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Alexander V. Pimenov[a]; Alexander G. Mitilineos[a]; Galina I. Pendinen[a]; Vladimir E. Chernov[a]; Alexander I. Lieberman[a]; Joseph L. Shmidt[b]; Huk Y. Cheh[c]
[a] Division of Electrophor, Inc, St. Petersburg, Russia [b] Electrophor, Inc, Dobbs Ferry, NY, U.S.A. [c] Duracell, Inc., Bethel, CT, U.S.A.

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THE ADSORPTION AND DEACTIVATION OF MICROORGANISMS BY ACTIVATED CARBON FIBER

Alexander V. Pimenov,[1] Alexander G. Mitilineos,[1] Galina I. Pendinen,[1] Vladimir E. Chernov,[1] Alexander I. Lieberman,[1] Joseph L. Shmidt,[2] and Huk Y. Cheh[3,*]

[1]Aquaphor Corporation, Division of Electrophor, Inc, St. Petersburg, Russia
[2]Electrophor, Inc, Dobbs Ferry, NY 10522, USA
[3]Department of Chemical Engineering and Applied Chemistry, Columbia University, New York, NY 10027, USA

ABSTRACT

The adsorption and deactivation of microorganisms by a new activated carbon fiber (Aqualen®) were investigated. Tests were conducted with *Escherichia coli*, *Agrobacterium tumefaciens,* and *Saccharomyces cerevisiae*. The esterase metabolic activities of the adsorbed microorganisms were measured and were found to diminish rapidly and cease completely in less than 24 hours. An adsorption column packed with Aqualen® was tested for bacteriostatic effects. No microbial growth was detected. An explanation of the microbial adsorption and deactivation is offered.

*Corresponding author. Current address: Duracell, Inc., Berkshire Corporate Park, Bethel, CT 06801, USA. Fax: 203-731-6556.

INTRODUCTION

Industrial applications of activated carbon adsorption are hindered by microbial contamination (1). The adhesion of microorganisms onto different solid materials and the influence of interfaces on microbial activities have been widely investigated (2,3). Solid surfaces influence microbial activity and lead to increased or decreased metabolic activity depending on the microbe, liquid-solid interface, and other environmental parameters.

A majority of microbes are inclined to attach to solid surfaces. The colonization of a solid surface by bacteria begins with the transport of microbes onto the surface. The transport occurs by diffusion, by electrophoretic motion toward the charged interface, by active motion of a motile bacterium, and by convective flow of the surrounding fluid. In active motion, a bacterium can encounter the surface directly with its pili or flagella.

The next step of bacteria colonization is the initial adhesion, which can be either reversible or irreversible, to the solid surface. Reversible adhesion occurs when bacterium can be removed from the surface by mild shear or by the bacterium's own mobility. The adhesion depends on the surface charges, hydrophilicity, and unevenness of the solid and the bacterium. It also depends on the physiological conditions of the surrounding media, such as ionic strength and pH. The initial adhesion can be assisted by contact with the pili or flagella that extend from the bacterium outer membrane. Pili are thin long polysaccharide polymers or protein aggregates that extend from the cell surface by as much as several microns or more (3,4).

The initial adhesion is followed by firm attachment. The adhered bacterium excretes surface-active compounds and forms special cell-surface structures, such as fibrils. A combination of the specific surface-active compounds and polymeric cell-surface structures strengthens the links between the bacterium and the solid surface. The attachment can take place in minutes or hours. It is strongly influenced by the presence of surface-active compounds (2).

The firm attachment is usually followed by surface colonization, which is characterized by growth and division of attached cells. When newly formed cells remain attached to the solid surface and to one another, microcolonies form. This is followed by the bacterial excretion of large quantities of lipopolysaccharides, leading to a sticky, viscous biofilm on the solid surface.

A portion of the adhered cells may de-adhere and move back into the liquid medium. The de-adhesion is influenced by the nature of the secreted surface-active compounds (2).

