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Inability of Microorganisms To Degrade Cellulose Acetate Reverse-Osmosis Membranes†

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Operational cellulose acetate reverse-osmosis membranes were examined for evidence of biological degradation. Numerous fungi and bacteria were isolated by direct and enrichment techniques. When tested, most of the fungi were active cellulose degraders, but none of the bacteria were. Neither fungi nor bacteria were able to degrade cellulose acetate membrane in vitro, although many fungi were able to degrade cellulose acetate membrane after it had been deacetylated. Organisms did not significantly degrade powdered cellulose acetate in pure or mixed cultures as measured by reduction in acetyl content or intrinsic viscosity or production of reducing sugars. Organisms did not affect the performance of cellulose triacetate fibers when incubated with them. The inability of the organisms to degrade cellulose acetate was attributed to the high degree of acetate substitution of the cellulose polymer. The rate of salt rejection decline was strongly correlated with chlorination of feed water and inversely with densities of microorganisms. These data suggest that microbial degradation of operational cellulose acetate reverse-osmosis membranes is unlikely.

The premature failure of cellulose acetate (CA) reverse-osmosis membranes, as manifested by a loss of salt rejection capacity and increase in water flux, has often been attributed to microbial degradation (6, 7, 12, 15, 16). CA of low to moderate degrees of acetate substitution has been shown to be susceptible to microbial degradation (6, 7, 22). However, episodes of microbe-induced membrane failure in operational reverse-osmosis modules have been poorly documented, and in many cases it is not clear whether abiotic factors were involved. Little is known about the microbial flora of operational reverse-osmosis membranes and whether microorganisms are in fact able to degrade the highly substituted CA presently used in modern membranes.

According to the *Reverse Osmosis Technical Manual* (23), cellulolytic microorganisms are capable of degrading CA membranes, resulting in marked declines in salt rejection capacity in less than 12 h. But membrane performance declines, whether acute or long term, can result from a variety of abiotic causes (23). If microorganisms are involved in membrane failure, (i) a cellulolytic microflora should be present that can utilize CA as a carbon and energy source, (ii) physical damage to the membrane should be attributable to microbial presence, and (iii) microbial occurrence and membrane failure should be positively correlated.

The ability to degrade cellulose is widespread among the higher fungi (20) and some groups of bacteria (24), but the ability of microorganisms to degrade substituted cellulose such as CA is less well known. Reese (22) reported that cellulose derivatives became more resistant to enzymatic attack with increasing degrees of acetate substitution. Generally one substitution per anhydroglucose unit (degree of substitution [DS] equal to 1.0) is thought to be sufficient to confer protection from enzymatic attack (8). However, the DS value represents an average, and a DS higher than 1.0 is required to insure at least one substitution per anhydroglucose unit.

Reese (22) found that a number of cellulolytic fungi were able to completely degrade a water-soluble CA with a DS of 0.76 through the action of cellobiose octaacetase. However, this enzyme was apparently only able to deacetylate short oligomers of CA. Organisms possessing cellobiose octaacetase were not able to degrade cellulose triacetate (CTA) with a DS of 2.86 and were not tested for their ability to degrade CA of intermediate degrees of substitution. Cantor and Mechalas (6) demonstrated that some bacteria were able to degrade CA membranes on nutrient

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agar, but the DS of the constituent CA was not stated, nor was the membrane present as the sole carbon source.

CAs of modern reverse-osmosis membranes generally have higher DS values than earlier membranes. Early membranes were commonly constructed of a single CA, usually CA 398-3 (DS of 2.45), whereas current membranes are generally a blend of CA (DS of 2.4 to 2.5) and CTA (DS of 2.86) according to Saltonstall (25) and King et al. (14). Some membranes are also constructed solely from CTA in a hollow configuration. Because of their high DS, it is doubtful that presently used CA membranes are susceptible to microbial attack. However, the ability of microorganisms to attack modern membranes is uncertain.

This study examines the ability of bacteria and fungi isolated from operational CA reverse-osmosis membranes to degrade CA and modern CA and CTA membranes.

MATERIALS AND METHODS

Membrane description. Microbial samples were taken from two types of CA reverse-osmosis membranes at the Roswell Test Facility of the Office of Water Research and Technology, U.S. Department of the Interior, Roswell, N.M. Spiral wound membranes consisted of a rectangular envelope of two separate membranes laminated to a dacron backing material. Manufacturers of the spiral wound modules included Purtech, Salt Lake City, Utah (membrane from Envirogenics Systems Co., El Monte, Calif.); Hydranautic Water Systems, Santa Barbara, Calif.; and Fluid Systems Div., Universal Oil Products, San Diego, Calif. Hollow fiber modules (Dow Chemical Co., Walnut Creek, Calif.) consisted of parallel hollow fibers embedded in resin at both ends.

