SYMCEL

IN SITU ANALYSIS OF SURFACE-ATTACHED BIOFILM USING MICROCALORIMETRY

INTRODUCTION

Biofilms are highly organized microbial communities that attach to surfaces and persist within a self-produced extracellular matrix. This mode of growth plays a central role in chronic infections, industrial contamination, and material degradation, making the study of biofilm formation and surface-associated activity a major focus across microbiology, medicine, and materials science[1]. A central challenge in this field is the development of standardized, reproducible, and physiologically relevant methods for quantifying biofilm behavior and evaluating antimicrobial or anti-adhesive strategies under realistic conditions[2].

Conventional biofilm assays—such as crystal violet staining, colony-forming unit (CFU) counting, and microscopic imaging—rely on endpoint measurements that provide limited insight into biofilm dynamics. These methods often require physical disruption or chemical extraction to quantify biomass or viability, which can alter biofilm structure and yield variable results. Furthermore, their reliance on optical properties or biomass proxies makes them poorly suited for complex or opaque media and surfaces. As a result, reproducibility between laboratories is often low, and the ability to compare biofilm responses to different materials or treatments is restricted[3].

Isothermal microcalorimetry (IMC) provides an alternative, non-destructive and label-free approach to studying microbial activity. By continuously measuring heat flow associated with metabolic processes, IMC enables real-time monitoring of biofilm development and response in treatments or interventions without disturbing the sample. When applied to surface-associated growth, this approach allows quantitative, in situ assessment of biofilm activity directly on the tested material—eliminating the need to remove cells from the surface or interfere with the biofilm microenvironment[4].

The combination of the BioSurface Technologies MicroWell™ system and Symcel's CalScreener™ enables standardized microcalorimetric assessment of surface-associated biofilms. This platform permits parallel testing of multiple materials and antimicrobial treatments under controlled conditions, with each measurement reflecting the total metabolic activity of the biofilm, including slow-growing subpopulations. The high sensitivity and reproducibility allow detection of subtle physiological differences between biofilms and treatments.

This approach provides a versatile and quantitative framework for evaluating antimicrobial coatings, surface chemistries, and treatment efficacy. Real-time, non-destructive monitoring offers mechanistic insight into biofilm tolerance and recovery processes that conventional endpoint assays may overlook. This application note demonstrates a standardized microcalorimetry-based method for comparative biofilm analysis across surfaces and antimicrobial conditions.

PROCEDURE

Experiments utilized *Pseudomonas aeruginosa* ATCC 9027 and *Staphylococcus aureus* ATCC 29213 to investigate the formation of Gram-negative and Gram-positive biofilms, respectively. Cultures were grown on TSA plates overnight at 37°C. Single colonies were transferred to 5 mL of TSB, incubated overnight at 37°C with shaking, and then adjusted spectrophotometrically to approximately 5×10^5 CFU/mL in fresh TSB. Biofilms were cultivated on stainless steel, brass, or HDPE coupons in the MicroWell™ system (BioSurface Technologies, US), partly submerged in 700 µL bacterial suspension in a 48-well plate, and incubated at 37°C for 24 h with 50 rpm of rotation. Post-incubation, coupons were rinsed in PBS in a 48-well plate, before being transferred to calVials containing 200 µL TSB or TSB with tobramycin (4-32 µg/mL), and sealed in titanium calorimetric cups for measurement. Calorimetric data were collected at 37°C over 24 h, recording metabolic activity (Fig1). Thermograms were analyzed using calData software for total and peak heat flow,

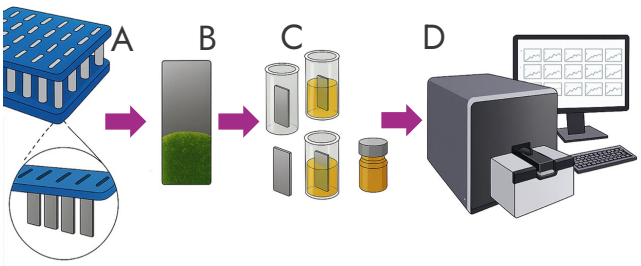

Figure 1 Simplified workflow for attached biofilm microcalorimetry assay. **A)** Pre-grown biofilm on the Microwell coupons over 24 hours. **B)** Rinse the coupons for planktonic cells. **C)** Fit the coupons to the calInserts, apply treatment, and seal the calVials, and **D)** measure continuously in the calScreener.

