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Evaluation of Biocide Efficacy by Microcalorimetric Online Monitoring of Bacterial Activity in Biofilms

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Introduction

In a process known as biofouling microbial cells are embedded in a matrix of extracellular polymeric substances (EPS) and form a slimy layer on surfaces, i. e. a biofilm, causing corrosion of constructional materials, heat loss in heat exchangers, or the decrease of product quality as in the paper industry [1, 2, 3]. In a biofilm matrix bacteria are largely protected against toxic substances such as antibiotics or biocides and the efficacy of their application cannot be as easily investigated as the treatments against free (planktonic) microorganisms [4]. Therefore, and since the costs for countermeasures against biofouling are high, there is obviously a need to use methods for evaluation of their efficacy in order to select antibiofilm compounds and to determine the appropriate dosage of application.

For this reason a microcalorimetric online test to directly determine microbial activity of unaltered, intact biofilms [5] was developed. This method allows to test the efficacy of antibiofilm agents as has been demonstrated in experiments with biofilms consisting of sulfate reducing (SRB) and chemoorganotrophic (COT) bacteria treated with a commercial biocide with glutaraldehyde as one of the active compounds (GLU). The investigated biofilms were produced in continuous culture on the surface of the flow-through gold tubing measuring cylinder of the calorimeter and, thus, online monitoring of changes in microbial activity during biocide treatment was possible.

Experimental

A Thermal Activity Monitor (TAM 2277) equipped with a flow-through measuring cylinder (2277-202) was used (Fig. 1). The tubings of the cylinder are made of 24 carat gold with an internal diameter of 1 mm. An external solution, with or without bacteria, was pumped via a peristaltic pump (flow-rate: 20 mL/h) through the tube. In the measuring cylinder the solution passed firstly through a heat-exchange coil for temperature equilibration before reaching the measuring position. Here the heat evolution in 0.6 mL of the tubing system was registered by Peltier elements. Calibration range for these

Figure 1. *View of a flow-through calorimetric unit.*

Fields of application

Life sciences

Instrument configuration

2277 Thermal Activtiy Monitor
2277-202 Combined Ampoule/ Flow Microcalorimetric Unit

References

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[2] Donlan, R.M., Pipes, T.L. and Yohe, T.L. (1994). Wat. Res. 28: 1497-1503.

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[5] von Rège, H. and Sand, W. (1998); Evaluation of biocide efficacy by microcalorimetric determination of microbial activity in biofilms; J. Microbiol. Meth. 33: 227-235.

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experiments was 0 - 300 µW. The limit of detectability was 0.5 µW and the base line stability was ± 0.3 µW. The thermostat was set to 30°C as operating temperature.

Either an anaerobic enrichment culture (containing SRB and COT) or an aerobic culture of *Vibrio natriegens* (DSM 759) were externally incubated at 30 °C as described elsewhere [5]. The respective culture solutions were continuously pumped (20 mL/h) through the flow-through calorimeter. Biofilm formation was traced by replacing the bacterial culture solution by a sterile one. This occurred at the end of the logarithmic growth-phase of the various test cultures (5 h for *Vibrio natriegens*, 48 h for the enrichment). The remaining heat output could be exclusively attributed to attached cells on the surface of the gold tubing in the measuring cylinder. Biocide was added to the continuous culture assays after the start of the experiment at about 46 h in case of *Vibrio natriegens* and at 120 h in case of the enrichment. At these times a constant heat output had indicated that a mature biofilm had developed. Control experiments aiming at the detection of surviving cells in the biofilm were run by replacing the biocide-containing by biocide-free nutrient solution followed by continuous heat-output recording.

