TABLE I. Comparison of Lambert absorption coefficient, $\alpha(\nu)$, values of water in the near-infrared region.

Wavenumber	Present study	Literature[a]	% Difference
7500	2.39	2.25	5.9
7600	1.72	1.67	2.9
7700	1.35	1.34	0.7
7800	1.19	1.13	5.0
7900	1.13	1.07	5.3
8000	1.17	1.11	5.1
8100	1.22	1.17	4.1
8200	1.28	1.22	4.7
8300	1.32	1.25	5.3
8400	1.33	1.25	6.0
8500	1.29	1.23	4.7
8600	1.25	1.18	5.6
8700	1.05	0.970	7.6
8800	0.592	0.548	7.4
8900	0.387	0.323	16.5
9000	0.326	0.232	28.8

[a] From Ref. 3.

straight line in the region of 7879.3 to 7908.6 cm^{-1}. The resultant spectrum is shown in Fig. 2.

The Lambert absorption coefficient $\alpha(\nu)$ can be calculated from the absorbance spectrum, $A(\nu)$, of Fig. 2 by using the following relationship:

$$\alpha(\nu) = 2.303A(\nu)/d \quad (1)$$

in which d is the pathlength of the cell. The pathlength of the cell was measured with a micrometer as 1.65 cm. Substitution of $A(\nu)$ and d in Eq. 1 produced $\alpha(\nu)$. In Table I, $\alpha(\nu)$ values of the present study are compared with those reported in the literature.[3] The agreement between the two sets of values is fairly good. It should be noted that in obtaining literature values of $\alpha(\nu)$, the reflection effects of the cell windows were eliminated empirically by first measuring the transmission of water in cells of two different pathlengths and then by ratioing the two transmissions. Had we corrected our $\alpha(\nu)$ values for reflection effects, the agreement between our $\alpha(\nu)$ values and the literature values in Table I would have been even better.

The refractive index of the cell windows is of interest to spectroscopists. The refractive index of the window material was calculated from the following relation:[5]

$$\Delta\nu = \tfrac{1}{2}nt \quad (2)$$

where $\Delta\nu$ is the average spacing of the interference fringes which were observed in the single-beam spectrum of the empty cell, n is the refractive index, and t is the thickness of the window. $\Delta\nu$ was calculated to be 34.17 cm^{-1}. The thickness of the window, t, was measured as 0.00914 cm with a micrometer. Substitution of $\Delta\nu$ and t in Eq. 2 gave a refractive index, n, value of 1.601 for the windows.

In summary, a simple NIR cell can be constructed by using Hewlett-Packard transparency as a window material. Such a cell can be used to measure NIR spectra of reasonably good quality. The cost of the HPTF cell is so low that it may be regarded as a disposable cell. HPTF as a window material is unbreakable, and the desired window shape can be cut by merely using a scissor.

ACKNOWLEDGMENT

I am grateful to Professor John E. Bertie of the Department of Chemistry of the University of Alberta for his allowance of generous time in his spectroscopy laboratory for experimental and computational work for this study.

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Multichannel ATR/FT-IR Spectrometer for On-Line Examination of Microbial Biofilms

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Index Headings: **FT-IR; Biofilms; Infrared spectroscopy; ATR spectroscopy; *Caulobacter*; PHA.**

INTRODUCTION

Microorganisms in aqueous environments attach to exposed surfaces, proliferate, excrete extracellular polymers, and form gelatinous layers termed biofilms.[1] By altering interfacial chemistry, biofilms produce both beneficial and detrimental effects in natural environments, industry, and medicine. For example, biofilms cause infections in patients as well as the failure of many implanted medical devices,[2,3] result in dental carries,[4] and create conditions favorable for corrosion of metal structures in industry.[5] In addition, biofilms are the source of free-floating microorganisms in high-purity water systems and can cause problems in the manufacture of semiconductor devices.[6] Biofilms beneficially degrade waste in water treatment plants[7] and ground water pollutants such as trichloroethylene.[8] Thus, instrumentation providing information about the function, physiology, and effects of biofilms is required to increase our understanding of these complex interactions between the biofilm and its environment.

