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Survival of Selected Bacterial Species in Sterilized Activated Carbon Filters and Biological Activated Carbon Filters

YVONNE ROLLINGER AND WOLFGANG DOTT*

Department of Hygiene, Technical University Berlin, 1000 Berlin 65, Federal Republic of Germany

The survival of selected hygienically relevant bacterial species in activated carbon (AC) filters on a bench scale was investigated. The results revealed that after inoculation of the test strains the previously sterilized AC adsorbed all bacteria (10^6 to 10^7). After a period of 6 to 13 days without countable bacteria in the effluent, the numbers of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Pseudomonas putida* increased up to 10^4 to 10^5 CFU/ml of effluent and 10^6 to 10^7 CFU/g of AC. When *Klebsiella pneumoniae* and *Streptococcus faecalis* were used, no growth in filters could be observed. The numbers of *E. coli*, *P. aeruginosa*, and *P. putida*, however, decreased immediately and showed no regrowth in nonsterile AC from a filter which had been continuously connected to running tap water for 2 months. Under these conditions an autochthonous microflora developed on the carbon surface which could be demonstrated by scanning electron microscopy and culturing methods (heterotrophic plate count). These bacteria reduced *E. coli*, *P. aeruginosa*, and *P. putida* densities in the effluent by a factor of more than 10^5 within 1 to 5 days. The hypothesis that antagonistic substances of the autochthonous microflora were responsible for the elimination of the artificial contamination could not be confirmed because less than 1% of the isolates of the autochthonous microflora were able to produce such substances as indicated by in vitro tests. Competition for limiting nutrients was thought to be the reason for the observed effects.

The use of granular activated carbon (GAC) in current water treatment processes as a further treatment step for the removal of organic contaminants has become a focus of attention owing to the increase in the number of microorganisms in its effluent. The growth of bacteria on activated carbon (AC) in water is an expected consequence of the favorable environment provided by this material. The adsorptive properties serve to enrich the organic substrate and can enhance the attachment of microorganisms.

Practical and laboratory experience has shown that it is impossible to operate a bacteria-free AC filter (1, 17, 22). Furthermore, it was found that the increased number of microorganisms in AC filters improved the removal capacity for organic compounds (7, 8, 10, 17). Therefore, the term biologically activated carbon (BAC) was introduced.

Our extensive examination of the bacteria found in BAC filters of different water plants revealed that special communities develop under working conditions. Most of the isolated species belonged to the genera *Pseudomonas*, *Flavobacterium*, *Acinetobacter*, and a so-called biotype I (12), a physiologically inactive gram-negative rod. Although there were high bacterial counts in the effluent of the BAC filters, we never found hygienically relevant species belonging to the family *Enterobacteriaceae*, *Pseudomonas aeruginosa*, or indicator organisms such as *Escherichia coli*, coliform bacteria, and fecal streptococci.

One explanation for this phenomenon was supposed to be the excessive densities of noncoliform bacteria which desensitize assay procedures (11, 13, 16, 18, 19). In laboratory tests conducted by Hutchinson et al. (16) suspensions of various antagonistic organisms were added to lactose tubes simultaneously with a suspension of *E. coli* and resulted in reduced coliform detection. But in these experiments the number of antagonists was about 100-fold higher than the coliform count. Similar problems were encountered in the

application of membrane filter techniques (11). These antagonistic relationships between the autochthonous bacteria and the indicator organisms should also play an important role in in situ situations, e.g., filter plants (3).

The purpose of this study was to examine the survival probability of selected species in GAC filters and furthermore to show the influence of the autochthonous microflora of the filter.

MATERIALS AND METHODS

AC filters. The AC filters consisted of glass tubes (8 in. [20 cm] high, 2 in. [5 cm] internal diameter) which were filled with 86.52 g of dry GAC (NORIT ROW 0.8 Supra). The upper part of the tube and the column were connected by a ground joint and could be removed for inoculation or taking carbon samples (Fig. 1). The filter system contained 195 ml of drinking water, which had been taken from the municipal water supply of Bonn. The filter effluent was connected to the inlet opening with silicone tubing. The hose pump circulated the water at a flow rate of 97 ml/min. The storage tank contained 500 ml of water, which was used to compensate the water loss during sampling. The sampling tap was dipped in 0.2% sodium azide solution to avoid retrograde contamination. The tests were performed at room temperature between 18 and 22°C. The whole filter system was sterilized by autoclaving.