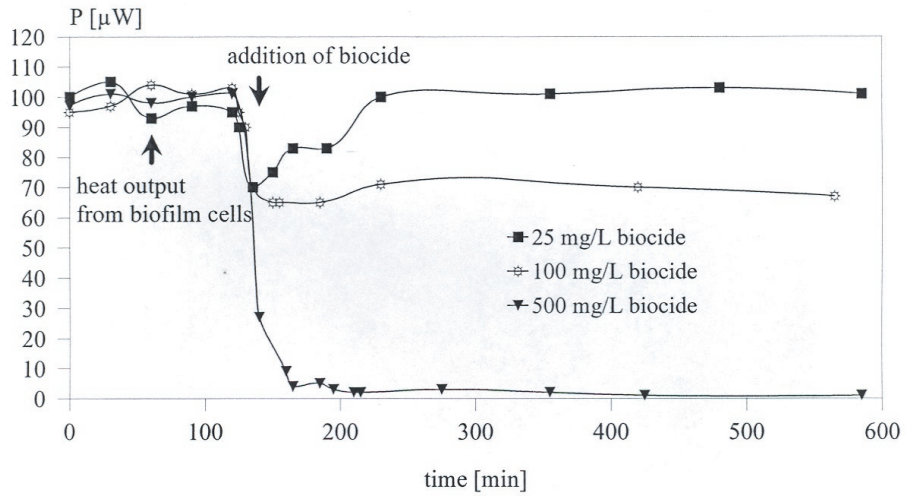

Figure 2. *Influence of biocide concentration (GLU) on an aerobic culture biofilm with Vibrio natriegens (P = thermal power) [5].*

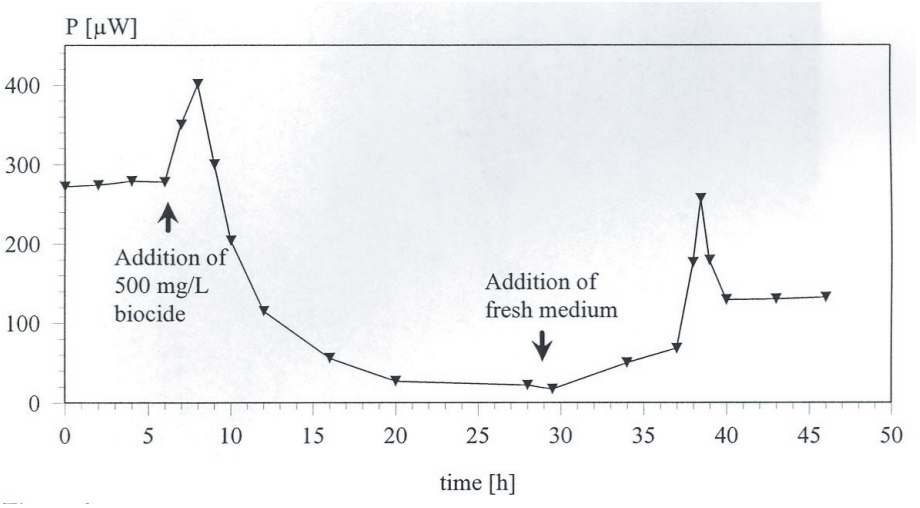

Figure 3. *Influence of biocide concentration (GLU) on an anaerobic mixed culture biofilm with SRB and COT (P = thermal power) [5].*

Results

A stable biofilm was obtained on the inner surface of the gold tubing of the flow-through cylinder with *Vibrio natriegens* after 46 h and for the enrichment culture after 120 h. The heat output amounted with *Vibrio* to 100 µW (Fig. 2), in case of the enrichment to 270 µW (Fig. 3).

With the *Vibrio*-biofilm and GLU the lowest biocide concentration resulted only in a slight and transient decrease of microbial activity (Fig. 2). With 100 mg/L GLU microbial activity decreased somewhat but remained during the whole experiment at around 70 µW. At the highest concentration microbial activity rapidly decreased to 3 µW and remained at that level. If in the latter case the biocide-containing medium was replaced by a biocide-free one, metabolic activity became restored to 85 µW after 10 h (not shown). Obviously, several bacteria survive the biocidal action (probably those which are deeply embedded in the biofilm and thus are protected) and start to regrow.

The biofilm of the enrichment culture was tested in the same way. In these experiments the biocide concentrations 25 and 100 mg/L remained without any influence on microbial activity (not shown). Only at a concentration of 500 mg/L GLU microbial activity (after a short transient increase) was strongly reduced to 3 µW after 23 h (Fig. 3). After nutrient solution exchange, biofilm regrowth also occurred. A heat output of 110 µW was noted after 18 h of consecutively culturing.

