APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Aug. 1982, p. 471–477
0099-2240/82/080471-07$02.00/0

Vol. 44, No. 2

Effects of Surface Area and Flow Rate on Marine Bacterial Growth in Activated Carbon Columns

ROBERT J. SHIMP[1]†* AND FREDERIC K. PFAENDER[2]

Curriculum in Marine Science[1] and Department of Environmental Sciences and Engineering,[2] University of North Carolina, Chapel Hill, North Carolina 27514

Received 12 November 1981/Accepted 27 April 1982

The colonization of granular activated carbon columns by bacteria can have both beneficial and potentially detrimental consequences. Bacterial growth on the carbon surface can remove adsorbed organics and thus partially regenerate the carbon bed. However, growth can also increase the levels of bacteria in the column effluents, which can adversely affect downstream uses of the treated water. This study of a sand column and several activated carbon columns demonstrated that considerable marine bacterial growth occurred in both sand and carbon columns and that this growth increased the number of bacteria in column effluents. Activated carbon supported approximately 50% more bacteria than did sand. Bacterial growth on activated carbon was reduced by increasing the flow rate through a carbon column and increasing the carbon particle size. Scanning electron micrographs showed that bacteria preferred to attach in the protected crevices on both the sand and carbon surface. The results of this study indicated that the colonization of activated carbon by marine bacteria was enhanced because of carbon's high surface area, its rough surface texture, and its ability to absorb organic materials.

The fact that bacteria readily colonize granular activated carbon has been clearly demonstrated (25), and the role of attached bacteria in the "bioregeneration" of granular activated carbon has been characterized in a number of studies (4, 15, 18, 23). However, the major factors which limit the growth of bacteria on activated carbon have not been identified. The purpose of this investigation was to examine the effects of the high exposed surface area of activated carbon and of carbon column flow rate on the growth of bacteria in activated carbon columns.

Bacterial attachment to a wide array of surfaces is a phenomenon that has been recognized for many years (26, 27). Indeed, the attachment of bacteria to surfaces is a widespread phenomenon (16) and plays an important role in the ecology of both freshwater and marine environments (12, 13). The ability of most surfaces to absorb organic materials is often proposed as one of the major reasons for microbial attachment (5, 7).

Activated carbon is a very adsorptive surface which is capable of removing a wide range of organic materials from solution (20). Bacteria which become attached to activated carbon can utilize adsorbed organic materials as growth substances (11). Thus, the high adsorptive capacity of activated carbon may create an enriched environment which could support a larger population of bacteria than a relatively nonadsorptive surface such as sand or coal. Shear forces induced by fluid flow past a surface can have a significant impact on the colonization of surfaces by preventing the attachment of bacteria or by shearing off developed biofilm (21). In comparison to other surfaces, activated carbon has a very high surface-area-to-volume ratio, due to its large proportion of internal pores and rough surface texture (22). This characteristic of carbon may make it a more favorable surface for colonization than other, more rounded surfaces such as sand.

The relative effects of adsorptivity, surface area, and flow rate on the growth of bacteria on activated carbon were examined in a 4-month study of bacterial numbers in the effluents from several bench-scale columns. Three different grain sizes of Nuclear WV-W activated carbon were used to test the effects of changing exposed surface area. Quartz sand was used as a relatively nonadsorptive surface to be compared with the adsorptive activated carbon. Two other carbon columns were run at flow rates different from the above columns to test the effects of different flows on colonization. After the col-

† Present address: Department of Environmental Sciences and Engineering, University of North Carolina, Chapel Hill, NC 27514.

umns reached a relative steady state with respect to bacterial counts, each column was disassembled, and the colonized surface was examined to determine bacterial numbers and metabolic activity on the carbon and sand and to characterize the mode and degree of bacterial attachment to the two surfaces by using scanning electron microscopy.

MATERIALS AND METHODS

Study site. This investigation was conducted at the Wrightsville Beach Test Facility in Wrightsville Beach, N.C. The purpose of the facility is to produce virtually particle-free seawater for use in reverse osmosis desalination pilot plants. Activated carbon is used in pretreatment to dechlorinate the seawater just before desalination. The growth of bacteria in the carbon columns of the facility may constitute a problem, since carbon column-derived bacterial particles may foul the reverse osmosis membranes.