BAC filters. BAC filters were obtained by connecting sterile columns continuously to running tap water of the municipal water system for 2 months and then installing them in the test set-up. The tap water contained no chlorine residuals.

Bacterial test strains. For the survival tests, the following strains were used: *E. coli* (ATCC 11229), *Klebsiella pneumoniae* (ATCC 4352), *P. aeruginosa* (ATCC 15442), *Pseudomonas putida* (DSM 50091), and *Streptococcus faecalis* subsp. *liquefaciens* (Hygiene-Institut Köln 1E1). All tests were performed in triplicate.

* Corresponding author.

Growth and inoculation of test strains. The test organisms were cultivated on nutrient agar medium (5) for 24 h at 25°C and suspended in 0.9% NaCl solution. The number of bacteria in the solution was determined as described below, and the suspension was diluted to obtain an initial bacterial concentration in the filter systems of 10^4 to 10^5 CFU/ml (inoculum, 10^6 to 10^7 CFU/13.5 ml). The accurate numerical data are given in Fig. 2 to 7.

Collection and treatment of water samples. Water samples (5 ml) were taken periodically over a period of 30 to 40 days. After sampling, the filter system was refilled with water from the reservoir. The water samples were collected in sterile test tubes and diluted in sterile 0.9% NaCl solution if necessary. Bacterial counts were determined by the spread plate technique and recorded as CFU per milliliter of sample. Pure cultures and bacteria of the filter communities were counted on nutrient agar.

E. coli was counted on Endo agar, and *P. aeruginosa* was counted on cetrimide agar. To identify *P. putida*, the colonies grown on nutrient agar were stamped on King B and gelatin agar (replica plating technique).

Collection of carbon samples. At the end of a test run about 1 g of AC was taken from the columns (depth of the filter bed, 1 in. [2.5 cm]) with a sterile spatula. The carbon was suspended in 4.5 ml of NaCl solution and shaken on a Whirli-Mix for 3 min. Bacterial counts in the supernatant were determined by the spread plate technique and calculated as CFU per gram of carbon. The studies were done on triplicate samples.

AC samples were further examined by scanning electron microscopy by the method of Tuschewitzki et al. (23).

Identification of bacterial filter community. Isolates of the filter effluent grown on the agar plates were physiologically characterized by the microtitration system as described previously (16). The details of the applied physiological test media and the identification procedure are given by Gehlen et al. (12).

Detection of inhibitory bacterial products. The isolates of the BAC community were screened for the production of substances inhibitory to the growth of *E. coli*, *P. aeruginosa*, or *P. putida*. Spread plates (5 days old) which had been used
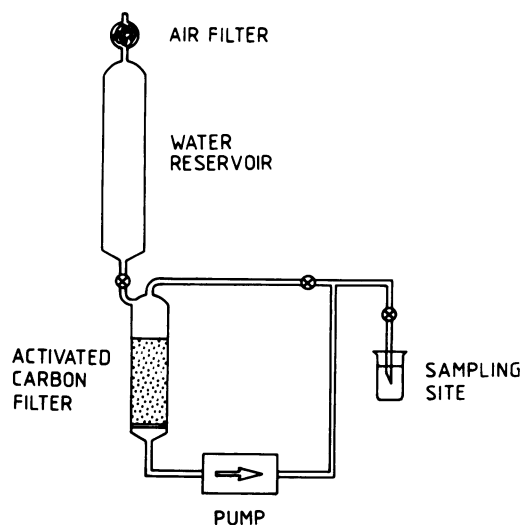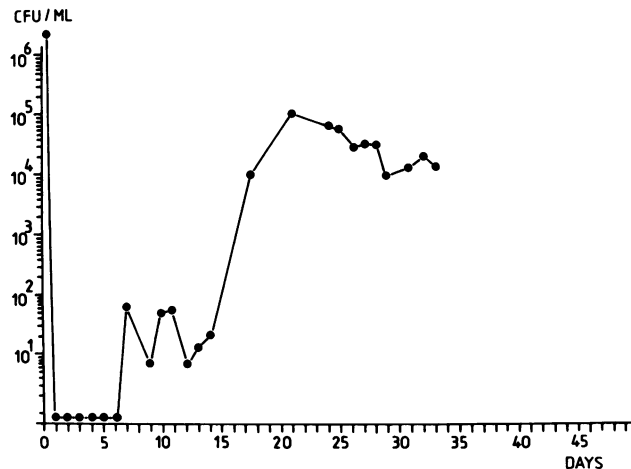