An increased phenol conversion by the microbes adsorbed on the granular activated carbon and the microbial activity of the microbes adsorbed on ion exchange resins have been reported (5,6). However, the effect of adhesion on the bacterial metabolism is uncertain. Van Loosdrecht et al. (3) stated, "There is no

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conclusive evidence that adhesion directly influences bacterial metabolism, in the sense that the bacteria undergo a structural change due to adhesion."

This paper describes our investigation on the adsorption and deactivation of different microorganisms including *Escherichia coli*, *Agrobacterium tumefaciens,* and *Saccharomyces cerevisiae* (yeast) onto Aqualen® activated carbon fiber. *E coli* was chosen because it is very often encountered in environmental applications. *A tumefaciens* and *S cerevisiae* were chosen because they are easily observed and photographed under the optical microscope.

EXPERIMENTAL AND RESULTS

Activated Carbon Fiber: Aqualen®

A new activated carbon fiber, Aqualen®, was developed and manufactured by the Aquaphor Corp (7,8). Aqualen® was produced by carbonizing and activating rayon fiber. Figure 1 shows an electron micrograph of an Aqualen® fiber. The outer surface of Aqualen® is mostly hydrophobic with a large number of meso- and macropores and crevices. The small diameter of the Aqualen® fiber provides a large surface area per unit weight (approximately 5000 cm^2/g) and is sufficient for a monolayer adsorption of approximately 10^{11} *E coli* cells per gram of fiber.

Physicochemical properties and the adsorption capacity of Aqualen® for a number of organic compounds, lead, and select proteins are shown in Table 1 (7). Table 1 shows that Aqualen® has a large adsorption capacity toward different organic compounds, especially the aromatics and enzymes. It also has significant cation- and anion-exchange capacities. Furthermore, more than one-half of the

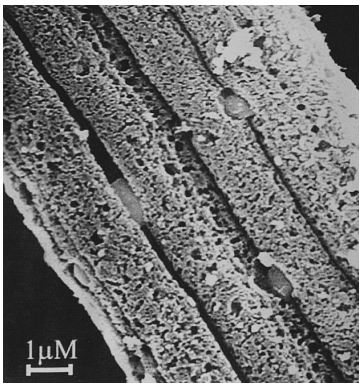

Figure 1. Electron micrograph of Aqualen® activated carbon fiber.

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Table 1. Physicochemical Properties and Adsorption Capacity of Aqualen® for Organic and Inorganic Compounds

Fiber diameter (μm)	6–10
Pore volume (cm^3/g)	0.5
Anion exchange capacity (mEq/g)	0.45
Cation exchange capacity (mEq/g)	0.75
Adsorption Capacity (mg/g)	
Lead	12.5
Phenol[a]	260[a] (160)[b]
Methylene Blue[a]	450[a] (250)[b]
Noradrenaline (M.W. 169)[c]	320
Hydrocortisone (M.W. 360) c	350
Vitamin B12 (M.W. 1350)[c]	32
Ribonuclease (M.W. 13 kd)[c]	170
Acetylcholinesterase (M.W. 360 kd)[c]	240

[a] One gram of Aqualen® was added to 450 mL of the model solution. The contact time was 12 hours under continuous stirring. The resulting solution was filtered through filter paper. The concentration was measured spectrophotometrically with a CF-46 spectrophotometer (LOMO Corp, St. Petersburg, Russia).
[b] One gram of Aqualen® was added to 450 mL of the model solution. The contact time was 120 seconds under continuous stirring. Other experimental conditions were the same as for phenol and methylene blue addition.
[c] One hundred milligrams of Aqualen® was added to 10 mL of the model solution. Other experimental conditions were the same as for phenol and methylene blue addition.

adsorption capacity of Aqualen® can be achieved in a matter of minutes, as compared to hours and days for the powdered and granular activated carbons (7). Also, the ion exchange capacity of Aqualen® is significantly higher than that for powdered and granular activated carbons (8).