Received 31 August 1992.
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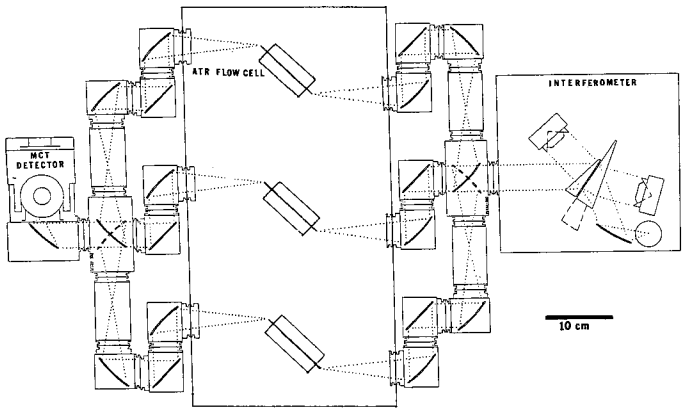

FIG. 1. The design of the instrument showing the optical system, interferometer, flow cell compartment, and MCT detector. All mirrors were gold coated.

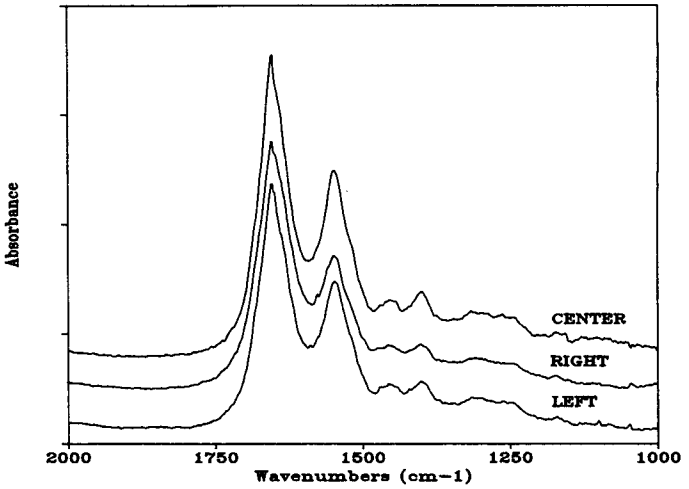

FIG. 2. Spectra of adsorbed BSA on germanium crystals demonstrating the reproducibility of the instrument for short-term experiments. The spectra were obtained from three channels and are presented on the same scale. The protein adsorbed from a 2% BSA in 0.15 M NaCl solution.

Attenuated total reflection/Fourier transform infrared spectroscopy (ATR/FT-IR) has provided *in situ* spectra of bacterial biofilms on germanium internal reflection elements (IRE).[9] The spectra contained information about functional groups of molecules located within approximately 1 μm of the IRE. Infrared absorption bands from macromolecules such as the amide linkages of proteins, ester bands of storage-product poly-β-hydroxyalkanoate (PHA), and C-O stretches of extracellular polymer material consisting primarily of polysaccharides detail the physiological state of microorganisms within the biofilm.

Experiments involving ATR/FT-IR analysis of biofilms are often time consuming, because biofilm development can require days. We have designed a multichannel FT-IR spectrometer to facilitate infrared studies of biofilms by providing simultaneous information from multiple channels, thus saving time because experiments can be performed in parallel instead of in sequence. Parallel studies eliminate physiological variations in the microorganisms and/or variation in concentration of solutes in the liquid phase which could occur in sequential experiments. Moreover, the multichannel FT-IR spectrometer can be used to examine the effect of different treatments applied to biofilms initially developed under identical conditions.

EXPERIMENTAL

The multichannel ATR/FT-IR spectrometer utilizing a mid-IR liquid-cooled source, a Transept III interferometer, an optical system containing collection and focusing mirrors for three channels, three flow cells, and a narrowband HgCdTe (MCT) detector is shown in Fig. 1 (KVB/Analect, Irvine, CA). The optical system was housed within purge tubes, and all mirrors were gold coated. A motorized carousel containing 90° four-inch-focus parabolic mirrors transferred the IR beam to the 90° four-inch-focus fixed mirrors within the right and left channels. An opening in the carousel allowed the IR radiation to pass to the center channel. All channels utilized plane mirrors and six-inch parabolic mirrors to focus the IR radiation onto and collect the light from the ATR crystals. The ATR flow cells were contained within a sample compartment. A second carousel focused the IR radiation onto the detector. The spectrometer was purged with air that had CO_2 and water vapor removed.

The ATR/FT-IR analyses were performed in flow cells (Harrick Scientific Corp., Ossining, NY) with germanium internal reflection elements (IREs), 50 mm × 10 mm × 2 mm and an entrance window cut at a 45° angle, wedged between two flow channel plates and sealed with Viton O-rings. The volume of each flow cell was 0.4 mL. Titanium foil masks were used to eliminate spectral interference from the O-rings. The flow cells were sterilized with ethylene oxide.