FIG. 2. Bacterial counts in the effluent of a GAC filter inoculated with *E. coli*. The first point of the curve was calculated from the inoculum: 4×10^8 CFU yielding an initial concentration of 2×10^6 CFU/ml. Water samples (5 ml) were taken over a period of 33 days. Colony counts were determined by the spread plate technique.

for counting bacteria were overlayered with the indicator organisms after the potential producers were killed with chloroform by the method of Means and Olson (21). Plates were incubated one further day at the optimum growth temperature of the indicator organism. Excretion of inhibitory substances was detected as an inhibition zone in the indicator lawn.

RESULTS

Survival and growth of pure bacterial cultures in sterilized AC filters. *E. coli*, *K. pneumoniae*, and *S. faecalis* as members of the sanitary indicator bacteria were selected for use in this study. *P. putida*, which had been frequently isolated from the effluent of GAC filters in water works, was selected because of its antagonism toward *E. coli* in mixed cultures (data not published), and *P. aeruginosa*, which could be found very rarely in the effluent of such filters, was chosen because of its taxonomic relation to *P. putida*. After inoculation of the sterile AC filters with different test strains (10^6 to 10^7 CFU), all bacteria were adsorbed within 6 h with the exception of *P. putida* which needed 4 days.

K. pneumoniae and *S. faecalis* did not colonize the filter system and died. During a period of 30 days, no bacteria could be found in the filter effluent or on the carbon surface as examined by scanning electron microscopy and culturing methods. In contrast *E. coli*, *P. aeruginosa*, and *P. putida* were able to colonize the filter system. Colony counts in the filter effluent showed a similar development independent of bacterial species (Fig. 2, 3, and 4). After a short period of adsorption, a period of 6 to 10 days followed during which no bacteria were found in the effluent. From days 6 to 20 the colony counts increased again to a constant level of 10^5 CFU/ml. On the carbon surface 3.8×10^6 *E. coli*, 2.0×10^7 *P. aeruginosa*, and 2.0×10^6 *P. putida* per g were found. The data indicate not only persistence but also growth of these three species.

Growth and characterization of autochthonous flora on AC. Two months after attachment of a sterile AC filter to the municipal water supply, a physiologically inactive, oligo-carbophilic population was established. The CFU level in the effluent was about 10^3 CFU/ml, and on the carbon 10^4
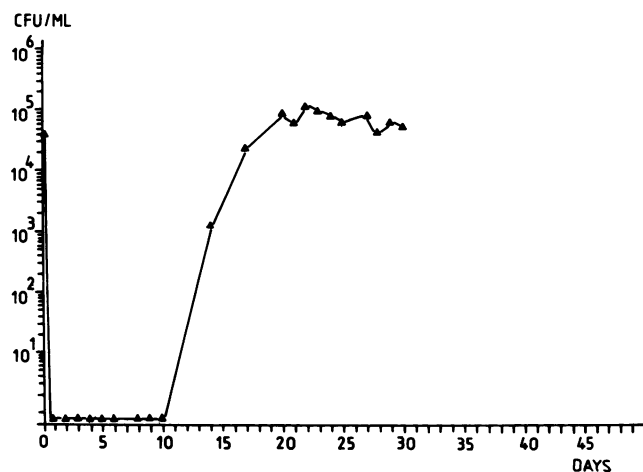

FIG. 1. Schematic diagram of the test equipment. Water was continuously circulated through the GAC column at a flow rate of 97 ml/min.

FIG. 3. Bacterial counts in the effluent of a GAC filter inoculated with *P. aeruginosa*. Inoculum, 8 × 10^6 CFU (see the legend to Fig. 2).

