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An automated system for biocide testing on biofilms*

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The paper presents and discusses a novel on-line real-time non-destructive continuous-flow system for biocide testing on industrial biofilms. This laboratory system is capable of monitoring changes in growth, accumulation and respiratory activity of biofilms in response to biocidal treatment. The system incorporates a fouling monitor for continuous measuring of the rate of biofilm accumulation (heat transfer resistance), a sensor for monitoring of microbial activity (oxygen meter for monitoring the rate of biofilm respiratory activity), and subsystems necessary for microbial life support and control of operation parameters. Examples of system operation and testing of oxidizing and non-oxidizing biocides are presented.

Keywords: biocide; biofilms; *Sphaerotilus natans*; heat transfer resistance; dissolved oxygen

Introduction

Control of biofilms in cooling water systems is an important component of any successful industrial water treatment program. Although most microorganisms in industrial systems are associated with surfaces, biofilms have historically received less attention than planktonic microorganisms. However, it has been shown that various biocides are less effective against sessile microorganisms than against free-floating dispersed cells [1,11,12]. The resistance of biofilms to antimicrobials, combined with their complex architecture and dynamic nature, make biofilms quite difficult to measure, monitor and control, and thus reduce the effectiveness of treatment strategies. Therefore, monitoring and control of biofilm accumulation is a challenging task to industry.

There are many known procedures for the determination of antimicrobial effectiveness on biofilms [4,8,9,21]. Success in selecting test methods depends on understanding which criteria are most important in a particular system, the ability to develop reproducible biofilm, and simulating applicable field conditions. The recent tendency in biofilm research is to go 'high-tech' using sophisticated techniques in order to understand structure, functions and response of biofilms on the microlevel, including confocal laser microscopy, spectrochemical, electrochemical and piezoelectric techniques [8,17]. However, in industrial applications, accurate information on the relative efficacy of biocides on biofilms is sufficient in most cases.

It is recognized in the water treatment industry that the most reliable data could be obtained via continuous, on-line fouling monitoring techniques. Generally, there are two types of continuous-flow systems currently used for the evaluation of biocidal efficacy on biofilms. Systems of the first type are usually a combination of a chemostat and a series of removable coupons (usually Robbins device, modified Robbins device, rectangular or tubular coupons) under continuous-flow and nutrient addition conditions. These systems utilize destructive techniques based on dry weight, plate counts, DNA, ATP, INT or other methods as a measure of accumulation or microbial activity [2,10,15,16,20]. Another system type is a continuous, on-line fouling monitoring technique [1,19,23]. Such a system is typically restricted to measuring the effects of the fouling deposit, with no evaluation of the biological components or direct measurement of biological accumulation. The mission of such a system is usually as field support of a biocide treatment program or as a process control program for mitigating the effects of biofouling/biocorrosion. The present use of fouling monitors in industrial applications focuses on methods incorporating changes in heat transfer resistance, differential pressure, fluid frictional resistance. Optical, acoustic, and other methods for indicating accumulation are in development, or are used in rare cases [1].

In general, there is a lack of information on the use of on-line biofilm monitoring techniques for biocide evaluations in defined laboratory conditions. Meanwhile, such an approach could be useful in understanding the mechanisms of biofilm accumulation, removal and inhibition, and could lead to the development of new biocides.

This paper presents a laboratory system which uses established industrial monitoring techniques for accurate monitoring of biocide impact on biofilms. The system incorporates a fouling monitor for continuous measuring of the rate of biofilm accumulation (heat transfer resistance), a sensor for monitoring of microbial activity (oxygen meter for monitoring of the rate of biofilm respiratory activity), and subsystems necessary for microbial life support and control of operation parameters.

Method development

Selection of components for system design

Analysis of available technical literature [1,5,19,23] showed that no one ideal monitor exists that will provide complete information on fouling accumulation, effects, and treatment efficacy. The approach chosen was to integrate specific established industrial on-line monitoring methods with specially developed operational procedures for biofilm

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development and testing, into a specialized laboratory system for biocide evaluation.

