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Assessment of Microbial Fouling in an Ocean Thermal Energy Conversion Experiment

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A project to investigate biofouling, under conditions relevant to ocean thermal energy conversion heat exchangers, was conducted during July through September 1977 at a site about 13 km north of St. Croix (U.S. Virgin Islands). Seawater was drawn from a depth of 20 m, within the surface mixed layer, through aluminum pipes (2.6 m long, 2.5-cm internal diameter) at flow velocities of about 0.9 and 1.8 m/s. The temperature of the seawater entering the mock heat exchanger units was between 27.8 and 28.6°C. After about 10 weeks of exposure to seawater, when their thermal conductivity was reported to be significantly impaired, the pipes were assayed for the accumulation of biological material on their inner surfaces. The extent of biofouling was very low and independent of flow velocity. Bacterial populations, determined from plate counts, were about 10^7 cells per cm^2. The ranges of mean areal densities for other biological components were: organic carbon, 18 to 27 $\mu g/cm^2$; organic nitrogen, 1.5 to 3.0 $\mu g/cm^2$; adenosine 5'-triphosphate, 4 to 28 ng/cm^2; carbohydrate (as glucose in the phenol assay), 3.8 to 7.0 $\mu g/cm^2$; chlorophyll *a*, 0.2 to 0.8 ng/cm^2. It was estimated from the adenosine 5'-triphosphate and nitrogen contents that the layer of live bacteria present after 10 weeks was only of the order of 1 μm thick. The C/N ratio of the biological material suggested the presence of extracellular polysaccharidic material. Such compounds, because of their water-retaining capacities, could account for the related increase in thermal resistance associated with the pipes. This possibility merits further investigation, but the current results emphasize the minor degree of biofouling which is likely to be permissible in ocean thermal energy conversion heat exchangers.

One conceivable way for man to harvest solar energy involves exploitation of the temperature difference which exists between the surface and deeper layers of the ocean (3, 25). The extraction of the potential energy from the ocean thermal gradient, or ocean thermal energy conversion (OTEC), depends chiefly on the efficient operation of heat exchanger units. Calculations indicate that even minor fouling of the heat exchanger surfaces will adversely affect thermal exchange and render the process uneconomic (1, 4, 28). In the summer of 1977, an operation was conducted near St. Croix (U.S. Virgin Islands) which represented the first successful attempt to experimentally investigate the relationship between biofouling, corrosion, and changes in thermal resistance associated with apparatuses which simulated heat exchanger units that might be used in OTEC plants (10). This communication reports on the quantitative aspects of the biofouling in the mock heat exchanger units.

MATERIALS AND METHODS

Mock heat exchanger units. The project was conducted from mid-July to the end of September 1977 aboard a barge located in waters about 3,600 m deep and nearly 13 km north of Christiansted, St. Croix. The mock heat exchanger units were erected on the deck of the barge, and they contained aluminum pipes (2.6 m long by 2.54-cm inside diameter) through which seawater flowed at a rate of either 0.9 or 1.8 m/s. The pipes were made of 6061-T6 aluminum alloy, and before use their interior surfaces were cleaned by brief exposures to dilute alkali and acid, interspersed and followed by washing with water (9). Four mock heat exchanger units were independently operated, and seawater for each unit was drawn from a depth of 20 m via a 7.6-cm-diameter plastic hose. Two of the units, containing only one pipe each, were used to measure changes in thermal resistance associated with the inner surfaces of the pipes at each flow rate (11, 15). These units were under the control of J. Fetkovich and his collaborators at Carnegie-Mellon University (CMU), Pittsburgh, Pa., and will therefore be called the CMU units. The pipes from the CMU units were only available for biofouling analyses at the end of the

10-week experiment. The other two mock heat exchanger units contained four pipes each. These were the University of Miami (UM) units from which pipes were periodically removed and replaced, to allow an examination of the progress of biofouling and corrosion throughout the 10-week experiment. When removing a pipe from the units, seawater was retained in the pipe by closing polyvinyl chloride ball valves at the ends of the pipe. After removal from the units, the pipes and valves were held vertically in a vise and cut into sections (2.5 cm long) with a pipe cutter. The pipes were kept full of seawater during the sectioning process, and the pipe sections were placed into filtered seawater (0.45-μm pore size, Millipore Corp.). Some of the sections were used immediately for biofouling analyses, whereas others were either frozen for later analysis or fixed for scanning electron microscopy.