Adsorption Properties of Aqualen® for Bacteria

The adsorption properties of Aqualen® for *E coli* are shown in Table 2. Aqualen® (0.1 to 2.0-mm loose fibers), distilled sterile water (pH 6.8, less than 0.001 mmol/L ionic strength), *E coli* (strain K12, Russian Culture Collection), *A tumefaciens* (strain 166, Russian Culture Collection), and *S cerevisiae* (strain

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P197/2D, Russian Culture Collection) were used in all experiments. One gram of Aqualen® was added to 450 mL of *E coli* suspension in a 500-mL glass beaker and was continuously stirred. The suspension was kept at 22°C. The adsorption equilibrium was reached in less than 10 minutes in all experiments. Then, the liquid was filtered and the filtrate was centrifuged. The *E coli* index (viable cells/L) was determined through the use of a standard dilution and agar plate sieving technique (9).

Bacteriostatic properties of Aqualen® were tested by cutting Aqualen® fibers 5 to 10 mm long and packing 13 grams of cut Aqualen® into a cylindrical polypropylene column of 50-mm diameter and 100 mL. Eight hundred liters of *E coli* 500 index suspension was pumped through the column at 150 mL/min with a peristaltic pump (Cole-Parmer, Chicago, IL, USA). The *E coli* index of the filtrate was less than 3. Ten liters of water was then pumped through the column at 150 mL/min to wash off bacteria from the tubing and connectors. The *E coli* index of the filtrate was less than 3. Then the column was placed in a 37°C oven (Pyrometer Corp, St. Petersburg, Russia). After 48 hours, the column was removed from the oven and 1 L of sterile distilled water was pumped through it at 150 mL/min. The *E coli* index of the filtrate was less than 3.

Table 2 shows that Aqualen® has a large adsorption capacity for *E coli*.

Observation of Microorganism Adhesion onto Aqualen®

Interactions of the *E coli*, *A tumefaciens*, and *S cerevisiae* with Aqualen® were investigated in visible and polarized light through an Olympus AN2-NAS microscope with Nomarski optics (Olympus Optical, Tokyo, Japan). A drop of distilled sterilized water was placed onto a microscope slide (25 × 75 mm, S8902, Sigma-Aldrich, St. Louis, MO, USA). A few Aqualen® fibers less than 0.5 mm long were added to the water drop. A cover glass (22 × 22 mm, S9802, Sigma Chemical Co, USA) was carefully placed on top of the microscope slide so that a uniform layer of liquid was formed. The microbial suspension was introduced into the layer of liquid between the lower and the upper glass covers. Interactions of the microbes with the Aqualen® fibers were photographed and observed under 455X magnification.

Table 2. Concentration of *Escherichia coli* in Solution after 10-minute Contact with Aqualen®

Initial Cell Index (Viable Cells/L)	100	200	300	500	1000	5000	10 000
E coli Aqualen®	<3	<3	<3	17	30	560	1050

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Cells in continuous Brownian motion were observed. The cells continuously approached and distanced themselves from the fiber. When the cells came within a certain distance from the Aqualen® fiber, they began to move toward it. After the cells reached the fiber surface, the adherence process began until a firm contact between the cell and the fiber surface was reached. The maximum distance between a cell and a fiber when the cell began its movement toward the fiber varied from approximately 300 μm for the *S cerevisiae* yeast cells to approximately 100 μm for *A tumefaciens* and *E coli*. The terminus of *E coli* and *A tumefaciens* attached to the fiber. No preference for the specific area attachment was observed for the *S cerevisiae* cells. The adherence time depended on the initial distance between the cell and the fiber. For *A tumefaciens,* the adherence time was approximately 2 to 3 seconds when the initial distance was 1 to 2 bacteria lengths, 8 to 12 seconds for 3 to 5 bacteria lengths, and 25 seconds for 10 bacteria lengths. The average adherence time varied from approximately 15 seconds for the *S cerevisiae* cells to approximately 10 seconds for the *E coli* cells. *A tumefaciens* and *S cerevisiae* exhibited firm attachment to the fiber surface. The *E coli* cells also exhibited a firm contact between the narrow end of the cell and the fiber, but the other unattached end of the rod swiveled back and forth.