Column design and operation. Eight bench-scale columns were constructed of Lucite tubing (2 in. [5.08 cm] diameter). Each column was filled with dry-heat-sterilized media (sand or activated carbon) and sealed to prevent air intrusion. Combined effluent from the pretreatment zeolite filters of the Wrightsville Beach Test Facility was fed to the columns from a single 30-gallon head tank. Flow rates were controlled by flowmeters (Dywer Instruments, Michigan City, Ind.) located in the effluent line of each column. Each effluent line was also equipped with a sample port, and each column was fitted with two glass tube manometers to measure headloss. All columns were designed to have an empty-bed contact time of 7 min.

Nuchar WV-W activated carbon (Westvaco Corp., Covington, Va.) was sieved to the following U.S. Standard mesh sizes: 8 by 14 (2.21 mm), 12 by 18 (1.50 mm), and 20 by 35 (0.83 mm). These classes supplied media for each of the three surface area columns, which were designated SA8×14, SA12×18, and SA20×35. Column SA12×18 was run in triplicate to give a measure of the variability between identical columns. Each column was run at a flow rate of 250 ml/min (162 liters/min per m^2). The method described by Heywood (8) was used to determine the external surface area of each class of material. Since this method does not account for internal pore area, we made the assumption that the external surface area represented that area available for colonization. Up to 50 particles of carbon were examined microscopically and dimensioned. The mean projected particle diameter was determined, and the external surface area was calculated on a per-gram basis. The method described by Heywood (8) accounts for surface texture and irregular particle shape through the use of coefficients described in the paper. Columns SA8×14, SA12×18, and SA20×35 each contained 946 g of carbon, which corresponds to 2.19×10^4, 4.5×10^4, and 7.7×10^4 cm^2 of exposed surface area, respectively.

The quartz sand used as a nonadsorptive surface was sieved to 12 by 18 mesh. The column, called "SAND," contained 2,610 g of sand and had a total exposed surface area of 4.3×10^4 cm^2. The colonization of the SAND column was compared with that of column SA12×18, which contained approximately the same amount of surface area.

Two other columns, designated FLOW2 and FLOW6, contained 12 by 18-mesh activated carbon and were run at 125 ml/min (81 liters/min per m^2) and 375 ml/min (243 liters/min per m^2), respectively. These columns contained 2.2×18^4 and 7.0×10^4 cm^2 of exposed surface area. Their colonization was compared with that of SA12×18, which was called FLOW4 during such comparisons.

The amount of carbon or sand in each column was adjusted to obtain a 7-min empty-bed contact time.

Headloss was measured daily in each column, and the flow rates were adjusted to correct for decreasing flow caused by a buildup of particulate matter in the columns.

Sampling. The following parameters were measured regularly in the feed and the effluents from each column: temperature, pH, dissolved oxygen, total bacteria, and colony-forming units (CFU) of viable bacteria.

pH measurements were made with a Fisher Accumet model 420 pH/ion meter (Fisher Scientific Co., Pittsburgh, Pa.) after standardization in seawater. Dissolved oxygen measurements were made with a Yellow Springs Instruments model 57 DO meter and model 5739 DO probe (Yellow Springs Instrument Co., Yellow Springs, Ohio) which was air standardized before each sampling period.

Total bacteria in column effluents were determined by the acridine orange direct count (AODC) method (9) on a Leitz Ortholux II fluorescence microscope (Leitz/Opto-Metric Div. of E. Leitz Inc., Rockleigh, N.J.) equipped with a Ploem illuminator.

CFU levels were measured with the spread plate technique of Buck and Cleverdon (2). Marine agar 2216 (Difco Laboratories, Detroit, Mich.) was used as the plating medium, and the plates were incubated for 2 weeks at 20°C.

The columns were sampled for all of the above parameters until a relatively steady state with respect to AODCs of cells developed for each column (approximately 4 months).

Column breakdown study. Upon completion of the effluent study, each column was drained and the medium was removed. Bulk samples of substratum from the SA8×14, SA12×18-2, SA20×35, SAND, FLOW2, and FLOW6 columns were taken from the top, middle, and bottom of each column bed for use in AODC, community metabolism, and scanning electron microscopy (SEM) analysis.