Bacteriological methods. A synthetic medium was routinely used which contained the following components: NaCl, 13.35 g; $MgSO_4 \cdot 7H_2O$, 3.45 g; $MgCl_2 \cdot 6H_2O$, 2.60 g; $CaCl_2 \cdot 2H_2O$, 0.74 g; KCl, 0.17 g; NH_4Cl, 0.1 g; trace metal solution (29), 1 ml; sodium acetate $\cdot 3H_2O$, 0.2 g; sodium DL-lactate, 0.2 g; D-glucose, 0.2 g; yeast extract (Difco), 0.5 g; proteose peptone (Difco), 1.0 g; distilled water, to 1 liter. The medium was adjusted to pH 7.6 to 7.8 and contained 1.5% agar (Difco). Biofouling layers were removed from the inner walls of the pipe sections by scraping into 5 ml of sterile seawater contained in a small sterile beaker with a sterile rubber policeman. The homogenized samples were then decimally diluted in sterile seawater. Triplicate portions (0.1 or 0.2 ml) from appropriate dilutions were spread onto solid media contained in petri plates. The petri plates were incubated for 7 days at room temperature (23 to 28°C) before counting bacterial colonies.

ATP extraction and assay. Biofouling layers were directly extracted for adenosine 5'-triphosphate (ATP) by adding pipe sections to 25 ml of extractant contained in 50-ml glass beakers. Extractants used were cold 0.6 N H_2SO_4 (22), boiling tris(hydroxymethyl)aminomethane(Tris) buffer (5-min extraction) (16), and 90% dimethyl sulfoxide in 0.01 M morpholinopropanesulfonic acid (MOPS) buffer at pH 7.4 (24). The dimethylsulfoxide-MOPS extraction was carried out at room temperature for 10 min, and 1-ml portions of the extract were diluted with 4 ml of MOPS buffer (0.01 M, pH 7.4) before freezing. Cold 0.6 N H_2SO_4 was routinely used and, after a 15-min extraction, the pipe sections were removed from the beakers and adhering material was rinsed into the extract with 5 ml of 0.6 N H_2SO_4. At this point the extract was either neutralized or treated with a cation-exchange resin to remove Al^{3+} ions (22). For resin treatment the extract was passed through a small plastic column (Quik Sep columns, Isolabs, Inc., Akron, Ohio) containing a 1-ml bed of Dowex 50 W-8 (H^+ form, 50 to 100 mesh size) which had been equilibrated with 0.6 N H_2SO_4. After washing with 2× 2-ml portions of 0.6 N H_2SO_4 the extract and washings were combined and then neutralized. The neutralization procedure was identical for both the resin-treated and untreated extracts: 1 ml of 1 M Tris was added, and the pH was adjusted to 7.8 with 3 N NaOH. Finally, the extract was brought to 50 ml, with distilled water, and frozen. ATP was assayed by luminescence produced in the luciferin-luciferase reaction (16), using a purified luciferin-luciferase preparation (DuPont Chemical Co., Wilmington, Del.) which was dissolved according to the manufacturer's instructions and then diluted with 9 volumes of potassium arsenate buffer (0.05 M, pH 7.4) containing 0.02 M $MgSO_4$. The bioluminescent assay was carried out in liquid scintillation vials. A 0.1-ml amount of luciferin-luciferase solution was added to 0.2 ml of sample. Bioluminescence was measured in an ATP photometer (model 3000, SAI, San Diego, Calif.), and the light emitted in the period 15 to 21 s after adding the luciferin-luciferase preparation was recorded. All samples were assayed with and without internal standards of ATP to correct for possible interfering substances in the extracts. Standard curves, using ATP dissolved in 0.02 M Tris (pH 7.8), were frequently run using a linear regression for a minimum of three different ATP concentrations. The ATP concentration in standard solutions was determined from its extinction at 259 nm.