The Heat Transfer Resistance (HTR) method of biofilm monitoring was selected as a prime monitoring technique. This method relies on detecting the increased HTR associated with the deposit. Various configurations are described in the literature [6,18,22], but all are based on inducing a heat load on a test section and monitoring the temperature change over this test section at a given flow rate. Temperature in the test section increases as a fouling deposit thermally insulates the surface and restricts transfer of the heat load. This system requires constant flow and induced heat load which is relevant to a real-life system if the system of interest includes a heat exchanger. The interpretation of HTR data is based on the fact that the overall heat transfer resistance consists of two components: conductive HTR and advective HTR. Conductive HTR is dependent on the thermal conductivity and generally increases as the biofilm accumulates. Advective HTR results from fluid motion or turbulence and generally decreases as biofilm accumulates, since the roughness of the biofilm increases turbulence in the interfacial region. Many results obtained with DATS Fouling Monitor in industry suggest that the assumption of correlation of biofilm accumulation with conductive and overall HTR appears valid [25]. The second method incorporated in the biofilm monitoring system was continuous measurement of dissolved oxygen (DO) in the water surrounding the biofilm that correlates with the metabolic activity of aerobic bacteria. Measurement of respiration rates of microorganisms was used previously for determination of relative effectiveness of biocidal activity of different biocides [3]. The concept behind the developed system for biocide testing was based on the fact that the system's make-up water was kept at constant oxygen saturation level (by continuous sparging of air) and constant pH and temperature. Thus, any changes in the dissolved oxygen concentration of the recirculating water were considered to occur due to biofilm activity. As an additional measure of biofilm activity, the measurement of pH of the circulating water was performed. It was anticipated that the change in pH would follow the DO curve because the CO_2 released during respiration would increase water acidity, and accordingly, would reduce the water pH.

System setup

A continuous-flow heat-exchange loop (HEL) was developed to establish and support biofilm growth (Figure 1). To produce an environment conducive to the production of a voluminous heavy biofilm, a biological growth reactor or chemostat was used. The reactor was a 5-gallon tank (11.0 liters working volume) equipped with stirrer, drilled and fitted with PVC piping and joints for inflow and overflow connections. The setup included three subsystems: (a) microbial life support; (b) measurement; and (c) control.

Microbial life support subsystem: The microbial life support subsystem was connected to the chemostat by nutrient feed (nutrient vessel, tygon tubing, pump, flow-breaker and valves), make-up feed (water tank, tygon tubing, pump, immersion heater, air sparging, floating valve), and chemical feed (reservoir, pump, tygon tubing, valves). The chemostat was also connected to a circulation pump that circulates liquid through the 316 stainless steel metal tube (61 cm in length and 1.59 cm in diameter) incorporated in a heat exchanger, which is a part of the DATS Fouling Monitor and simulates the actual heat loading present in industrial heat exchangers. In order to dissipate the heat from the heat exchanger, an additional chilled water cooling system, consisting of a cooling coil connected to a refrigerated circulating bath, was introduced (Lauda RC20, Brinkmann Instruments, Westbury, NY, USA).

Measurement subsystem: The major elements of this subsystem are the DATS II Fouling Monitor from Bridger Scientific (Sandwich, MA, USA) [7], and a TBI-Bailey (Carson City, NV, USA) TB 701 analytical controlling transmitter coupled with a dissolved oxygen probe TB234 and pH probe TB 551. The sensor elements, ie oxygen sensor and pH-probe, were introduced into the chemostat. Temperature probes located in the heat exchanger area measured the water and heater block temperatures, while a sensitive flow meter measured the water flow velocity through the steel tube. The HTR was automatically calculated from these measurements and heat load. Through an analytical controlling transmitter, 4–20 mA signals from the DO and pH probes were sent to the auxiliary input channels in the Data Acquisition System. Thus, DO and pH levels were easily monitored and logged along with temperatures and other fouling monitor data. Data were collected continuously by a personal computer. A customized LabVIEW based software interface simulated the configuration of the operating conditions, collected, stored and displayed the data. Variables were scanned every 15 s, and the average recorded every 3–60 min in a data file, which was automatically transferred to Excel for analysis and graphing.