Metabolic Activities of Microorganisms Adsorbed on Aqualen®

The metabolic activity of the *E coli*, *A tumefaciens,* and *S cerevisiae* adsorbed on Aqualen® was investigated by measuring the organism esterase activity through the intracellular fluorescein produced from fluorescein diacetate via the esterase reaction. Presence of the esterase activity allows one to differentiate live from dead microorganisms (10).

Fluorescence was observed under ultraviolet polarized light through the Lumam-I microscope with a photodiode and a digital voltmeter (LOMO Corp, St. Petersburg, Russia). One hundred milligrams of fluorescein diacetate (Sigma Chemical Co.) was dissolved in 10 mL of acetone, and 0.2 mL of the solution was diluted with 100 mL of distilled water. An aliquot of 0.05 mL of the resulting solution was added to the Aqualen® that had microbes adsorbed on it. The results of the fluorescein analysis are shown in Figure 2, which shows the diminishing esterase activity of *E coli*, *A tumefaciens,* and *S cerevisiae* during the first 24 hours of attachment after adhesion.

Cell death rate can be estimated by:

$$X = X_0 \exp(-ke^{-E_a/RT}t) \quad (1)$$

where X is the cell weight at a given time; X_0 is the cell weight at time zero; k is the cell death constant; R is the universal gas constant; T is absolute temperature; and t is time. Typical activation energy E_a for cell death is approximately 60 kcal/mole (11).

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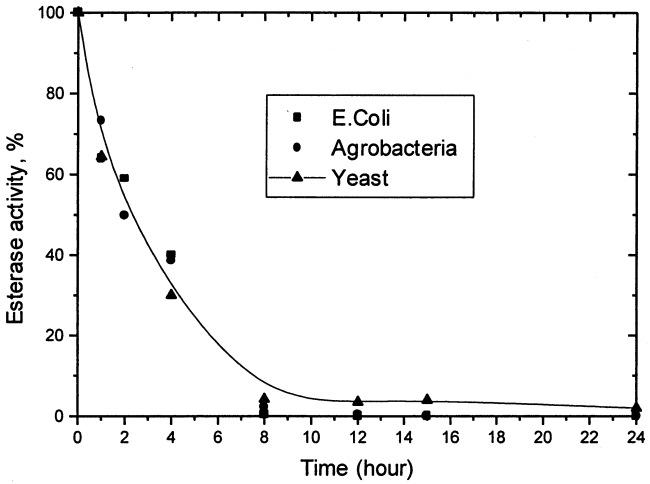

Figure 2. Comparison of the esterase activities of the *E coli*, *A tumefaciens,* and *S cerevisiae* cells adsorbed on Aqualen® during the first 24 hours and theoretical approximation of the death rate for the adsorbed microorganisms.

Figure 2 shows the diminishing esterase activity of *E coli*, *A tumefaciens,* and *S cerevisiae* cells during the first 24 hours of attachment. Both experimental results and theoretical approximations for the depleted esterase activity of the adsorbed cells are shown in Figure 2. A theoretical approximation of the death rate

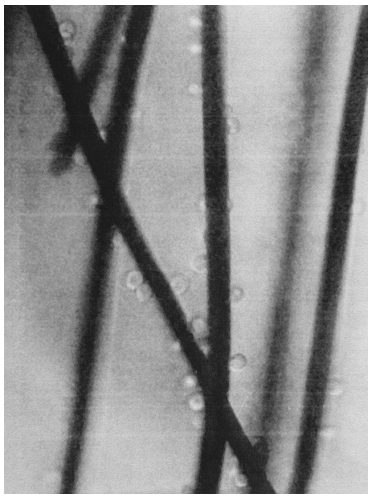

Figure 3. *S cerevisiae* adsorbed on Aqualen®.