Carbon and nitrogen determinations. Organic residues were removed from the glassware and aluminum foil, used in these analyses, by heating them at 450°C for 24 h. The fouling layers were loosened by exposing the pipe sections to HCl fumes. The sections were placed in porcelain crucibles, or onto watch glasses, and then into a 2-liter beaker. A 20-ml beaker containing concentrated HCl was then placed in the 2-liter beaker, which was covered with a large watch glass. After exposure for up to 18 h, the pipe sections were removed from the HCl fumes and dried at 110°C. The fouling layers were scraped, with a scalpel, onto pieces of aluminum foil, which was then rolled up and stored in a desiccator. Inorganic CN contents were determined on samples which had been combusted at 500°C for 18 h. The CN contents of samples were determined with a Perkin-Elmer model 240 elemental analyzer, using acetanilide as the standard.

Carbohydrate analyses. Carbohydrate contents of the fouling layers were determined by the phenol and anthrone methods using the procedures of Herbert et al. (13). For these procedures the fouling layers were removed by scraping either with or without prior exposure to HCl fumes. D-Ribose and D-glucose were used as standards, and the spectra and extinction values were obtained with a Unicam S.P. 800 spectrophotometer, using 1-cm-path length silica cuvettes.

Other methods. Chlorophyll *a* contents were determined fluorometrically using essentially the procedures of Strickland and Parsons (32). Biofouling films, removed by scraping with a rubber policeman, were collected by filtration on glass fiber filters (Whatman GFF). The filters were stored frozen in the dark until analyzed with a Turner fluorometer.

Pipe sections for scanning electron microscopy were fixed in filtered seawater (0.45 μm, Millipore Corp.) containing 2% glutaraldehyde, rinsed in seawater, transferred to distilled water via a graded series of diluted seawater, and dehydrated by passage through acetone-water, acetone, acetone-xylene, xylene. Sections were stored desiccated in vacuo until fractured for scanning electron microscopy: fragments were

mounted on stubs and sputter-coated with gold (about 20 nm thick). Micrographs were taken with a JEOL model 35 scanning electron microscope.

RESULTS

Site characteristics. Site characteristics will be reported in detail elsewhere (Aftring et al., manuscript in preparation), but the surface waters at the site had low nutrient and microbial loads and were relatively constant in their characteristics (15). The temperature range for the water during the project was 27.8 to 28.6°C.

C, N, and carbohydrate contents. Fouling layers for CN analysis were removed from the pipe sections by repeated exposure to HCl fumes and, after drying, scraping with a scalpel. One treatment removed about 80% and two treatments removed over 90% of the C and N that could be recovered in three treatments. Even though the fouling layers were exposed to HCl fumes, they still contained an inorganic C fraction which was not removed by combustion at 500°C. The average inorganic contents, of material recovered in two HCl treatments, were about 10% for both C and N (Table 1). As a practical approach the CN analyses were restricted to material recovered in two HCl exposures but, to compensate for material still left on the inner surfaces of the pipe sections, the data were not corrected for the inorganic component. The results for the accumulation of C and N in the fouling layers, at both seawater flow rates, are compiled in Table 1.

Fouling layers for carbohydrate analysis were initially removed, as for the CN analyses, by repeated exposure of the pipe sections to HCl fumes followed by drying and scraping. Material removed by the second and especially the third treatments often produced a colloidal suspension during the phenol assay that interfered with the spectrophotometric measurement. The suspension was not removed by filtration (glass fiber filters, 0.1-μm pore size) or sedimented by centrifugation (20,000 × *g* for 10 min). The occurrence of the colloidal suspension seemed to correlate with the presence of aluminum filings removed from the pipe sections by repeated scraping. To avoid this problem, the carbohydrate assays were restricted to material removed by one scraping and without exposure to HCl fumes since similar recoveries were obtained even if this treatment was omitted (Table 2). The carbohydrate contents (phenol method) of the fouling layers at both flow rates increased to similar levels after 10 weeks (Table 2). Quantitative estimations were not carried out with the anthrone procedure, but qualitatively the colors obtained in the assay with fouling material were indicative of the presence of hexoses rather than pentoses (13).