For AODC analysis, triplicate 2.5-g samples of the medium were placed in 250 ml of filtered seawater and homogenized for 10 min at the highest speed of a Waring blender to free attached bacteria. The homogenized samples were then filtered through a 3-μm Nuclepore filter to remove larger carbon particles which interfered with the AODC analysis. The filtrate was fixed with formaldehyde and was later analyzed by the usual AODC procedure. Homogenization does not remove all cells from the carbon surface. However, since each sample was treated in the same manner, the fractions recovered should be comparable, even though the reported numbers represent conservative estimates of the actual counts present on the carbon surface.

Heterotrophic activity of the bacteria attached to the surface of the carbon or sand was determined by measuring the mineralization of a trace concentration

of a ^{14}C-amino acids mixture representative of an algal protein hydrolysate (New England Nuclear Corp., Boston, Mass.). Triplicate 2.0-g samples of medium from the top, middle, and bottom of each column were placed in 40-ml Pierce septum vials with 20 ml of sterile filtered seawater from the zeolite filters of the pretreatment plant. A 40-μl volume of ^{14}C-amino acids mixture (1 μCi/ml) was added to each vial, and the vials were incubated for 30 min. Control samples to determine non-biological production of $^{14}CO_2$ were prepared by killing the bacteria on the medium in a fourth sample with 0.25 M $HgCl_2$. After incubation, metabolism was terminated with $HgCl_2$, and the samples were acidified with 2 N H_2SO_4 to liberate CO_2 from solution. $^{14}CO_2$ was trapped on a piece of 1 N KOH-soaked filter paper for 4 h. The paper was placed in 10 ml of Aquasol II scintillation fluid, and the radioactivity was counted on a Packard Tri-carb model 3320 scintillation counter (Packard Instrument Co., Inc., Rockville, Md.). Turnover rates for the amino acids were calculated in the manner discussed by Azam and Holm-Hansen (1): as long as the concentration of substrate added is negligible compared with ambient levels, the turnover rate (reciprocal of turnover time) can be directly measured from the fraction of added label metabolized to $^{14}CO_2$ divided by the incubation time.

Samples from the top 5 cm of columns SA12×18, SAND, FLOW2, and FLOW6 as well as sterile sand and sterile 12×18 carbon were fixed for SEM analysis in 2% (vol/vol) glutaraldehyde and prepared for viewing as described by Paerl and Shimp (17). The fixed, dried samples were examined on an Etec Autoscan scanning electron microscope.

RESULTS

The column study was started on December 3, 1980 and was completed April 10, 1981. During this period, the temperature of the seawater passed through the columns varied from 7.5 to 16.5°C, and the pH varied from 7.72 to 7.99. Neither of these parameters was appreciably affected by the passage of the seawater through the columns.

The measurements of dissolved oxygen in the feed and effluents from the columns showed that dissolved oxygen was reduced during passage through the activated carbon columns. There was only a slight reduction in the SAND column. This decline in dissolved oxygen was probably the result of both chemical adsorption of oxygen by the carbon itself (19) and bacterial metabolism on the carbon surface.

AODC results in the feed and effluents are shown in Fig. 1 and 2. With the exception of day 1, the three replicate SA12 × 18 columns showed little variability (coefficient of variation was less than 10%). Colonization appeared to be greater on the carbon than on the sand (Fig. 1). Increasing flow rates (shown in Fig. 2) appeared to reduce the effluent AODC levels from the columns.

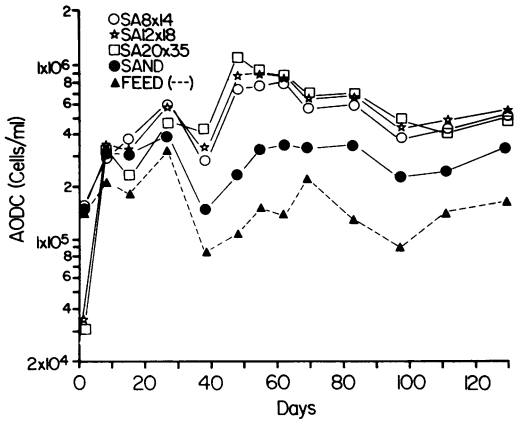

FIG. 1. Total cell counts (AODC) in effluent from the surface area and sand test columns.