Control subsystem: There were several levels of controls in the present design of the system. The DATS controller provided control of the flow rate, heat load or wall temperature. Through the water chiller (RC20), stability of the recirculation water temperature was achieved. Overflow tubing in the chemostat secured the water working volume. The presence of a floating valve, in combination with continuous sparging and temperature control in the make-up reservoir made it possible to supply the system with aerated water of constant temperature.

The program was formed in such a way that the system functioned continuously without interruptions for several weeks. Only biofilm growth in the tank, with possible clogging of the outlet tubing, could cause unforeseen maintenance problems. Regular maintenance, including nutrient preparation, make-up and nutrient flow rates check-up and calibration, check-up of pumps, tygon tubing conditions, etc, should be provided on a daily/weekly basis.

Growth conditions

The sheathed *Sphaerotilus natans* (ATCC 15291), which is known to reside on heat exchanger surfaces in cooling water systems and papermaking machines, and is also associated with sewage treatment process upsets (such as the bulking of activated sludge), was selected for biofilm growth. This organism was successfully grown as a biofilm in several studies [16,24]. Details of inocula preparation

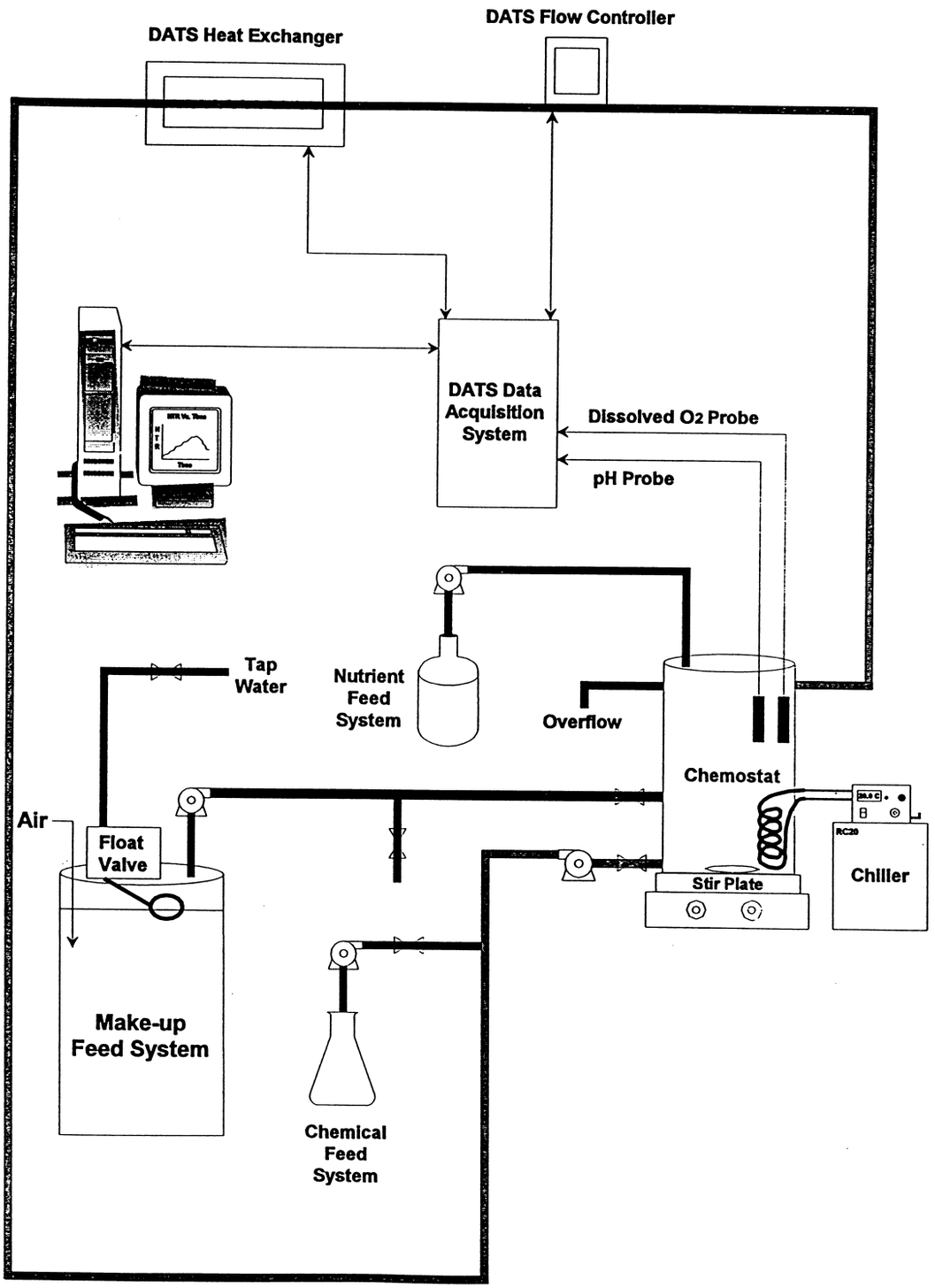

Figure 1 An automated system for biofilm monitoring.

and growth conditions in the static system were described earlier [13]. Prepared inocula were pumped into the microbial growth reactor and usually allowed to sit at room temperature overnight. The next day the make-up water (Clinton tap) and nutrient (CGY media) were started. The selection of initial growth conditions and system parameters was based on previous experience, laboratory limitations, geometric