Single-sided interferograms were collected at 4 cm^{-1} (256 scans) resolution. All interferograms were Fourier processed with the use of the 1024-point Mertz phase correction method[10] and a Norton-Beer apodization function.[11] Stability tests were performed by generating 100% transmission lines prior to each experiment to ensure temperature stability and proper mirror and flow cell alignment. The temperature was stable to ±2°C. After a stable air background was obtained, sterile high-purity water was pumped through the ATR flow cell and water spectra were collected. After approximately 3 h, the water band stabilized, and growing bacterial cells or protein solutions were pumped through the flow cell. The ratios of the transformed water and sample interferograms to the transformed stable air background interferogram produced the transmission spectra, which were subsequently converted to absorbance. The stable water spectrum was interactively subtracted from the biofilm spectra. Water vapor subtraction and baseline correction were also performed when necessary.

In the protein studies, 2% bovine serum albumin (BSA) fraction V (Calbiochem, La Jolla, CA) in 0.15 M NaCl at pH 6.9 was pumped through the flow cells at a flow rate of 2 mL·min^{-1} for 3 h, followed by a one-hour sterile water rinse.

In the biofilm study, the biological medium for monitoring biofilm development was prepared with the use of 18-MΩ·cm water and contained the following ingredients: 1.0 mM KH_2PO_4, 0.28 mM glucose, 0.24 mM

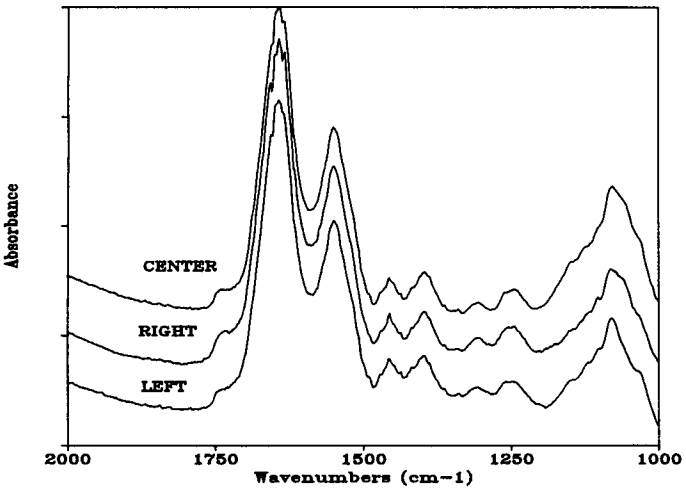

FIG. 3. Spectra of three *Caulobacter crescentus* biofilms grown on Ge crystals for each channel. The spectra were obtained under identical growth conditions and are presented on the same scale.

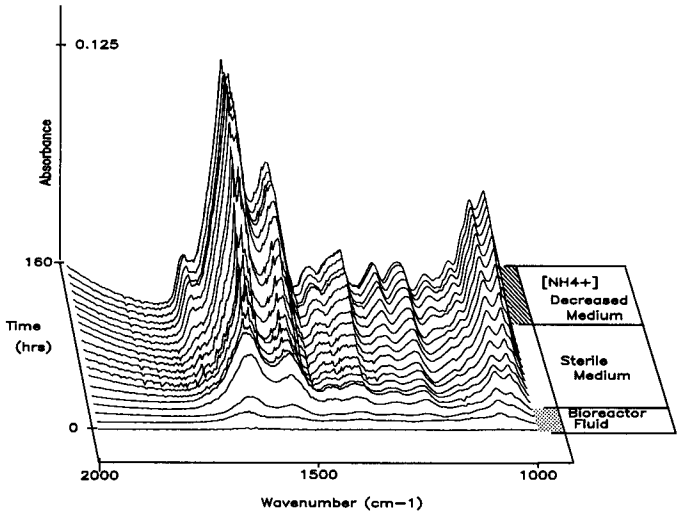

FIG. 4. Three-dimensional plot showing biofilm spectra vs. time. The instrument monitors the formation of the *Caulobacter subvibriodes* biofilm and the effect of a liquid-phase perturbation as described in the text. The different solutions are presented on the right, and time is presented on the left side of the plot.