CFU/g were counted. Most isolates of the filter community (50%) were aerobic, gram-negative, oxidase-negative, catalase-positive, motile rods (0.5 to 0.8 by 2 to 3 μm) which had relatively restricted metabolic capacity, including the inability to utilize sugars and sugar alcohols, to hydrolase gelatin and starch, to decarboxylase amino acids, and to reduce nitrate or nitrite. They were grouped together and designated as biotype I (12), indicating their predominant occurrence in the aquatic environment (Table 1). Additional isolates belonged to the genera *Acinetobacter*, *Pseudomonas*, and *Enterobacter* and to the group of *Moraxella*-like bacteria.

If such a BAC filter was connected to the test equipment (Fig. 1), the bacterial counts increased to 10^5 CFU/ml of effluent and 10^6 CFU/g of carbon.

Isolates producing antagonistic substances. A total of 2,758 isolated bacterial colonies were examined with respect to the production of inhibiting substances against *E. coli*, *P. aeruginosa*, or *P. putida*. Less than 1% of the bacteria which had originated from the filter community were able to inhibit the growth of *E. coli* and *P. putida* in vitro (Table 2). The zones of inhibition were only about 1 mm in diameter. In neither of the test cases was *P. aeruginosa* seen to be sensitive.
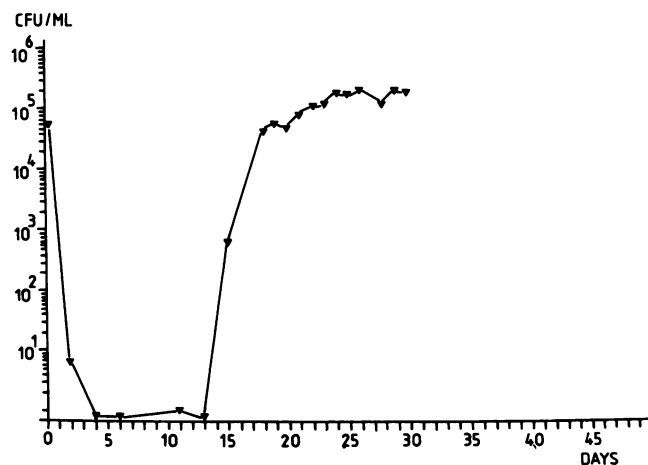

FIG. 4. Bacterial counts in the effluent of a GAC filter inoculated with *P. putida*. Inoculum, 2 × 10^7 CFU (see the legend to Fig. 2).

TABLE 1. Qualitative and quantitative composition of the bacterial flora in the effluent of a BAC column

Bacterial species	No. of isolates (%)
All	40 (100.0)
Biotype I	20 (50.0)
Acinetobacter sp.	5 (12.5)
Moraxella-like bacteria	4 (10.0)
Pseudomonas sp.	2 (5.0)
Enterobacter sp.	2 (5.0)
Unidentified bacteria	7 (17.5)

Survival of *E. coli*, *P. aeruginosa*, and *P. putida* on BAC. *E. coli*, *P. aeruginosa*, and *P. putida* failed to survive on BAC. After inoculation, the colony counts in the filter effluent dropped beyond the level of detection until the end of the examination period. The decline extended from 1 to 10 days depending on the bacterial strain used (Fig. 5, 6, and 7). Even carbon samples were negative for the three bacteria. On the other hand, colony counts of the filter population stayed at a constant level.

DISCUSSION

The results presented here demonstrate that *E. coli*, *P. aeruginosa*, and *P. putida* proliferated on sterile AC filters. Each of the bacterial species increased to 10^5 CFU/ml in the filter effluent and to 10^6 to 10^7 CFU/g of carbon. Further growth was not limited by the available surface area of the carbon since scanning electron microscopy indicated that the surface was only barely covered with bacterial cells. The nutrient concentration of the tap water in the closed filter system was probably the main limiting factor.

Camper et al. (3) used a quite similar filter model and demonstrated that pathogenic bacteria such as *Yersinia enterocolitica*, *Salmonella typhimurium*, and *E. coli* colonized and grew on granular activated carbon. The extent of colonization and the survival time of the pathogens was reduced in the presence of an autochthonous bacterial community.

