

# Kinetics of biofilm formation by drinking water-isolated *Penicillium expansum*

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The biofilms constitute one of the major microbial problems in drinking water distribution systems (DWDS) that most contributes to the deterioration of drinking water (DW) quality. Bacteria are generally dominant population in DW biofilms due to their high growth rates, small size, adaptation capacities and the ability to produce extracellular polymeric substances. Apart from other microorganisms the filamentous fungi may also be present. Very few reports on filamentous fungal biofilms can be found in the literature, probably because these microorganisms cannot fit completely within restrictive biofilm definitions based on bacteria. However, fungi are especially adapted for growth on surfaces, as evidenced by their absorptive nutrition mode, their secretion of extracellular enzymes to digest complex molecules, and apical hyphal growth. Fungi are therefore excellent candidates for biofilm formation but this aspect is still poorly understood. Furthermore, there is no quantitative, fast, and reliable method for the determination of fungal viability, especially for those of the filamentous type.

The purpose of this work was to assess the kinetics of biofilm formation by *Penicillium expansum*, a species commonly detected at the DWDS of Braga, using microtiter plates for biofilm formation and diverse methods to quantify biofilm production. The biofilms were analysed in terms of biomass and metabolic activity, and their morphology and structure were characterized by epifluorescence microscopic analysis (in situ). The biomass adhered on the inner walls of the wells was quantified by crystal violet (CV) staining. The biofilm metabolic activity was assessed by resazurin, fluorescein diacetate (FDA) and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) methods. The epifluorescence microscopic analyses were performed with calcofluor white, FUN1, FDA and acridine orange stains.

The results of biofilm accumulation along time obtained by different methods of quantification showed the typical sigmoidal curve of biofilm formation. It was possible to distinguish the different phases of biofilm formation (induction, exponential, stationary, and sloughing off). The different methods used to assess the biofilm metabolic activity provided similar results. The biofilm mass and metabolic activity results were correlated (an increase in biofilm productivity increased biofilm metabolic activity). Additionally, the microscopic analysis of filamentous fungi biofilms allowed to identify the involvement of conidia on initial adhesion (4 h), germling formation (8 h), initial monolayer formation (12 h), a monolayer of intertwined hyphae (24 h), mycelial development, hyphal layering, hyphal bundling, and development of the mature biofilm (> 48 h).

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