Discussion

For testing biocide efficacy many techniques are in use. However, plate counts and MPN-techniques remain to be the most widely used ones because of their low cost and the unequivocal results concerning the killing effect of the biocidal agents. The results presented here indicate that microcalorimetry has considerable advantages over those techniques. Although the latter is primarily more expensive than the classical methods, it may replace the former, because it allows to measure rapidly, even online, an inhibition of microbial activity by biocidal action. Furthermore, in combination with the classical techniques it becomes possible to differentiate between killing or inhibition as a result of a biocidal action. Even a remaining effect, due to adsorbed biocide to the biofilm matrix, becomes detectable. Thus, the time span until a further biocide application becomes necessary, can be determined. This may be of considerable importance for industries such as paper industry, where biocide actions can only reduce but not totally remove microbial biofilm-bound activity.

Conclusion

Microcalorimetry allows by rapid and online tests to screen biocides for their efficacy under a manifold of conditions. However, until now, it is not possible to test biofilms on other surfaces than gold with the flow-through system. Because of the direct response of the instrument to any heat evolution, the experimental time becomes considerably reduced. In times, where economical considerations together with environmental responsibility are of utmost importance, the novel technique will surely find its place in the biocide business.

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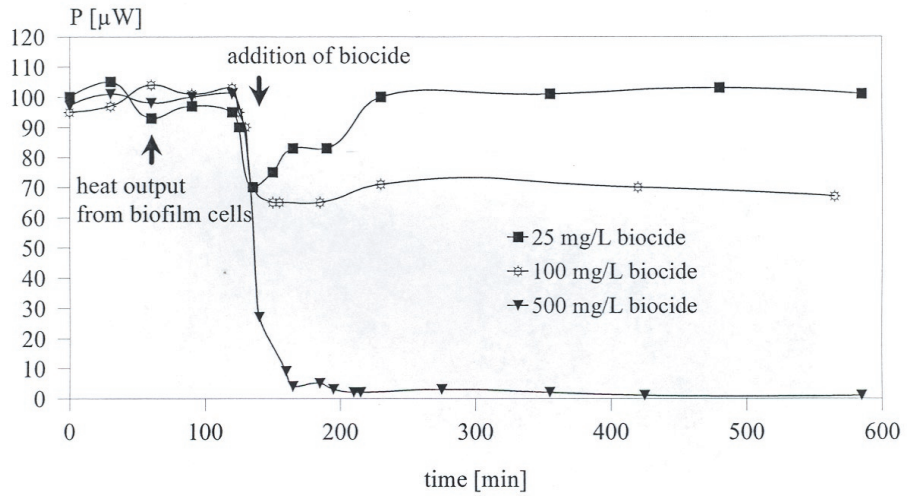

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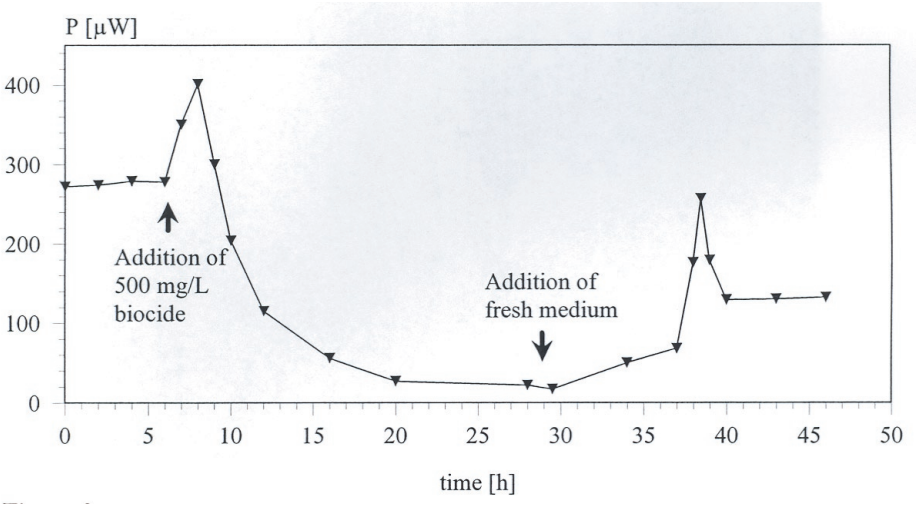

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