CFU counts demonstrated more variability (coefficient of variation for the three SA12×18 replicates was 15 to 25%) than the AODC and did not reveal the same patterns of colonization as the AODC measurements. In fact, effluent CFU levels were approximately the same in all of the columns throughout most of the study. However, the CFU measurements clearly showed that bacteria did indeed colonize all of the columns. Feed CFU levels were approximately 1×10^3 CFU/ml, and effluent levels from all of the columns were nearly 8×10^4 CFU/ml after the first 30 days of operation.

Results from the column breakdown study are shown in Fig. 3. An F-test analysis of variance was made for all of the data. According to the results of that testing, AODC levels (Fig. 3A) for the top samples from all of the carbon columns were significantly different from middle samples from the columns ($P \ll 0.01$). In the SAND column, the difference was less pronounced but

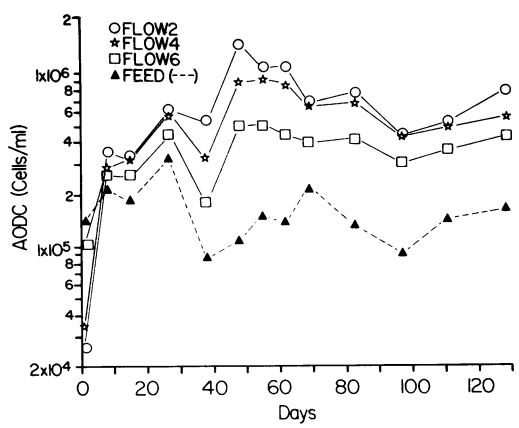

FIG. 2. Total cell counts (AODC) in effluent from the flow rate test columns.

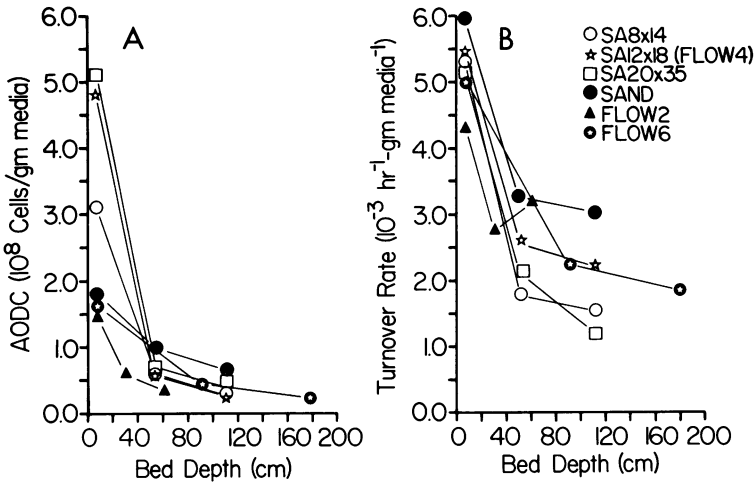

FIG. 3. Results of the test column breakdown study. (A) Total cell counts (AODC) per gram of media; (B) amino acids turnover rate (equal to the fraction of added label metabolized to CO_2 divided by the incubation time).

significant at $0.01 < P < 0.025$. There were no significant differences between the middle and bottom AODC counts for any of the columns and no trends with respect to surface area and flow rate at those locations.

Comparing the AODC levels in the top samples alone, the AODC per gram levels for SA8×14 were significantly smaller than for both SA12×18 and SA20×35 ($P \ll 0.01$), but SA12×18 was not significantly different from SA20×35. Colonization on the SAND column was significantly less than on SA12×18 ($P \ll 0.01$). Colonization on FLOW6 was significantly less than on FLOW4 (SA12×18). However, FLOW2 and FLOW6 were not significantly different.

Amino acids turnover rate results (Fig. 3B) showed a trend of decreasing metabolic activity with depth, but did not reflect any of the differences between columns that were apparent from the AODC data.