size of the system components, and the desire to promote a growth of biofilm. Shifting of growth conditions from planktonic growth to attached filamentous growth was obtained by lowering media concentrations to 5% or less and maintaining dilution rates higher than the maximum specific rate (according to [24]). Selected test conditions are shown in Table 1.

Table 1 *Sphaerotilus natans* biofilm growth conditions

Inoculum	*S. natans*
pH	7.2–8.2
Circ. water temperature	75°F
Wall temperature	85°F
Make-up water	Clinton tap 150–200 ml min^{-1}
Substrate	CGY media 0.5–1.0 ml min^{-1}
Water flow	1–5 f.p.s.
Half-life	45 min

Monitoring of biocide efficacy

Monitoring of biocide efficacy was performed on already established biofilm. The treatment procedure was designed in such a manner that the first treatment would be performed at the point where the slope of HTR rises to the level of $2.0–2.5 \times 10^{-3}$ h-sq ft-F btu^{-1}. Two biocides were tested in the biofilm monitoring system: non-oxidizing and oxidizing biocide. During these runs the system function was tested at constant conditions. The wall temperature was kept at 85°F, the flow rate at 3 fps, and nutrients were fed at 0.5–1.0 ml min^{-1} level. Make-up and discharge rates were kept at 170 ml min^{-1}, and the dilution was close to 0.9. Biocide treatment was carried out by: an initial slug dose injected to overcome biocide demand, followed by a continuous 3-h maintenance treatment in concentrations, calculated per make-up water. Such treatment was repeated for 3 consecutive days, every 24 h.

Results and discussion

Biofilm growth

There are many parameters that are important for successful biofilm growth. Parameters such as water/surface temperature, flow rate, and nutrient level were of great importance in the current design. The results of testing and optimization of these parameters are discussed further.

