ABSTRACT

Huang, Zhen. Ph.D., Purdue University, May 2010. Characterization of Biofilm Structure and Composition. Major Professor: M. Katherine Banks.

Five stages are involved in biofilm formation in an aqueous environment: initial reversible attachment (Stage 1), irreversible attachment (Stage 2), microcolony formation (Stage 3), biofilm maturation (Stage 4) and steady state growth (Stage 5). To determine the effect of nano-scale biofilm surface roughness on hydrodynamic conditions above biofilm surface, the HBL thickness of two pure culture biofilms, *Pseudomonas aeruginosa* and *Nitrosomonas europaea*, (sampled in Stages 3 and 5) were evaluated using micro-particle image velocimetry (μ-PIV) for various free stream velocities within the laminar regime. The results indicate that the rougher biofilm surface (*N. europaea* biofilm) significantly increased the effect of HBL thickness. No effect was observed for biofilm roughness on the coefficient of friction.

Atomic force microscopy (AFM) has been widely used to evaluate microbial surfaces properties due to its ability to quantify surface stiffness and interactive forces under in-situ conditions and to produce high resolution three-dimensional images. The physiological structure of the biofilm surface (morphology, roughness, spring constant, hydrophobicity and charge properties) was evaluated using AFM with CH_3/COOH/NH_2 functionalized tips at different growth stages. The results indicate that the pure culture *Pseudomonas aeruginosa* biofilm underwent profound morphology changes during biofilm formation. The surface roughness reached a maximum level in Stage 3,

decreased in Stage 4 due to the accumulation of EPS, and stabilized in Stage 5. Surface stiffness increased through Stage 3 to early Stage 5 and then reached a plateau, which was likely due to cell accumulation and phenotypical changes. Biofilm surface physicochemical properties (hydrophobicity and charge properties) were significantly altered in Stage 4 although relatively stable in both Stages 3 and 5. These results indicate that the composition, structure, and chemical properties of a biofilm is highly dependent on the growth stage of the biological matrix.