Bacterial populations and ATP and chlorophyll *a* contents. The areal densities of bacteria in the fouling films, after the 10-week exposure, were about 10^7 cells per cm^2 at both flow rates in both the UM and CMU pipes. Only a limited examination by scanning electron microscopy of the fouling material was performed, and nothing very distinctive was discernible even after fouling. Long, thin (<0.1-μm diameter) interlacing filaments were present, and in some prints they appeared to be embedded in a matrix resembling extracellular slime (Fig. 1).

The extraction and assay of ATP from the fouling films presented an unusual problem because of the presence of aluminum and its corrosion products. Ice-cold 0.6 N H_2SO_4 was usually a more efficient and certainly a more reliable extractant than either boiling Tris or dimethyl sulfoxide-MOPS buffers: other workers have also found that the recovery of ATP from solid or particulate materials is favored by an acidic rather than a neutral pH (12, 18, 22). ATP was

TABLE 1. *Carbon and nitrogen contents of fouling layers*[a]

Flow rate (m/s)	Exposure (days)	Nitrogen (μg/cm²)	Carbon (μg/cm²)
0.9	71	2.3 (0.1)	24 (2)
	71 (CMU)	2.5 (0.2)	27 (4)
	71 (CMU)[b]	0.3 (0.3)	2 (0.6)
1.8	72	1.5 (0.3)	18 (2)
	72 (CMU)	3.0 (0.4)	27 (4)
	72 (CMU)[b]	0.4 (0.1)	3 (0.5)

[a] Material recovered from pipe sections (2.5 cm long) after two exposures to HCl fumes. The results are not corrected for inorganic CN contents and are the means of at least three samples, with standard deviations shown in parentheses.

[b] Material remaining after combustion at 500°C for 18 h, i.e., inorganic C and N.

TABLE 2. *Carbohydrate content of fouling layers*

Flow rate (m/s)	Exposure (days)	Carbohydrate recovered (μg/cm²)[a]	
		A	B
0.9	71	3.9 (0.0)	3.7 (0.6)
	71 (CMU)	7.0 (0.9)	ND[b]
1.8	72	3.8 (0.5)	ND
	72 (CMU)	5.4 (0.4)	5.2 (0.3)

[a] Material recovered from 2.5-cm-long pipe sections: A, No exposure to HCl fumes; B, after exposure to HCl fumes. Results expressed as micrograms of glucose. Standard deviations are given in parentheses.

[b] ND, Not determined.

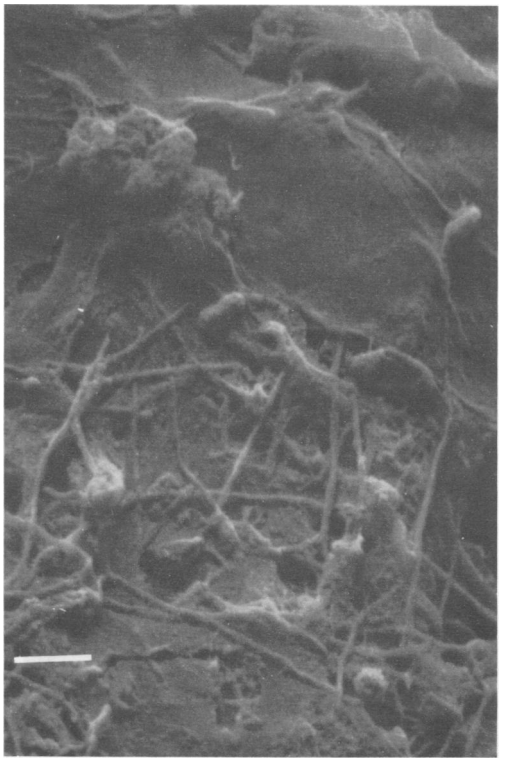

FIG. 1. *Scanning electron micrograph of biofouling film after 71-day exposure to seawater flowing at 0.9 m/s (CMU pipe). Bar = 1 μm.*

probably bound to the corrosion products at neutrality, and even though the H_2SO_4 prevented this it promoted the dissolution of metallic ions from the pipe sections. Unless the acidic extract was passed through a column of cation-exchange resin, before neutralization, a copious white precipitate was formed. Extracts containing this precipitate, which was probably mainly $Al(OH)_3$, gave erratic results in the bioluminescent assay for ATP. Clarification of the extracts by centrifugation merely sedimented the ATP, presumably because of its adsorption to the $Al(OH)_3$ (Table 3). Resin-treated extracts after neutralization were, at the worst, only faintly opalescent and presented no problems in the luciferin-luciferase assay (Table 3).