Effect of temperature: The first series of experiments was designed to define the system parameter stability, limitations and operation range in 'no-growth' and 'growth' conditions in the designed configuration. The first task was to define temperatures for optimal biofilm growth. It was known that the optimal temperature for *S. natans* is 75–85°F and that the DATS fouling monitor can operate in two different modes: (a) constant heat load (usually used mode); and (b) constant wall temperature (the heat load is automatically adjusted by the DATS controller to compensate for the insulating effect of the biofilm). From 'no growth' tests it was found that the bulk water temperature changed much more slowly than the block and wall temperatures with increasing heat load, and that the working volume and flow rate had a less significant effect on the temperature parameters than the heat load. It was demonstrated that due to the insulating effect of grown biofilm, the wall temperature but not water temperature was the limiting parameter in biofilm growth at the designed configuration. The conclusion was made to continue biofilm growth experimentation under constant wall temperature conditions.

Effect of flow rate: The test (Figure 2) involved varying the flow rate (from 1 to 5 fps) to determine its impact on biofilm growth and to optimize conditions for biofilm growth. The test demonstrated that the HTR was responding adequately to changes in flow velocity. In general, when the flow rate was increased, sloughing occurred. The sloughing event was also confirmed by the increase in the bulk water turbidity corresponding to HTR decline. The significant regrowth was observed several hours after each sloughing. It was found that the higher flow velocity caused more significant sloughing. Additional experiments demonstrated that the optimal biofilm growth could be obtained at a flow rate of 3 fps which is typical for heat exchangers in many industrial applications. This flow rate was selected for further experiments.

Typical biofilm growth: Figure 3 shows typical patterns of HTR, DO and pH changes corresponding to attached growth of *S. natans*. The HTR curve showed significant exponential growth, while the DO level reduced sharply, according to strong respiration of the biofilm organisms in the presence of nutrients. The pH curve followed the DO curve. Nutrient addition and stop events had a very strong effect on biofilm growth. There was no growth in the system without appropriate nutrient addition. On the other hand, an excessive nutrient addition supported undesired planktonic growth and increased halogen demand of the bulk water which was a negative factor. Nutrient addition was optimized at 0.5–1.0 ml min^{-1} of CGY medium. The stop of nutrient addition was followed by a reduction in respiration rate. Reduced respiration initiated an increase in aqueous dissolved oxygen concentration due to saturation of the make-up water with oxygen. The theoretical exponential curve, calculated from an assumption that there is no biofilm in the system, and based on the half-life calculations, shows that about 5 h would be required for the system to go from zero to almost 100% DO, as well as to achieve the make-up water pH 8.2. These parameters would stay at that level without biofilm. In the presence of biofilm, it took about 20 h to reach a steady dissolved oxygen level of about 85%, probably corresponding to endogenous respiration only. As soon as the nutrient pump was turned on again, DO and pH levels sharply decreased to the level they had before the nutrient was stopped. Somewhat different changes happened to HTR level: a sharp decrease in the first several hours was followed by a slow steady decrease which continued until the nutrient was turned on. At this point, HTR began to increase, but at a pace slower than during initial growth phase in the first 72 h. Biofilm, probably 'takes time' to recover from nutrient starvation. Evidently, an erosion of biofilm takes place without nutrient, followed by biofilm regrowth when nutrient is restored.

Effect of biocide: Figure 4 demonstrates the typical effect of an effective biocide on biofilm. In general, the behavior of the curve is similar to the nutrient stop: there are three portions on most curves. The first part usually