normalized to controls. Each condition was tested in triplicate, including untreated, antibiotic-treated, and negative controls. The biofilm inhibitor effect and bacteriocidal effect of treatment have been calculated as Biofilm Reduction Effect: $(max_{Heat}/start_{Heat}) \times total_{Heat}$

RESULTS

The metabolic activity of *P. aeruginosa* biofilms during regrowth following surface colonization differed modestly across materials. Biofilms pre-grown on brass exhibited a slightly reduced metabolic signal relative to those grown on stainless steel and HDPE, whereas no measurable difference was observed between stainless steel and HDPE. Despite this early reduction on brass, the cumulative heat output during untreated regrowth was comparable across all surfaces, indicating that *P. aeruginosa*

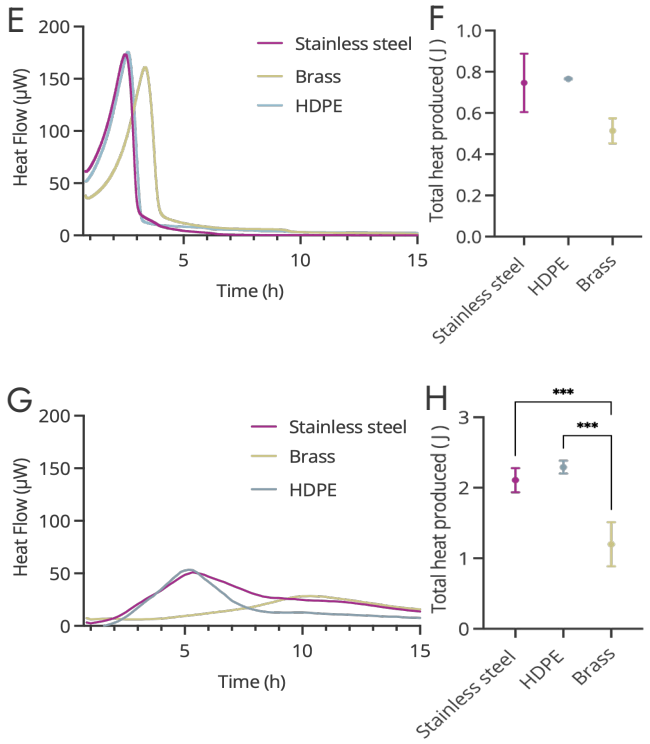

Figure 2 Metabolism of pre-grown biofilms on coupons of stainless steel, brass, or HDPE. **E)** Thermogram from *P. aeruginosa* biofilms **F)** Total heat from coupons with *P. aeruginosa* biofilms **G)** metabolic thermogram from *S. aureus* biofilms **H)** Total heat from coupons with *S. aureus* biofilms

recovered similarly regardless of initial material-associated metabolic suppression. In contrast, *Staphylococcus aureus* demonstrated a marked sensitivity to brass. Biofilms established on brass displayed substantially lower heat production throughout the regrowth phase compared to those formed on stainless steel or HDPE. The reduced metabolic activity was sustained over time, indicating that brass exerted a more pronounced inhibitory effect on *S. aureus* than on *P. aeruginosa* (Fig. 2). These findings suggest that species-specific susceptibility to brass-associated surface effects exists. Exposure of 24-h *P. aeruginosa* biofilms to increasing concentrations of tobramycin resulted in a clear dose-dependent decline in metabolic heat production for all tested surfaces. Biofilms formed on stainless steel showed progressive inhibition of metabolic activity with escalating antibiotic concentrations. Brass-grown biofilms exhibited a more pronounced reduction in heat flow at higher tobramycin doses compared to stainless steel and HDPE, but measurable metabolic activity persisted even at 32 µg/mL, indicating incomplete eradication. Biofilms on HDPE followed a similar dose-response pattern, with reductions in peak heat flow and total heat output consistent with antibiotic-mediated inhibition (Fig. 3).