The recovery of ATP during acidification and resin treatment was very variable. When standard amounts commensurate with the ATP contents of the fouling films were used the recoveries ranged between 6 and 52%, with a mean value of 20% ($n = 6$). The results for ATP and chlorophyll *a* contents of the fouling films in pipes exposed for 10 weeks are shown in Table 4.

TABLE 3. *Effect of cation-exchange treatment on ATP recovery by acid extraction*[a]

Treatment	ATP recovered (μg)
(A) Acidified, then neutralized	2.91 (0.41)
(B) Acidified in presence of Al ring, neutralized, centrifuged to remove $Al(OH)_3$[b]	0.02 (0.02)
(C) As for (B) but resin treated before neutralizing[c]	0.63 (0.03)

[a] 5-μg quantities of ATP added to 25-ml portions of ice-cold 0.6 N H_2SO_4 and treated as shown before assaying for ATP recovery. The results are means from three separate experiments, with standard deviations in parentheses.

[b] Pipe sections (2.5 cm long) exposed to flowing seawater for about 10 weeks, stored frozen, and then dried at 100°C.

[c] Resin = 1-ml bed of Dowex 50 W-8 (H^+ form, 50 to 100 mesh).

TABLE 4. *ATP and chlorophyll a contents of the fouling layers*[a]

Flow rate (m/s)	Exposure (days)	ATP[b] (ng/cm²)	Chlorophyll *a* (ng/cm²)
0.9	71	11 (3)	0.8 (0.2)
	71 (CMU)	20 (6)	0.4 (0.2)
1.8	72	4 (3)	0.2 (0.1)
	72 (CMU)	28 (8)	0.4 (0.1)

[a] Results are means of four samples, with standard deviations shown in parentheses, and are based on the amounts recovered from 2.5-cm-long pipe sections.

[b] Based on a 20% recovery of standard ATP during acidification and resin treatment.

DISCUSSION

Theoretical calculations indicate that static layers of water only 25 to 50 μm thick will significantly impair heat transfer efficiencies in OTEC heat exchangers (1, 4, 28). Fetkovich and his colleagues have developed a method for determining changes in thermal resistance (R_f) associated with the inner surfaces of metallic pipes which contain flowing seawater and thereby mimic OTEC heat exchangers (10). The average thickness of the fouling layer is calculated from R_f values by assuming it has the thermal conductivity of seawater (10). The fouling layer has been equated with biofouling (10) even though there was no experimental evidence for this assumption. This report describes the first attempt to quantify biofouling under conditions relevant to OTEC heat exchangers.

Most of the biofouling data for the pipes exposed for about 10 weeks are compiled in Table 5. The CMU pipes were slightly more fouled than the UM pipes, but the accumulation was apparently independent of the flow rate. The average fouling layer thicknesses, calculated from R_f measurements, were 55 and 35 μm at

TABLE 5. *Biofouling after 10 weeks of exposure to seawater*[a]

Flow rate (m/s)	Heat exchanger unit	Carbon ($\mu g/cm^2$)	Nitrogen ($\mu g/cm^2$)	Carbohydrate[b] ($\mu g/cm^2$)	ATP (ng/cm^2)	Chlorophyll (ng/cm^2)
1.8	CMU	27	3.0	5.4	28	0.4
0.9	CMU	27	2.5	7.0	20	0.4
1.8	UM	18	1.6	3.8	4	0.2
0.9	UM	24	2.2	3.9	11	0.8

[a] Based on mean amounts recovered from 2.5-cm-long pipe sections.
[b] As micrograms of glucose.