In contrast to our results, Botzenhart and Kufferath (2) and Dickgiesser and Rittweger (4) demonstrated a decline in *E. coli* counts in sterile tap water. However, they did not supply nutrients in their tests, in contrast to our system which was refilled with fresh water after each sampling.

The increase of *P. aeruginosa* in sterile drinking water is well documented (2, 4). Botzenhart and Kufferath (2) noticed a 100-fold increase to 10^6 CFU/ml during 4 days, which is consistent with the present results. *P. aeruginosa* seems to use the substrates more efficiently than *E. coli* and *P. putida*, which results in the 10-fold-higher colony counts.

K. pneumoniae and *S. faecalis* did not survive in the low-nutrient filter system. Botzenhart and Kufferath (2), however, demonstrated an increase in *K. pneumoniae* in sterile tap water, and Dickgiesser and Rittweger (4) found no change in *S. faecalis* counts.

TABLE 2. Inhibitory activity of the filter community

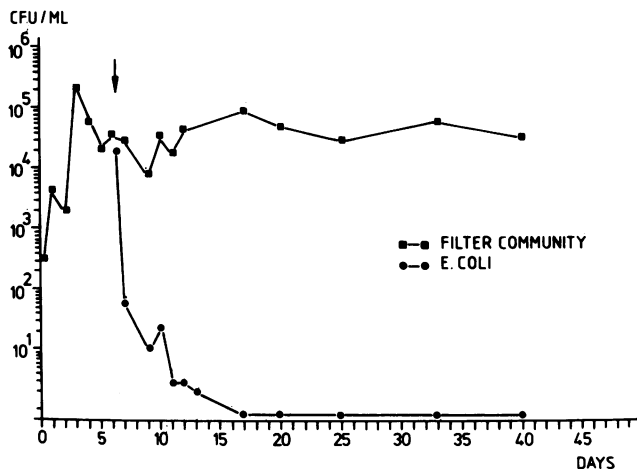| Indicator organism	No. of colonies examined	No. of colonies with inhibition zone	% Colonies with inhibition zone
E. coli	1,582	11	0.7
P. aeruginosa	501	0	0
P. putida	675	1	0.15

FIG. 5. Stable heterotrophic plate count bacterial population in the effluent of a BAC filter. The arrow indicates the inoculation with E. coli (4 × 10^6 CFU) (see the legend to Fig. 2).

FIG. 7. Stable heterotrophic plate count bacterial population in the effluent of a BAC filter. The arrows indicate two subsequent inoculations with P. putida (4 × 10^6 CFU and 8 × 10^6 CFU, respectively) (see the legend to Fig. 2).

E. coli, P. aeruginosa, and P. putida, which grew on sterile AC filters, failed to proliferate on BAC filters. These results indicate that not only the medium (water), pH, temperature, and other physical factors are responsible for the survival of indicator bacteria but also the presence or absence of other bacteria of the biotope which can act as antagonists. Genera which have been implicated as being antagonistic toward coliform bacteria include Bacillus, Pseudomonas, Flavobacterium, Moraxella, Arthrobacter, Actinomyces, Sarcina, Micrococcus, and yeasts (9, 14–16, 18–21, 24).

