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Imaging and quantification of Aspergillus niger biofilms using confocal laser scanning microscopy

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ABSTRACTS – Posters

Imaging and quantification of *Aspergillus niger* biofilms using confocal laser scanning microscopy

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Biofilms are a heterogeneous aggregate of microorganisms that adhere to a surface and are enclosed in extracellular polymeric substances (EPS). The purpose of this project is to assess cell viability and quantify the biofilm features of *Aspergillus niger* biofilm. To study filamentous fungal biofilms that are representative of those in the built environment, we established a method for engineering biofilms in a controlled reactor under low-shearing force on a glass coverslip. *A. niger* is being studied because it is ubiquitous and a model organism. Cell viability quantification in *A. niger* biofilms has not been reported, thus we are comparing two different methods to determine which is optimal. One method utilizes the LIVE/DEAD Yeast Viability Kit containing FUN1 cell stain that exhibits orange-red fluorescent intravacuolar structures in metabolically active cells, while dead cells fluorescence green-yellow. The second method involves using the LIVE/ DEAD BacLight Bacterial Viability kit containing SYTO9, a green fluorescent stain with a capacity to penetrate the active cell walls, and Propidium Iodide (PI), a red fluorescent stain that penetrates the damaged cell membrane. Confocal microscopy and the computer program COMSTAT 2.1 are being used to visualize fluorescent labelled cells and quantitating biofilms structures. Biofilms were stained using both methods and were compared for reliability. The combination of nucleic acid stains SYTO9 & PI is more reliable for imaging and live-dead cells differentiation. The center portion of the biofilm contained more live cells when compared to the edge portion. Also, the edge portion contained conidiogenous cells and conidiophores. The viability of the edge portion was not homogenous, a majority of the biofilm cell was viable, but a minority of red fluorescent nonviable cells was also noted. Once the statistical analysis is completed, this protocol will be employed to test the efficacy of different anti-fungal biofilm agents.