NH_4Cl, 0.051 mM $MgSO_4$, 0.017 mM $CaCl_2$, and 0.25% (volume : volume) Wolfe's mineral solution.[12] All chemicals were reagent grade. The medium was titrated to pH 7.2 and sterilized by filtration (0.2-μm pore size). The flow system consisted of a 350-mL bioreactor, silicone tubing, three pumps, the flow cells, and medium and waste reservoirs; details are described elsewhere.[9]

RESULTS AND DISCUSSION

The water-subtracted spectra of BSA adsorbed onto Ge IRE from each channel are presented in Fig. 2 (identical scale). These spectra demonstrate the reproducibility of the three-channel instrument in a 4-h experiment. The rate of adsorption was similar for each channel (not shown). The spectra are consistent with the results obtained by Chittur *et al.* under similar conditions.[13] The spectra contained bands at 1652, 1548, 1452, 1402, and 1307–1245 cm^{-1} that were assigned to the amide I, amide II, CH_2 scissor, C-O (carboxylate ion), and amide III vibrational modes, respectively.[13] Many of the original methodologies developed for ATR/FT-IR monitoring of protein adsorption were adopted for our biofilm studies.

The reproducibility of biofilm spectra was examined in an experiment where *Caulobacter cresentus* formed biofilms under identical conditions on the surface of three Ge IREs. The final spectra of the 160-h biofilms from each channel are presented on the same scale in Fig. 3. The bands at 1738, 1648, 1548, 1456, 1398, 1308, 1250, and 1080 are attributed to the C=O stretch, amide I, amide II, CH_2 scissor, carboxylate ion, amide III (two bands), and C-O stretch vibrational modes, respectfully.[9] These results demonstrate the reproducibility of the biofilm spectra from biofilms grown in parallel. The reproducibility of this long-term experiment is similar to that of the 4-h protein study (Fig. 2).

Typical results of an ATR-FT/IR biofilm monitoring experiment from a single channel are presented in Fig. 4. In this experiment, *Caulobacter subvibriodes* cells were grown in a bioreactor, and the bioreactor solution containing bacteria and medium was pumped through the flow cells. By 8 h, an amide I band at 1648 cm^{-1} and an amide II band at 1548 cm^{-1}, probably resulting from amide linkages of proteins associated with a *C. subvibriodes* biofilm, were detected. In addition, a band was present at 1080 cm^{-1} caused by the formation of an abiotic phosphate film and/or the C-O stretch of carbohydrates and alcohols found in the RNA, the DNA, the cell envelope and/or the extracellular polymers of the bacteria within the biofilm. As the biofilms developed, other bands at 1456, 1398, and 1308/1250 cm^{-1} were detected and attributed to the CH_2 scissor, the C-O stretch of the carboxylate ion, and the amide III vibrational modes, respectively.[9] These bands are possibly due to bacterial proteins and are also observed in the BSA spectra. After 48 h, sterile medium was pumped through the flow cells. Sterile medium delivers more nutrients to the biofilm than the bioreactor fluid, thus stimulating rapid growth. The biofilm growth was detected by the increase in the intensity of all the IR absorbance bands. On the fourth day of the experiment, the ammonium ion concentration of the growth medium was decreased from 2.4×10^{-3} M to 0.5×10^{-3} M in order to stimulate production of PHA, a carbon storage product, in the *C. subvibriodes* cells. The production of PHA was detected as the C=O stretch (esters) at 1738 cm^{-1}. PHA polymers are of interest because they are being investigated as possible replacements for non-biodegradable plastics and they can be utilized as a biomarker for nutritional status.

These results prove that the three-channel ATR/FT-IR spectrometer can be used to obtain reproducible spectra of biofilms. The spectrometer allows replication of spectra and examination of effects of different treatments to biofilms that are initially established under similar conditions, and it can be expanded to contain more channels. The spectrometer will be used to facilitate ATR/FT-IR studies involving the effects of liquid-phase perturbations on the activity and physiology of the microorganisms within biofilms and to provide valuable insight into the interaction between these dynamic living entities, the surface, and their aqueous environments.

ACKNOWLEDGMENTS

The research was supported by the Office of Navy Research (N00014-88-k-0012), National Aeronautics and Space Administration (NAS8-38493), Department of Energy (DE-F605-87ER-75379), and National Science Foundation (CHE-8718057). The optical layout of the motorized beam transport was designed by Les Asato and Carlos Pareja of KVB/Analect. In addition, the authors would like to thank J. P. Alarie for his contribution.

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