Scanning electron micrographs for the SAND and SA12×18 columns are shown in Fig. 4. Bacterial growth appeared as light-colored globular masses which consisted of individual cells imbedded in extracellular material. Colonization on the SAND column appeared to be limited to the cracks and crevices in the medium surface (Fig. 4B). Column SA12×18, on the other hand, appeared to be more heavily covered with cells (Fig. 4E). The microcolonies on the carbon also appeared more dense than on the sand. Close-ups at 2,000× (Fig. 4C and F) showed that the colonized areas of both sand and carbon were covered with 1- to 2-μm cells buried in mats in fibrous extracellular material.

Micrographs (200×) for the different flow rate columns, FLOW2, FLOW4 (SA12×18), and FLOW6 are shown in Fig. 5. Colonization appeared to be greatest on column FLOW2 (Fig. 5A) and least on FLOW6 (Fig. 5C). The FLOW6 picture shows several large areas of uncolonized surface, mostly on exposed faces of the carbon granule, whereas column FLOW2 was more evenly covered with cells. The extent of colonization of column FLOW4 appeared to be somewhere in between that of the other two columns.

DISCUSSION

Bacteria colonized both the activated carbon and the sand used in the column study. Effluent AODCs were at least three times higher than feed levels for most of the study (Fig. 1 and 2). Differences between effluent and feed CFU levels were even more pronounced. At times, effluent CFU counts were more than two orders of magnitude higher than feed counts. Total cell counts on the media were on the order of 10^8 cells per g. Finally, the SEM micrographs clearly show attached bacteria on the surface of both carbon and sand particles.

As shown by the results of the breakdown study, the majority of the cells in each column were attached to the media in the top 10 to 20 cm of each bed (Fig. 3A). Although significant colonization occurred in the rest of the bed, there were between 2 and 10 times more cells in the top samples than in samples from the middle and bottom of each bed.

Bacteria colonized activated carbon to a greater extent than sand. Effluent AODC levels

