Isothermal Microcalorimetry and Biofilms: Available Options to Measure Biofilm Metabolism

Keywords: Biofilms, metabolism, isothermal microcalorimetry, flow-through calorimetry

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BACKGROUND

Biofilms are defined as "bacteria growing on a surface as single or multi-layered communities" [1]. Over the past several years the importance of biofilms in research has been increasingly growing as scientists realized that many, if not a large majority, of microorganisms exist naturally as biofilms [2]. Indeed, biofilms studies are common in water science (natural waters or waste waters), environmental studies (biofilm formation on rocks), material science (anti-fouling surfaces) and finally medical or biomedical studies (infections, implantology).

The study of biofilms has evolved with the development of many staining, molecular, or microscopy techniques. However, most of these techniques are destructive and therefore only provide endpoint measurements [3]. Consequently, the metabolism and the dynamic behavior of biofilm remains mostly inaccessible to researchers.

In this context, isothermal microcalorimetry is a valuable tool enabling investigation of development and metabolic activities of growing or mature biofilms. Several techniques combined with an isothermal calorimeter can be used to study biofilms and this application note aims at summarizing these techniques and providing some tips and tricks.

BIOFILMS ON BEADS

Porous glass beads of different diameters and porosity can be used as a surface to grow biofilm on. The glass beads are inexpensive, readily available from many different manufacturers, and can be easily sterilized. In this case, the biofilm is produced outside of the calorimeter by incubating the beads within a bacterial culture. The biofilm will develop overnight (or over a few days for slow growing organisms) on the beads. After a biofilm has formed, the non-adherent cells (bacteria not belonging to the biofilm) are removed by washing the beads in sterile buffer (PBS or saline solutions are most commonly used). At this stage beads can be exposed to different treatment (i.e.: sterilization, antimicrobials). After treatment, the effect on the biofilm is monitored by placing the beads inside a calorimeter vial containing a suitable growth medium and placing this vial into the isothermal microcalorimeter. In this case, the measurement is rather indirect as the calorimeter measures the growth of surviving biofilm cells in liquid medium without focusing on these surviving cells growing as biofilm or as planktonic cells [4–6].

Alternatively, the development of a biofilm onto beads can be followed by directly incubating beads and a low inoculum size of bacteria with growth medium. In this case, one will take care to use small diameter beads enabling the vial to be filled with many beads thereby increasing the surface to volume ratio in the vials. Again, under these conditions, it remains difficult to deconvolute the contribution of the biofilm (attached to glass beads) from the planktonic cells [7].

FLOW-THROUGH-SYSTEMS

Although there are commercial flow-through devices, many researchers have made their own flow through systems (example in Figure 1). Growth of a biofilm within a liquid medium as well as a surface for growth is achievable in a calorimeter with flow-through systems. In some cases, the surface of interest can be the tubing itself [8]. After the biofilm has grown in the measurement cells, the flow- through system circulates fresh medium (or buffer) and flushes the non-adherent (i.e., planktonic) cells out of the system. When the planktonic cells have been flushed out, only the biofilm remains for a period of time while the cells start another growth cycle. At this point, the biofilm's metabolic heat production can be measured (Figure 2). After the non-adherent cells start growing again the process can be repeated, if necessary.

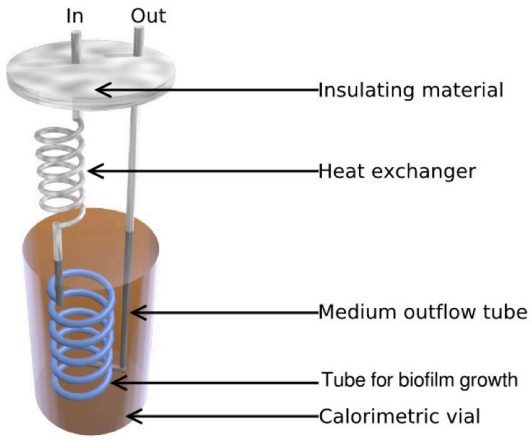

Figure 1: Possible design of a flow-through system inspired and redrawn from Said et al 2015 [8]. See complete setup details in reference 8.