Antagonism may be attributed to nutritional competition, injury, or chemical antibiosis. Means and Olson (21), for example, showed that 22% of the isolates which were screened from a chlorinated water system were able to produce bacteriocinlike substances inhibitory to the growth of at least one of three selected coliforms. On the other hand, LeChevallier and McFeters (18, 19), who investigated the interaction between standard plate count bacteria in drinking water and coliforms, could not detect any bacteriocins or antibiotics. Consistent with the present results, they noticed
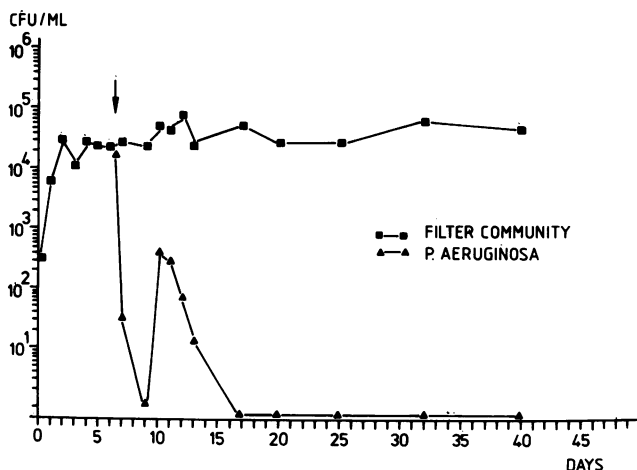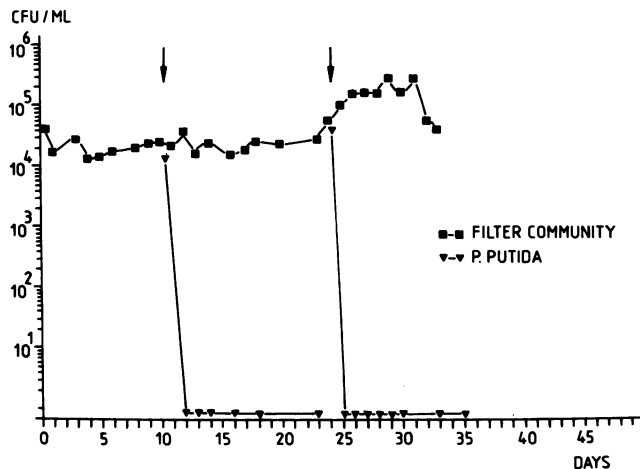
an increase in the noncoliform organisms of 10^4 to 10^5 CFU/ml of tap water and a decrease in coliform organisms of more than a factor of 10^3 within 8 days. These authors indicated that coliforms have higher growth requirements than the heterotrophic plate count bacteria, and competition for nutrients was hypothesized to be responsible for coliform suppression. On BAC, suppression of the test organisms is probably based on nutritional competition, too, in which the naturally occurring filter community is better adapted to the use of the low nutrient supply of the water. Since the present in vitro tests showed that less than 1% of the bacteria isolated from the filter effluent produced substances inhibitory to the growth of the test strains, ammensalism does not seem to play an important role in their suppression. Furthermore, dilution by the tap water and adsorption by the carbon lessen the influence of antagonistic substances.

Data presented in this report indicate that the survival and growth of indicator organisms and enteric pathogens on BAC filters used during water treatment is not probable and that the autochthonous microbial filter community plays an important role in the elimination of these contaminants.

LITERATURE CITED

1. Bernhardt, H. 1970. Entkeimung von Aktivkohlefiltern durch Erwärmung. Schriften. Ver. Wasser. Boden. Lufthyg. 31: 165–169.
2. Botzenhart, K., and R. Kufferath. 1976. Über die Vermehrung verschiedener Enterobacteriaceae sowie Pseudomonas aeruginosa and Alcaligenes spec. in destilliertem Wasser, entionisiertem Wasser, Leitungswasser und Mineralsalzlösung. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe B 163:470–485.
3. Camper, A. K., M. W. LeChevallier, S. C. Broadaway, and G. A. McFeters. 1985. Growth and persistence of pathogens on granular activated carbon filters. Appl. Environ. Microbiol. 50:1378–1382.
4. Dickgiesser, N., and F. Rittweger. 1979. Untersuchung über das Verhalten von grampositiven Kokken und gramnegativen

FIG. 6. Stable heterotrophic plate count bacterial population in the effluent of a BAC filter. The arrow indicates the inoculation with P. aeruginosa (4 × 10^6 CFU) (see the legend to Fig. 2).

Bakterien in Aqua bidest und Leitungswasser bei verschiedenen Temperaturen und unterschiedlichen Ausgangskeimzahlen. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe B 169:308–319.

5. **Dott, W.** 1980. Qualitative and quantitative examination of bacteria found in aquatic habitats. I. Communication: selective recording of bacteria during isolation on different solid media. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe B 170:93–98.

6. **Dott, W., and E. Thofern.** 1980. Qualitative and quantitative examination of bacteria found in aquatic habitats. II. Communication: application of miniaturized multitest systems for identification and biochemical typing of bacteria using a multi-point method. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe B 170:99–107.

