

# ABSTRACT

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**Title of bachelor thesis:** Overview of methodological approaches leading to qualitative and quantitative microbial biofilm evaluation

**Background:** The aim of this work was to compose an overview of methodical approaches leading to qualitative and quantitative evaluation of microbial biofilms. To optimize the methods leading to preparation of mounts with biofilm-forming microorganisms evaluated by epifluorescence or confocal laser scanning microscopy.

**Methods:** Optimization of microbial biofilm staining procedures for subsequent evaluation by epifluorescence microscopy. Formation of microbial biofilms – cultivation of microorganisms in various cultivation media in a microtiter plate, preparation of solutions with fluorescent dyes. Observation of coloured, dispersed biofilms by fluorescence microscope.

**Results:** Using fluorescence microscopy, optimal target concentrations of fluorescent dyes in various combinations were verified. The combination of Syto 9 (SYTO™ 9 Green Fluorescent Nucleic Acid Stain) and propidium iodide was confirmed to be effective for the determination of viability of biofilm-forming microbial cells. The combination of fluorescein isothiocyanate (FITC) conjugated to concavalin A (ConA), (FITC-ConA) together with calcofluor white was confirmed to be effective for the detection of biofilm-forming yeast and biofilm polysaccharide matrix. For the study of bacterial biofilms by microscopy the combination of FITC-ConA and Hoechst 33342 was effective.

**Conclusions:** In the theoretical part of this work a brief overview of methodical approaches used for the study of microbial biofilms was included. The methodical approaches that led to the successful fluorescent microscopic evidence of microbial biofilms are described in the experimental part of this work.

**Key words:** microbial biofilm, biofilm-forming microorganisms, quantification of adherent microbes, quantification of biofilm matrix