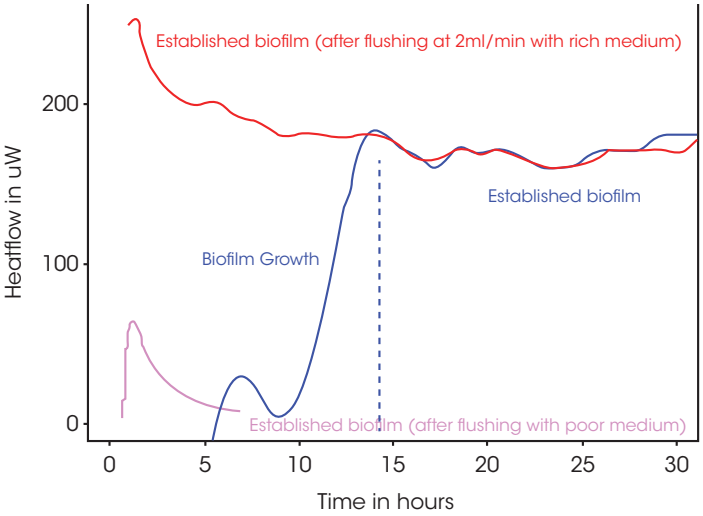

Figure 2: Results obtained using a flow-through system inserted into a TAM 2277 and growing the Staphylococcus aureus biofilm in the tested tubing. Results show the growth and establishment of the biofilm (blue), the activity of an established biofilm (red) and the activity of an established biofilm exposed to poor medium (i.e., PBS – pink). Results were taken from Said et al 2015 [8] and redrawn using datathief III.

Such approaches are much more direct compared to beads as the medium flowing through the system can be enriched with specific carbon sources, antimicrobials, or other compounds of interest. The effect on the biofilm can be directly observed and does not rely on the ability of biofilm cell regrowth, as is the case for beads. Unfortunately, common drawbacks of this type of system are low throughput and some of these systems are difficult to sterilize.

SOLID-MEDIA: THE "SANDWICH" APPROACH

The formation and activity of a biofilm on a surface in a liquid medium is often difficult to assess due to the presence of planktonic cell, that needs to be removed for accurate measurements. In addition, other popular techniques such as microscopy are only usable on flat and / or transparent materials. In this context, use of solid media applied directly on the surface of interest (usually a disk of small diameter fitting in the calorimeter vials) is a valuable asset. In practice, the surface of interest can be freshly inoculated with a minimal amount of inoculum (no more than 10 µL) with 10'000 to 100'000 CFU. After inoculation, the disk is placed on the solid medium previously prepared in the calorimeter vial with the inoculated / treated side facing the medium (Figure 3). The biofilm will then develop in-between the solid medium and the inoculated surface. As a result, the biofilm will develop directly in contact of the surface of interest. In the context of antimicrobial surfaces, growth will be either delayed, or inhibited [9,10]. See Figure 4 for example of results.

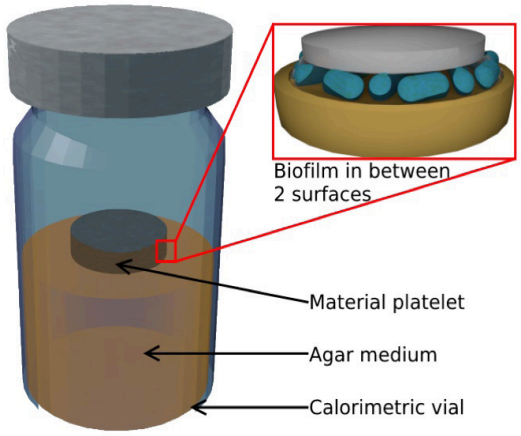

Figure 3: Cartoon showing the "sandwich" approach.