7. **Eberhardt, M.** 1971. Untersuchungen zur optimalen Kombination von Adsorption, Filtration und biologischer Reinigung. Veröff. Bereich Lehrstuhl Wasserchem. Univ. Karlsruhe 5: 358–379.

8. **Eberhardt, M., S. Madsen, and H. Sontheimer.** 1975. Untersuchungen zur Verwendung biologisch arbeitender Aktivkohlefilter bei der Trinkwasseraufbereitung. Gas Wasserfach Wasser Abwasser 116:245–247.

9. **Fischer, G.** 1950. The antagonistic effect of aerobic sporulating bacteria on coli-aerogenes group. Z. Immunitätsforsch. Exp. Ther. 107:16–22.

10. **Fischer, K., and D. Bardtke.** 1981. Auswirkungen der biologischen Besiedlung auf die Adsorption von Aktivkohle und auf deren Anwendung in der weitergehenden Abwasserreinigung. Gas Wasserfach Wasser Abwasser 122:58–64.

11. **Franzblau, S. G., B. J. Hinnebusch, L. M. Kelley, and N. A. Sinclair.** 1984. Effect of noncoliforms on coliform detection in potable groundwater: improved recovery with an anaerobic membrane filter technique. Appl. Environ. Microbiol. 48:142–148.

12. **Gehlen, M., H. J. Trampisch, and W. Dott.** 1985. Physiological characterization of heterotrophic bacterial communities from selected aquatic environments. Microb. Ecol. 11:205–219.

13. **Geldreich, E. E., H. D. Nash, D. J. Reasoner, and R. H. Taylor.** 1972. The necessity of controlling bacterial populations in potable waters: community water supply. J. Am. Water Works Assoc. 64:596–602.

14. **Herson, D. S.** 1980. Identification of coliform antagonists, p. 153–160. *In* Proceedings of the 8th American Water Works Association Water Quality Technology Conference. American Water Works Association, Denver.

15. **Herson, D. S., and H. T. Victoreen.** 1980. Hindrance of coliform recovery by turbidity and non-coliforms. EPA 600/2-80-097. U.S. Environmental Protection Agency, Washington, D.C.

16. **Hutchinson, D., R. H. Weaver, and M. Scherago.** 1943. The incidence and significance of microorganisms antagonistic to *Escherichia coli* in water. J. Bacteriol. 45:29–33.

17. **Klotz, M., P. Werner, and R. Schweisfurth.** 1976. Mikrobiologische Untersuchungen zur Trinkwasseraufbereitung. Forschung und Entwicklung in der Wasser werkspraxis. Wiss. Ber. Unter. Planungen Stadtwerke Wiesbaden AG. ESWE Band 3:75–82.

18. **LeChevallier, M. W., and G. A. McFeters.** 1985. Interactions between heterotrophic plate count bacteria and coliform organisms. Appl. Environ. Microbiol. 49:1338–1341.

19. **LeChevallier, M. W., and G. A. McFeters.** 1985. Enumeration of injured coliforms in drinking water. J. Am. Water Works Assoc. 77:81–87.

20. **McCabe, L. J., I. M. Symons, R. D. Lee, and G. G. Robeck.** 1970. Survey of community water supply systems. J. Am. Water Works Assoc. 62:670–687.

21. **Means, E. G., and B. H. Olson.** 1981. Coliform inhibition by bacteriocin-like substances in drinking water distribution systems. Appl. Environ. Microbiol. 42:506–512.

22. **Thielemann, H.** 1969. Die Beseitigung geruchs- und geschmacksintensiver Substanzen im Trinkwasser (Uferfiltrat) der Stadt Meseburg—eine hygienische Forderung. Z. Gesamte Hyg. 15:180–183.

23. **Tuschewitzki, G. J., P. Werner, and W. Dott.** 1983. Besiedlung und mineralische Ablagerungen auf Materialien zur Trinkwasseraufbereitung. Gas Wasserfach Wasser Abwasser 124:521–526.

24. **Weaver, R. H., and T. Boiter.** 1951. Antibiotic-producing species of bacillus from well water. Trans. K. Acad. Sci. 13:183–188.