CONCLUSION

This study demonstrates that microcalorimetry provides a robust and reproducible framework for quantifying biofilm metabolism directly on material surfaces, without requiring biofilm disruption or removal. The assay reliably resolved surface-dependent growth differences, with brass exhibiting inhibitory effects on biofilm regrowth—especially for *S. aureus*—while stainless steel and HDPE supported comparable metabolic recovery. In addition, dose-dependent inhibition of *P. aeruginosa* by tobramycin was clearly captured, although residual metabolic activity persisted even at the highest concentration examined, underscoring the capacity of surface-associated biofilms to

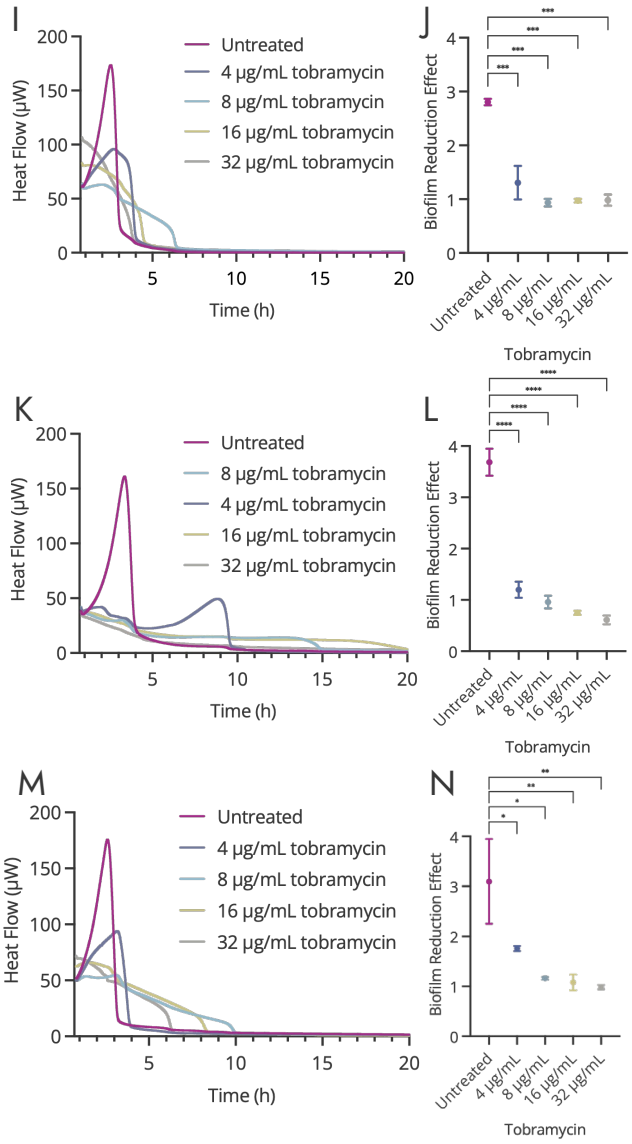

Figure 3 Treatment with tobramycin of pre-grown *P. aeruginosa* biofilms on different materials. **I)** Metabolic thermogram of biofilm on stainless steel treated with increasing doses of tobramycin. **J)** Biofilm reduction effect of tobramycin on stainless steel-bound biofilm. **K)** Thermogram of biofilm on brass treated with tobramycin **L)** Biofilm reduction effect of tobramycin on brass-bound biofilm **M)** Thermogram of biofilm on HDPE treated with tobramycin **N)** Biofilm reduction effect of tobramycin on HDPE-bound biofilm

tolerate antimicrobial exposure. Together, these findings highlight the value of in situ microcalorimetry for detecting nuanced biofilm-material interactions and antimicrobial susceptibility profiles. The standardized format, continuous readout, and low variability across replicates provide a sensitive and scalable platform for assessing biofilm responses under physiologically relevant conditions. This approach offers a useful tool for comparative evaluation of biomaterials, coatings, and antibiofilm strategies where dynamic and non-destructive characterization of surface-associated microbial activity is required.

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