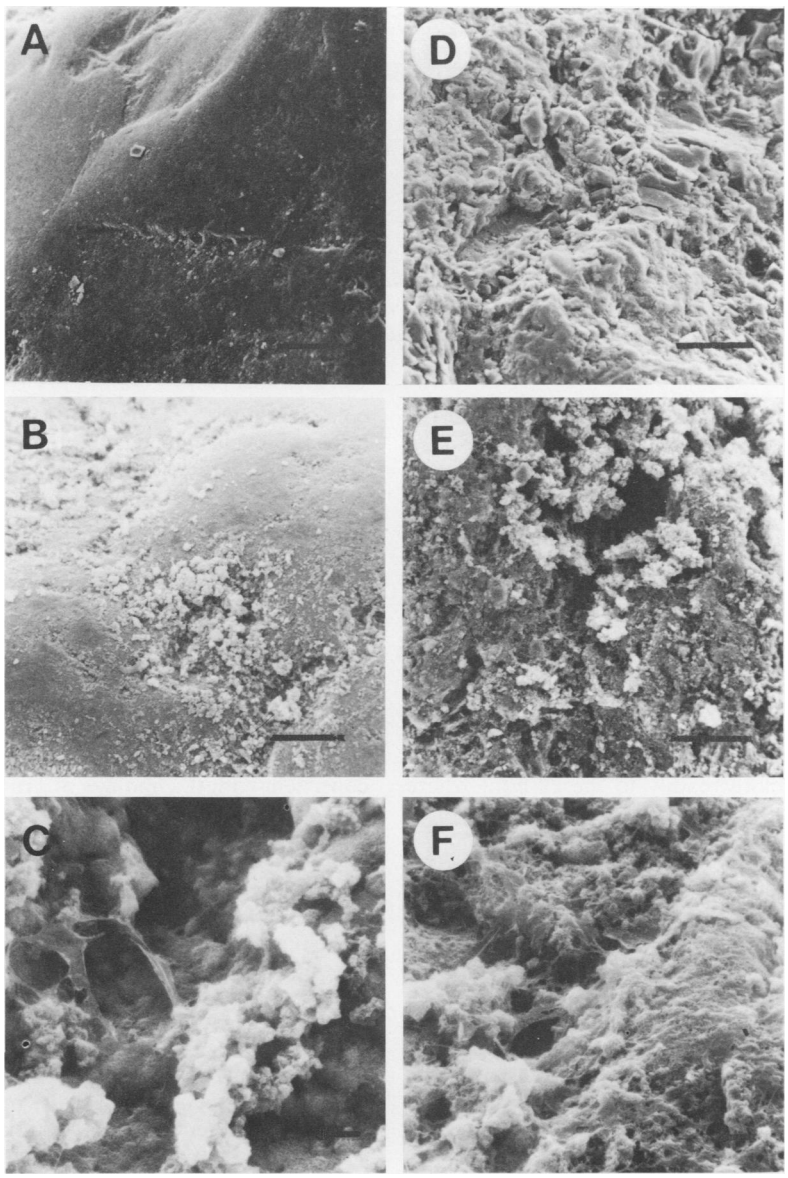

FIG. 4. Scanning electron micrographs illustrating the similarities and differences between the colonization of quartz sand and SA12×18 activated carbon. (A) Sterile sand, with relatively smooth surface texture; (B) colonized sand, bacteria congregated in surface fissures; (C) close-ups of colonized sand, showing extent of extracellular secretions; (D) sterile activated carbon with rough surface texture; (E) bacterial colonization on SA12×18 activated carbon was widespread and appears to exceed that of sand; (F) close-up of SA12×18 activated carbon is similar to (C) in that cells secreted considerable amounts of extracellular material. Bars in A, B, D, and E are 50 μm. Bars in C and F are 2 μm.

in the SA12×18 columns were two to three times higher than effluent levels from the SAND columns for every sampling date past day 40 (Fig. 1). This result is supported by the column breakdown AODC data (Fig. 3A) and the SEM micrographs (Fig. 4). There are several explanations for this phenomenon.

The total organic carbon (TOC) levels in the feed were relatively low (1 to 3 mg/liter), so substrate adsorption by the two media (sand and carbon) may have enhanced colonization by concentrating organic material. The large capacity of activated carbon to adsorb organic material may provide richer substrate conditions near

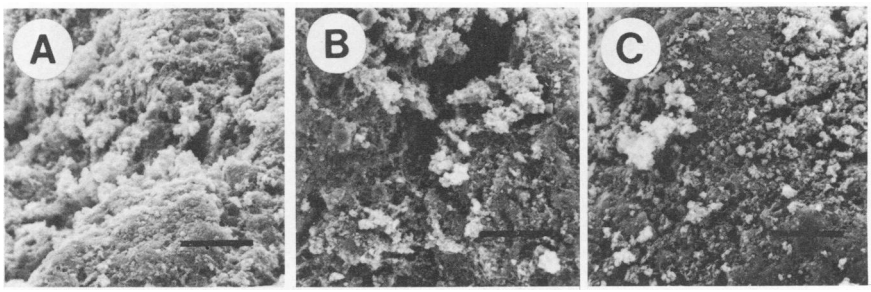

FIG. 5. Scanning electron micrographs comparing the colonization of the different flow rate columns. (A) FLOW2; (B) FLOW4; (C) FLOW6. Note that the extent of colonization appears to decrease as the flow rate increases. Bars are 50 μm.

the carbon surface than would be found near the surface of the relatively nonadsorptive sand.

Another reason that bacteria colonized activated carbon more heavily than sand is related to the physical structure of the medium. Activated carbon has a considerably rougher surface texture than sand (Fig. 4A and D). According to Meadows (14) and Weise and Rheinheimer (25), attached bacteria colonize the indentations and surface fissures of marine sand grains, presumably to escape the adverse effects of fluid shear. This is also evident from the SEM micrographs (Fig. 4B). Since activated carbon has a more irregular surface than sand, bacteria would logically attach more readily to the carbon, where the number of protected crevices is higher than on the sand particles.

Increasing surface area (decreasing grain size) exerted a small positive effect on the bacterial colonization of activated carbon. Effluent AODC levels on SA20×35 were slightly, but consistently, higher than those in SA8×14 effluents for most of the study (Fig. 1). It is doubtful, however, that even with replication, these data would stand alone. However, the total counts on the medium itself (Fig. 3A) also show the same trend. The differences between SA20×35 and SA8×14 AODCs on the carbon surface were highly significant ($P \ll 0.01$).