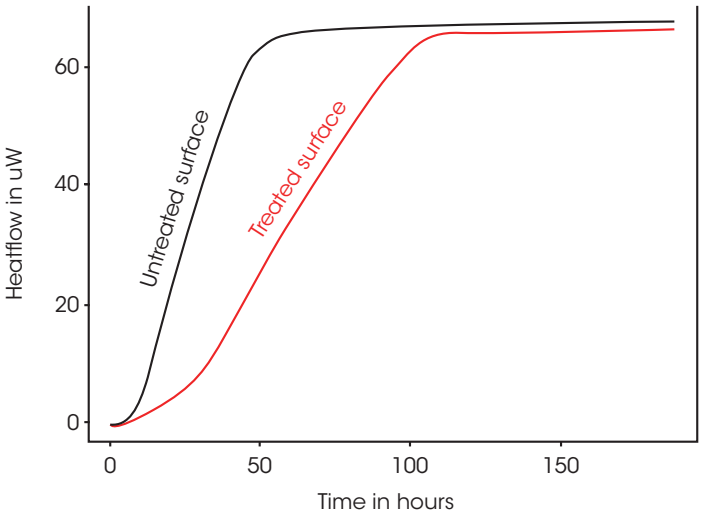

Figure 4: Results showing the evolution of heat resulting from the growth of bacterial on antimicrobial treated surface and a non-treated control. The samples were incubated at 37°C in an 8-channel TAM air.

SOLID MEDIA: "THE FILTER" APPROACH

Biofilms can also be formed on filters [11]. Nylon filter is cut to fit into the calorimetry vials and sterilized. Following this initial preparation, the filters are inoculated using a dense liquid culture and then placed over a Petri dish until a biofilm has formed on top [12]. It is difficult to assess the maturity of a biofilm. However, when no changes in surface coverage of the filter and no visible changes in the biofilm appearance are observed, one can reasonably assume to have a mature biofilm. Using sterile tweezers and aseptic technique, the filters are transferred into calorimetry vials containing solid slanted medium (Figures 5-6). For the first run it is recommended to use only fresh medium as a positive control to record basic biofilm metabolism. After the signal has returned to baseline, the filter can be transferred into a vial containing slanted medium added with the compound of choice (antimicrobial, QS inhibiting or other compounds).

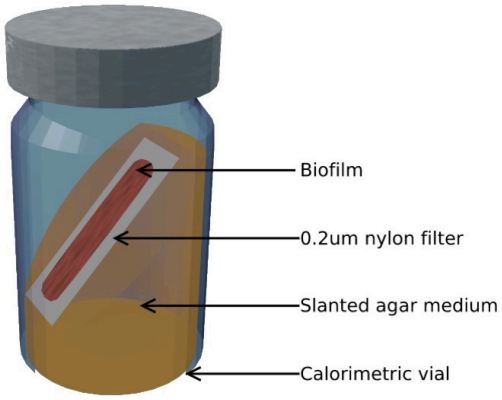

Figure 5: Sketch showing the filter approach. Note that in this approach filters can be re-used and transferred to other medium with different conditions.

The advantage of such approach is that filters can be used several times and transferred easily from one condition to another. Except for the flow-through system described above, biofilms are usually used only once because they are difficult to handle without destroying their structure. Considering the heterogeneity of biofilm (and the potential variations resulting from such heterogeneity), it is very helpful to compare the very same biofilm under different conditions.