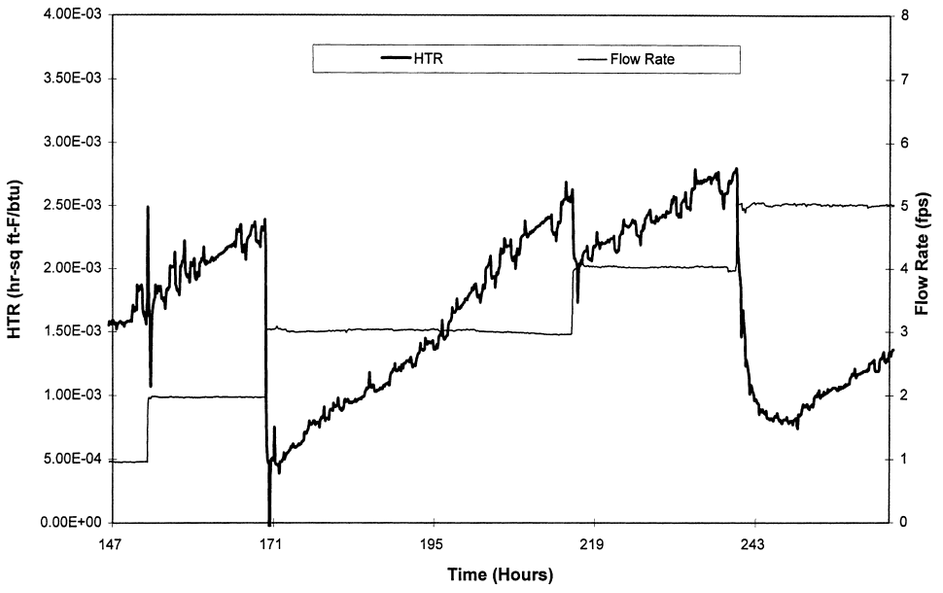

Figure 2 Effect of flow rate on the growth and sloughing of *Sphaerotilus natans* biofilm. Sharp declines in HTR values correspond to biofilm sloughing events.

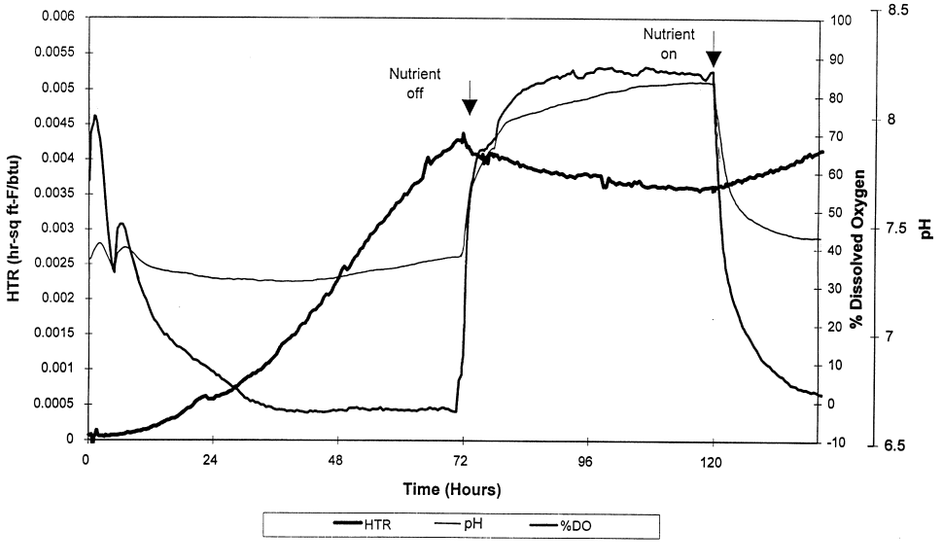

Figure 3 Typical biofilm growth of *Sphaerotilus natans* biofilm under described conditions. Arrows correspond to the start and stop of nutrient addition.

shows the decrease in HTR level and increase of DO and pH, probably corresponding to erosion or removal of biofilm and a cessation or reduction in respiration. The second part of the curve could show a plateau in parameter value, corresponding to suppression of biofilm growth (some level of kill). The third part shows an increase in HTR, DO, and pH levels, corresponding to biofilm regrowth. It was anticipated, that depending upon the nature of the biocide, the mechanism of its activity and concentration, the shape of HTR, DO and pH curves would indicate the biofilm response to the biocide. By comparing these curves to curves obtained previously, a definition of biocidal efficacy can be obtained. In general, HTR data are produced only by biofilm grown on the heated portion of the tube (15.2 cm in length), while biofilm covers most of the system surface, and DO and pH data are the response from the whole system.