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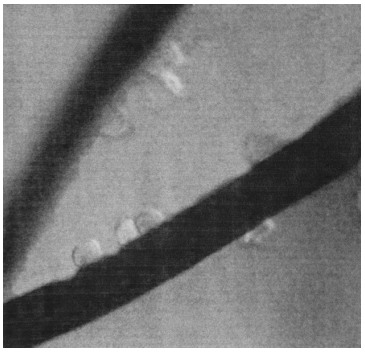

Figure 4. Deformation of *S cerevisiae* adsorbed on Aqualen® after 12 hours.

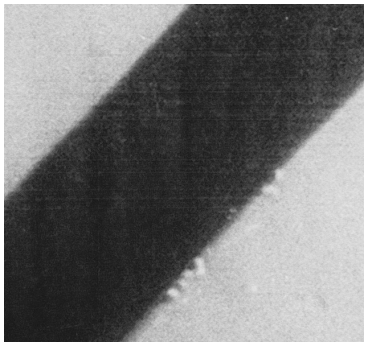

Figure 5. *A tumefaciens* adsorbed on Aqualen®.

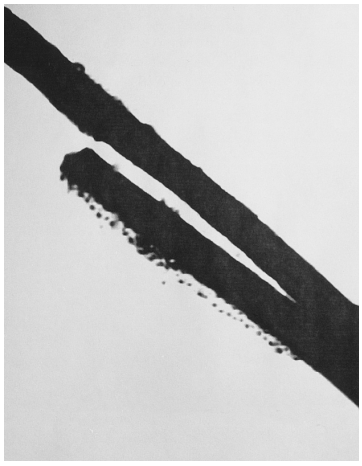

Figure 6. *E coli* adsorbed on Aqualen®.

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for the adsorbed microorganisms is shown as a solid line. By fitting Eq. (1) to the death rate of cells, we found k was 0.50 h^{-1} for all tested microorganisms. Because the experimental results for different types of microorganisms were very similar, the mechanism of depleting the esterase activity is probably the same for these cells.

Figures 3 to 6 are micrographs of different microorganisms adsorbed on Aqualen®. The yeast cells are the easiest to distinguish mainly because of their large o.d. (> 8 μm). Figure 3 shows that a significant contact area exists between the yeast cell and the fiber. Figure 4 shows that the adsorbed yeast cells undergo a significant deformation that leads to eventual membrane rupture. Figures 5 and 6 show that both *A tumefaciens* and *E coli* aggregate on the Aqualen® surface.

CONCLUSIONS

The exact mechanism of the microbial adsorption and deactivation is unknown. Microorganisms are one of the most versatile life forms in that they are able to adapt to a wide variety of environmental conditions. This adaptation is accomplished by the reorganization of macromolecular structure, the induction and/or repression of enzyme systems, and the reallocation of materials in cellular metabolic pools. Our results indicate that the adsorbed microorganism is "tricked" into an enhanced production and excretion of different extracellular compounds, including polysaccharides, lipids, fatty acids, peptides, and other biosurfactants, to promote adhesion and subsequent colonization. Activated carbon fiber adsorb these compounds away from the microbial membrane may starve the microbe at the same time by adsorbing nutrients, or a part of the lipid outer membrane may be adsorbed right onto the Aqualen® surface, enhancing the removal of biosurfactants and other compounds away from the microbe and weakening the structural integrity of the normally resilient and rigid microbial membrane.

Contacting a suspension of the microorganisms with the activated carbon fiber allows the removal and deactivation of microorganisms. These effects were achieved without the use of strong chemical and/or biological means. The esterase activity of the adsorbed *E coli*, *A tumefaciens,* and *S cerevisiae* ceased completely in less than 24 hours. The cell death rate was similar among these microorganisms. The experiments provided conclusive evidence that adhesion of microbes onto the activated carbon fiber directly influences bacterial metabolism because the bacteria undergo a structural change due to their adhesion on the activated carbon fiber.

Bacteriostatic testing of the adsorption bed comprised of activated carbon fiber showed strong bacteriostatic and bactericidal properties of Aqualen® toward *E coli.*

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