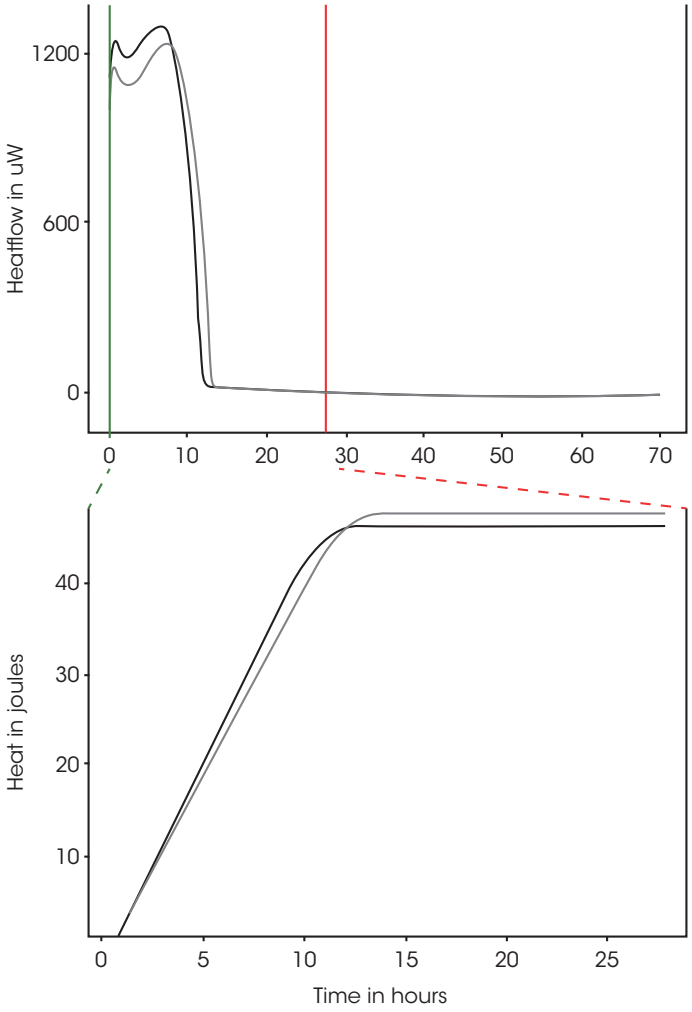

Figure 6: Results of 2 duplicates M.smegmatis biofilms on filters and incubated at 37°C in an 8-channel TAM air.

TOWARD BIOFILM ENERGETICS

The metabolism and bio-energetic profile of biofilms is a complicated topic and calorimetry data can be complemented by respiration data, O_2 consumption or CO_2 production. In sealed glass vials such measurements are made practical using parallel samples that are prepared in the same way but incubated in an oven. The O_2 and CO_2 concentration variations in the headspace over time can be recorded using laser absorption spectroscopy (TDLAS) [13,14]. Alternatively, oxygen optrodes have also been used successfully [15,16].

CONCLUSION

Isothermal microcalorimetry using 4 or 20 ml vials or a flow-through system offers several options to assess biofilm metabolism. Although the filter and sandwich approach are more convenient in larger vials (20 ml option) they can be used in 4 mL vials as well. The possibility to combine microcalorimetry with other techniques remains attractive as isothermal microcalorimetry is non-destructive and offers further options to investigate biofilm bioenergetics.

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REFERENCES

1. Hall-Stoodley, L.; Costerton, J.; Stoodley, P. Bacterial biofilms: from the natural environment to infectious diseases. Nat. Rev. Microbiol. 2004, 2, 95–108, doi:10.1038/nrmicro821.

2. Sutherland, I. W. Biofilm exopolysaccharides: A strong and sticky framework. Microbiology 2001, 147, 3–9.

3. Flemming, H. C.; Wingender, J. The biofilm matrix. Nat. Rev. Microbiol. 2010, 8, 623–633.

4. Tafin, U. F.; Corvec, S.; Betrisey, B.; Zimmerli, W.; Trampuz, A. Role of rifampin against Propionibacterium acnes biofilm in vitro and in an experimental foreign-body infection model. Antimicrob. Agents Chemother. 2012, 56, 1885–1891, doi:10.1128/AAC.05552-11.