Since it appears that the surface of the colonized activated carbon was not completely covered with cells (Fig. 4E), it is unlikely that surface area itself limited microbial growth. Rather, the slight surface area enhancement observed is probably due to smaller carbon grain size in column SA20×35. Li and Digiano (11) hypothesized that decreasing particle diameter decreases the diffusional pathlength of organic substrates from the internal pores of the carbon to the biofilm and hence increases the availability of adsorbed substrate to attached cells. Thus, growth would be somewhat enhanced in small-grain systems such as SA20×35.

Increasing the flow rate through a carbon column decreased the number of bacteria in the column effluents. Column FLOW6 had lower total cell levels in its effluent than either FLOW2 or FLOW4 (Fig. 2). Additionally, the SEM micrographs (Fig. 5) indicate, at least qualitatively, that the biofilm was more luxuriant and more evenly dispersed on carbon from the low flow rate FLOW2 column than on carbon from either of the other two columns. It should be noted that these micrographs show only a small part of a single carbon grain and cannot be regarded alone as convincing evidence of higher colonization in lower flow rate systems. However, they do reinforce the column study data. Lower cell counts in higher flow regimes are consistent with the findings of Trulear and Characklis (21) that higher flow rate systems have less extensive steady state biofilms because of higher reentrainment of the biofilm by high fluid shear.

One seeming contradiction in the results of this study concerns the amino acids turnover experiment (Fig. 3B). Although the results of this experiment reflected the strong gradient of colonization within a particular column, the turnover rates did not show any significant differences between columns. This was not, however, an unexpected result. In marine waters, not all components of the bacterial community are equally active (10). Thus, the metabolic activity of the community is dependent on the proportion of active cells, not on total numbers. Metabolic activity is also a function of available substrate concentrations (10). Bacteria at the head of each column are being exposed to the same incoming nutrient conditions. Therefore, the activities observed were probably more a function of the nature of the community and nutrients in the influent than the physical characteristics of the individual columns.

Besides their qualitative value as indicators of population density on different media and in different flow regimes, the scanning electron micrographs give a clear indication of the means by which bacteria attach to solid surfaces. Most of the cells attached to the carbon did not

produce small amounts of extracellular material. Instead, they imbedded themselves in a thick fibrous mat of material on the medium surface (Fig. 4F). Strands of polymer appeared to span macropores and completely cover micropores in the carbon surface. Whether or not this coverage affects the adsorptive properties of activated carbon is not clear. Li and Digiano (11) and Peel and Benedek (18) believe that organic molecules easily diffuse through biofilms that surround carbon particles and that the biofilm does not affect adsorption.

The nature of attachment to sand was similar to that for carbon (Fig. 4C and D). In the densely colonized crevices of the sand grain, bacteria appeared to have secreted considerable amounts of extracellular material. The material secreted by attached marine pseudomonads has been identified as an acidic muco-polysaccharide consisting of glucose, galactose, mannose, and galacturonic acid subunits (3). The material is a fibrous reticular substance which is stretched from the bacteria to the attachment medium (6).

The results of this study indicate that colonization of activated carbon by bacteria is influenced by a number of factors, including flow rate, carbon grain size, and surface texture. Bacterial colonization of carbon can be extensive and leads to the development of a biofilm consisting of considerable amounts of extracellular polymeric material which holds cells tightly to the surface.

ACKNOWLEDGMENTS

This work was supported by grant no. 14-34-0001-9507 from the U.S. Department of the Interior, Office of Water Resources Technology.