Non-oxidizing biocide: Isothiazolone at 4 ppm active was used in this test. It was known that, by mechanism of action, this biocide influences respiration. Figure 5 shows that the first treatment with this biocide was effective in reducing HTR (removing some biofilm), and increasing the DO level to about 70% of the level of saturation. However, within 24 h, evident regrowth occurred, and the second treatment was much less effective than the first. The effect from the third treatment was less effective than the second. From this and other experiments an observation was made that the biocidal efficacy of non-oxidizing biocides diminishes with every subsequent treatment. The reasons for this

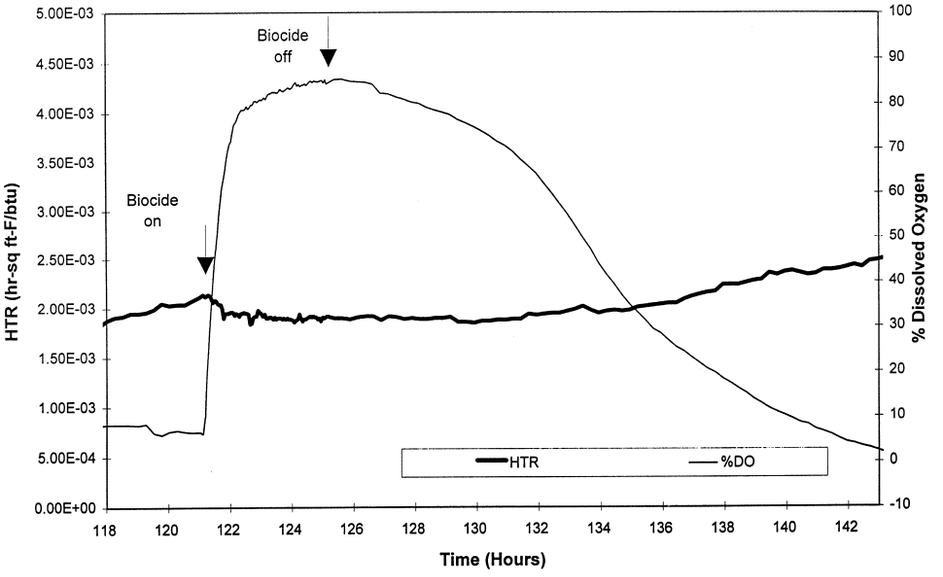

Figure 4 Effect of biocide treatment on *Sphaerotilus natans* biofilm. Arrows correspond to the start and stop of biocide addition.

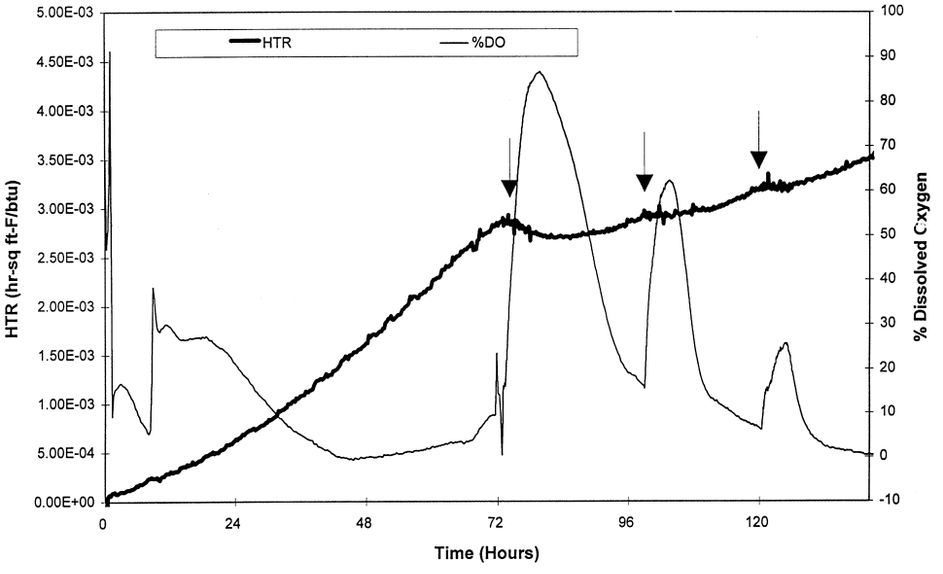

Figure 5 Testing biocidal efficacy of non-oxidizing biocide (isothiazolone). Arrows correspond to the start of biocide treatment for 3 consecutive days.