5. Corvec, S.; Tafin, U. F.; Betrisey, B.; Borens, O.; Trampuz, A. Activities of fosfomycin, tigecycline, colistin, and gentamicin against extended-spectrum-lactamase-producing escherichia coli in a foreign-body infection model. Antimicrob. Agents Chemother. 2013, 57, 1421–1427, doi:10.1128/AAC.01718-12.

6. Maiolo, E. M.; Tafin, U. F.; Borens, O.; Trampuz, A. Activities of fluconazole, caspofungin, anidulafungin, and amphotericin b on planktonic and biofilm candida species determined by microcalorimetry. Antimicrob. Agents Chemother. 2014, 58, 2709–2717, doi:10.1128/AAC.00057-14.

7. Hauser-Gerspach, I.; Kulik, E. M.; Weiger, R.; Decker, E.-M.; Von Ohle, C.; Meyer, J. Adhesion of Streptococcus sanguinis to dental implant and restorative materials in vitro. Dent. Mater. J. 2007, 26, 361–366, doi:10.4012/dmj.26.361.

8. Said, J.; Walker, M.; Parsons, D.; Stapleton, P.; Beezer, A. E.; Gaisford, S. Development of a flow system for studying biofilm formation on medical devices with microcalorimetry. Methods 2015, 76, 35–40, doi:10.1016/j.ymeth.2014.12.002.

9. Braissant, O.; Chavanne, P.; De Wild, M.; Pieles, U.; Stevanovic, S.; Schumacher, R.; Straumann, L.; Wirz, D.; Gruner, P.; Bachmann, A.; Bonkat, G. Novel microcalorimetric assay for antibacterial activity of implant coatings: The cases of silver-doped hydroxyapatite and calcium hydroxide. J. Biomed. Mater. Res. - Part B Appl. Biomater. 2015, 103, doi:10.1002/jbm.b.33294.

10. Guimond-Lischer, S.; Ren, Q.; Braissant, O.; Gruner, P.; Wampfler, B.; Maniura-Weber, K. Vacuum plasma sprayed coatings using ionic silver doped hydroxyapatite powder to prevent bacterial infection of bone implants. Biointerphases 2016, 11, doi:10.1116/1.4943225.

11. Merritt, J. H.; Kadouri, D. E.; O'Toole, G. A. Growing and analyzing static biofilms. Curr. Protoc. Microbiol. 2011, doi:10.1002/9780471729259.mc01b01s22.

12. Solokhina, A.; Brückner, D.; Bonkat, G.; Braissant, O. Metabolic activity of mature biofilms of Mycobacterium tuberculosis and other non-tuberculous mycobacteria. Sci. Rep. 2017, 7, doi:10.1038/s41598-017-10019-4.

13. Brueckner, D.; Solokhina, A.; Krähenbühl, S.; Braissant, O. A combined application of tunable diode laser absorption spectroscopy and isothermal micro-calorimetry for calorespirometric analysis. J. Microbiol. Methods 2017, 139, 210–214, doi:10.1016/j.mimet.2017.06.012.

14. Brueckner, D.; Roesti, D.; Zuber, U. G.; Schmidt, R.; Kraehenbuehl, S.; Bonkat, G.; Braissant, O. Comparison of Tunable Diode Laser Absorption Spectroscopy and Isothermal Micro-calorimetry for Non-invasive Detection of Microbial Growth in Media Fills. Sci. Rep. 2016, 6, doi:10.1038/srep27894.

15. Regan, M. D.; Gosline, J. M.; Richards, J. G. A simple and affordable calorespirometer for assessing the metabolic rates of fishes. J. Exp. Biol. 2013, 216, 4507 LP-4513.

16. Itoga, N. K.; Hansen, L. D. Gas-phase optrode measurements of oxygen in calorimetric vessels. Thermochim. Acta 2009, 490, 78–81.

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