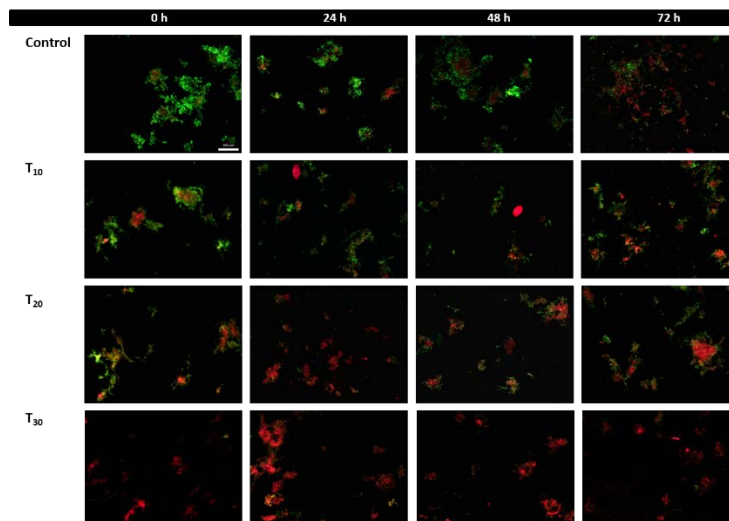


Figure 3. Epifluorescences images of bench-scale assay from control unit (0 mg/gMLSS) and 10, 20 and 30 mg/gMLSS concentration of triclosan (T10, T20 and T30) at the beginning of treatment (0 h) and after 24 h, 48 h and 72 h of biocide treatment. Live/dead cell viability assay showing live cells stained with SYTO-BC (green) and dead cells stained with PI (red), with the magnification of 100 X (scale bars = 100 μ m) for all images.

ACKNOWLEDGEMENTS

This study was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UIDB/04469/2020 unit, and by LABBELS – Associate Laboratory in Biotechnology, Bioengineering and Microelectromechanical Systems, LA/P/0029/2020.

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