fact are not clear. There are several possible explanations: (1) an increase in biomass affects biofilm response; (2) physiological acclimation/adaptation of the biofilm cells to biocides; (3) a reduction in the biocide transport permeating the biofilm due to extensive growth of biofilm; (4) an improving transport of nutrients to living cells following treatment with biocides, etc.

Oxidizing biocide: A slow-release oxidizing biocide containing methylethylhydantoin, bromine and chlorine was dosed at initial concentrations of 10 ppm as total Cl_2. Results of the biocidal treatmens are shown in Figure 6. In the case with oxidizing biocide, suppression of biofilm accumulation was followed by fast regrowth. On the other hand, reduction in respiration response of biofilm to the second and third treatments was less expressed than in the experiment with non-oxidizing biocides. Probably, these experimental results reflect the difference in biocidal mechanisms between non-oxidizing and oxidizing biocides. Additional data on the comparative performance of oxidizing biocides can be found in [14]. On-going efforts are directed towards understanding and interpreting results, as well as towards testing of new biocides and their blends.

Conclusions

(1) The design and monitoring methodology of biofilm monitoring system proved to be successful.
(2) On-line measurements of heat transfer resistance, dis-

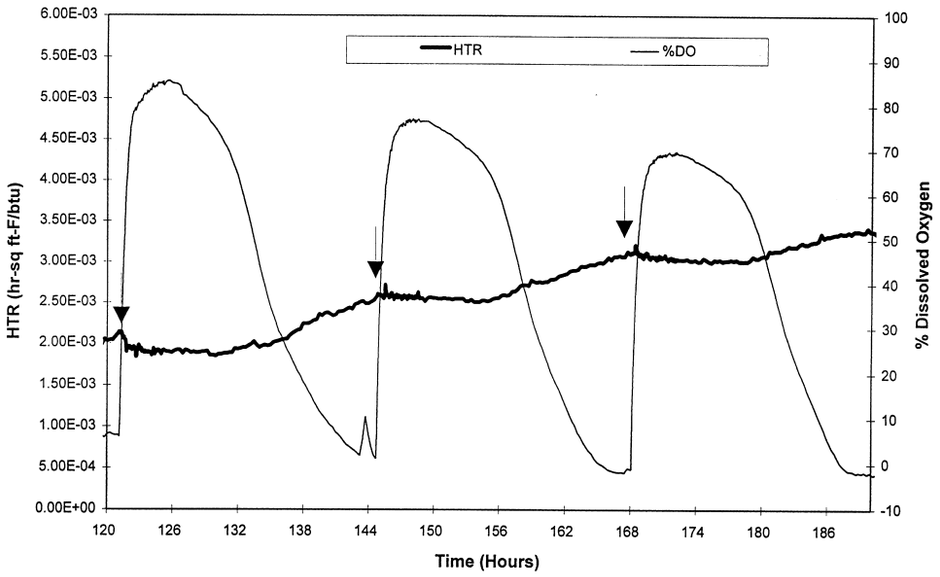

Figure 6 Testing biocidal efficacy of oxidizing biocide (halohydantoin). Arrows correspond to the start of biocide treatment for 3 consecutive days.

solved oxygen, and pH data provided quantitative information on biofilm accumulation, removal, and biofilm microbial activity.

(3) This technique demonstrated the capability to detect and record, in real time, the impact of the biocide treatment on biofilm growth.

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