LITERATURE CITED

1. **Azam, F., and O. Holm-Hansen.** 1973. Use of tritiated substrates in the study of heterotrophy in seawater. Mar. Biol. **23**:191–196.
2. **Buck, J. D., and R. C. Cleverdon.** 1970. The spread plate method for the enumeration of marine bacteria. Limnol. Oceanogr. **5**:78–80.
3. **Corpe, W. A.** 1970. An acid polysaccharide produced by a primary film forming bacterium. Dev. Ind. Microbiol. **11**:402–412.
4. **Digiano, F. A.** 1979. General considerations in assessing the beneficial aspects of microbial activity on GAC. Presented at the U.S. Environmental Protection Agency International Conference on Practical Applications of Adsorption Techniques in Drinking Water, Preston, Va.
5. **Fletcher, M.** 1979. The attachment of bacteria to surfaces in aquatic environments, p. 97–108. *In* D. C. Ellwood, J. Melling, and P. Rutter (ed.), Adhesion of microorganisms to surfaces. Academic Press, Inc., London.
6. **Fletcher, M., and G. D. Floodgate.** 1973. An electron microscopic demonstration of an acidic polysaccharide involved in the adhesion of marine bacterium to solid surfaces. J. Gen. Microbiol. **74**:325–334.
7. **Heukelekian, H., and A. Heller.** 1940. Relation between food concentration and surface for bacterial growth. J. Bacteriol. **40**:547–558.
8. **Heywood, H.** 1947. The scope of particle size analysis and standardization. Inst. Chem. Eng. London Trans. **25**:14–24.
9. **Hobbie, J. E., R. J. Daley, and S. Jasper.** 1977. Use of Nuclepore filters for counting bacteria by fluorescence microscopy. Appl. Environ. Microbiol. **33**:1225–1228.
10. **Jannasch, H. W.** 1967. Growth of marine bacteria at limiting concentrations of organic carbon in seawater. Limnol. Oceanogr. **12**:264–271.
11. **Li, A. Y., and F. A. Digiano.** 1980. The availability of sorbed substrate for microbial degradation on granular activated carbon. Annu. Water Pollut. Control Fed. Conf. 53rd, Las Vegas, Nev., Sept. 23–Oct. 3.
12. **Marshall, K. C., R. Stout, and R. Mitchell.** 1971. Selective sorption of bacteria from seawater. Can. J. Microbiol. **17**:1413–1416.
13. **Marszalek, D. S., S. M. Gerchakov, and L. R. Udey.** 1979. Influence of substrate composition on marine microfouling. Appl. Environ. Microbiol. **38**:987–995.
14. **Meadows, P. S.** 1966. Microorganisms attached to marine and freshwater sand grains. Nature (London) **212**:1059–1060.
15. **Miller, G. W., and R. G. Rice.** 1978. European water treatment practices—the promise of biological activated carbon. Civil Eng. (N.Y.) **48**:81–83.
16. **Paerl, H. W.** 1975. Microbial attachment to particles in marine and freshwater ecosystems. Microb. Ecol. **2**:73–83.
17. **Pearl, H. W., and S. L. Shimp.** 1973. Preparation of filtered plankton and detritus for study with scanning electron microscopy. Limnol. Oceanogr. **18**:802–805.
18. **Peel, R., and A. Benedek.** 1977. The modelling of activated carbon adsorbers in the presence of biooxidation. AIChE Symp. Ser. **73**:25–35.
19. **Probes, R., J. J. Pyhea, and W. E. Kidon.** 1975. Interaction of activated carbon with dissolved oxygen. AIChE J. **21**:1200–1204.
20. **Symons, J. M.** 1978. Removal of specific organic compounds with activated carbon, p. A9–A21. *In* Interim treatment guide for controlling organic contaminants in drinking water using GAC. U.S. Environmental Protection Agency, Cincinnati, Ohio.
21. **Trulear, M. G., and W. G. Characklis.** 1980. Dynamics of biofilm processes, p. 838–853. Proc. 34th Ind. Waste Conf., Purdue University, West LaFayette, Ind.
22. **Weber, W. J., Jr.** 1972. Physicochemical processes for water quality control, p. 239. Interscience Publishers, Inc., New York.
23. **Weber, W. J., Jr., L. D. Friedman, and R. Bloom, Jr.** 1973. Biologically extended physicochemical treatment, p. 612–617. Proc. 6th Int. Conf. Int. Assoc. Water Pollut. Res.
24. **Weber, W. J., Jr., M. Pirabazari, and G. L. Melson.** 1978. Biological growth on activated carbon: an investigation by SEM. Environ. Sci. Technol. **12**:817–819.
25. **Weise, W., and G. Rheinheimer.** 1978. Scanning electron microscopy and epifluorescence investigation of bacterial colonization of marine sand sediments. Microb. Ecol. **4**:175–188.
26. **Zobell, C. E.** 1943. The effect of solid surfaces upon bacterial activity. J. Bacteriol. **46**:39–56.
27. **Zobell, C. E., and E. C. Allen.** 1935. The significance of marine bacteria in the fouling of submerged surfaces. J. Bacteriol. **29**:239–251.