flow velocities of 0.9 and 1.8 m/s, respectively (14, 15). The development of thermal resistance at 0.9 m/s was unusual, in comparison to previous data (10, 11), because it increased linearly from the beginning of the experiment to its termination and at a faster rate than at 1.8 m/s (15). The CMU pipe at the lower flow rate was also corrosively pitted over 50% of its inner surface (26). However, the CMU pipe at the higher flow velocity had minor pitting and exhibited an increasing rate of R_f development with time of exposure to seawater (15, 26), similar to that of earlier studies (10, 11). Detailed comparisons of biofouling data with fouling layer thicknesses estimated from R_f values are therefore restricted to the 1.8-m/s flow velocity. The calculated composition of a 35-μm-thick bacterial film is shown in Table 6 together with extrapolations of the biofouling data, into cellular layers, for the CMU pipe at 1.8 m/s. Photosynthetic microbes made an insignificant contribution to the biofouling layers, based on published chlorophyll *a*/carbon ratios (27). Biofouling layer thicknesses derived from bacterial populations were very low, but plate counts often severely underestimate real populations (17). Extrapolations from the ATP (19, 21) and N (23) data gave similar results but a 1-μm-thick film is minor in contrast to the 35-μm estimate from R_f measurements (14, 15). La Motta (21) measured the ATP content of biological films of varying thicknesses which developed in a biological film reactor with "high fluid velocities." From this relationship the ATP content of the biofouling layer corresponds to a thickness of about 1.4 μm, very similar to thicknesses calculated from ATP and N in Table 6. The calculations in Table 6 do not consider extracellular material, an important component in bacterial fouling (6, 8). Material resembling extracellular slime was evident in some of the electron micrographs. The difference in thickness of the biofouling film as estimated from C content, in contrast to N or ATP content, is probably due to extracellular polysaccharidic material (6, 8). The reactive material in the carbohydrate assay represented only about 15% (by weight) of the postulated extracellular carbon, but some components of extracellular polymers (e.g., amino acids, lipids, uronic acids) are unreactive or weakly reactive in the phenol method (5, 13). Extracellular polysaccharides are expanded structures (2) often associated with bacteria growing on rocks in fast-flowing streams (7). Some polysaccharides and glycoproteins form aqueous gels at very low concentrations, thus entraining a large volume of water on a weight basis (30, 31). Evidence for the effect of carbohydrate on R_f can be found in the carbohydrate content of the biofouling layer at 0.9 m/s. This is the only value at 0.9 m/s which exceeds that at 1.8 m/s (CMU units), despite the approximately 60% difference in the fouling layer thicknesses calculated from R_f measurements of these pipes. If the dry-to-wet weight ratio of the extracellular slime is only 0.01 to 0.02, rather than 0.22 (23) for intracellular material (Table 6), the estimated thickness of the biofouling layer, at 1.8 m/s, swells to about 15 to 30 μm, sufficient to account for the observed thermal resistance. Clearly, further work is needed to understand the biological component of the fouling layer and its relationship to R_f measurements, especially since corrosion products seem to contribute minimally to thermal resistance (11, 26).

TABLE 6. *Calculated composition of a 35-μm-thick bacterial film and the estimated thickness of the biofouling layer in the 1.8-m/s CMU pipe*[a]

Component	Calculated amt in 35-μm layer[a] (per cm^2)	Amt detected in 1.8-m/s CMU pipe (per cm^2)	Estimated biofouling layer thickness in 1.8-m/s CMU pipe (μm)
Carbon	430 μg	27 μg	2.3
Nitrogen	120 μg	3 μg	0.9
ATP	850 ng	28 ng	1.2
Cell no.	1×10^{10}–2×10^{10}	1×10^7	0.04–0.09

[a] Calculations based on the following factors for bacterial composition: density, 1.1 g/cm^3 (20); dry-to-wet weight ratio of 0.22, carbon-to-dry weight ratio of 0.5, carbon to nitrogen ratio of 3.6 (23); ATP content, 0.1% of dry weight (19); cell volume, 0.09 to 0.28 μm^3 (33).

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