The membranes in this study were composed of CA, CTA, or mixtures of CA and CTA with various degrees of acetate content. The Dow (15.2 by 121.9 cm, CTA of 43.5 to 43.7% [wt/wt] acetyl), Hydranautics (21.6 by 101.6 cm, 50:50 blend of CA of 39.8% [wt/wt] acetyl and CTA of 43.5% [wt/wt] acetyl), and Fluid Systems (30.5 by 152.4 cm, CA of 40.0% [wt/wt] acetyl) modules were commercial units tested at the Roswell Test Facility. The Purtech (6.4 by 30.5 cm, 50:50 blend of CA of 39.8% [wt/wt] acetyl and CTA of 43.2% [wt/wt] acetyl) modules were small sacrificial units placed in 10 locations within the plant to sample different areas for microbial contamination. Feed water to most modules was a total dissolved solids blend (3,000 mg/liter) of Roswell city water and brackish well water adjusted to a target pH of 5.5 to 5.8 with H_2SO_4. Other feed water pretreatments included a combination of the following: filtration through a manganese green sand bed, an activated carbon bed, or a mixed bed of both manganese green sand and activated carbon and chemical treatment with $(NaPO_3)_6$, NaOCl, or $Na_2S_2O_3$.

Microbial enumeration and isolation. After various periods of operation (Table 1), individual filter modules were opened under aseptic conditions to expose the membrane surface. For the Dow module, small bundles of fibers (1 to 2 cm long) were removed from the outside edge at the feed and product ends. Bundles of fibers were also removed from the same locations near the central core of the module. For bacterial enumeration, fibers from each of the sample points were placed in sterile petri dishes, shaken to separate the fibers, covered with molten (≅45°C) nutrient agar (BBL Microbiology Systems, Cockeysville, Md.), and swirled to further separate the fibers and disseminate any organisms present. The bacteria plates were incubated at 30°C, and microbial counts were expressed as CFU per square centimeter of membrane surface.

Fungi were isolated from the hollow fiber membranes by placing small bundles of fibers on plates of half-strength cornmeal agar (Difco Laboratories, Detroit, Mich.), 2% malt extract agar (Difco), and CA agar (6). The fungal media contained 25 mg each of penicillin and streptomycin per liter to inhibit bacterial growth. Plates were incubated for 10 to 15 days at 25°C.

Bacteria were enumerated from spiral wound membranes by swabbing 2-cm^2 areas of feed and product surfaces with sterile cotton. Swabs from the feed side of the leaves were taken directly from the membrane surface, and swabs from the product side were taken from the dacron backing. The swabs were placed in 2 ml of pH 7 sterile phosphate buffer and agitated on a Vortex mixer, and 1-ml samples were used for nutrient agar pour plates. Colonies developing at 25°C were

TABLE 1. Operational parameters and performance statistics of reverse-osmosis modules

Module	Total h of operation	Operating pressure (MPa)	Salt rejection			Mean feed water residual Cl (mg/liter)	Days until culture[a]
			Starting (%)	Ending (%)	Decline (%/day)		
Dow I	2,723	1.72	96.10	57.62	-1.74×10^{-1}	0.67	1
Dow IIa	7,872	5.17	95.38	94.63	-3.09×10^{-3}	0.00	ND
Dow IIa[b]	1,656	1.72	95.53	92.34	-3.03×10^{-2}	0.90	ND
Dow IIb	4,533	1.72	96.49	95.71	-4.01×10^{-3}	0.00	1
Dow III	5,874	1.72	93.75	90.82	-1.10×10^{-2}	0.62	11
Hydranautics	898	3.03	95.05	77.93	-3.30×10^{-1}	0.86	14
Fluid Systems	338	2.96	92.79	88.52	-6.24×10^{-1}	1.12	90

[a] Number of days from unit shutdown until cultures were taken. ND, Microbial cultures or salt rejection decline not determined.

[b] Unit was shut down for 1 day for the attachment of chlorination equipment and was promptly restarted.

counted and expressed as CFU per square centimeter. Fungi were isolated from the spiral wound membranes by placing membrane pieces (approximately 1 cm^2) feed side down on agar plates as described above for the hollow-fiber membranes.

Enrichment cultures were also made from both membrane types for bacterial and fungal isolations. The media were as described above. Membrane samples were also enriched in liquid culture with the CA medium of Cantor and Mechalas (6), basal salts-yeast extract medium (BSY) of Sinclair (27), basal medium (BM) plus 0.05% yeast extract (Difco), BM plus 0.5% reprecipitated CA (CA 398-3; Eastman Chemical Co., Rochester, N.Y.), and Hutchinson (HUT) medium as described by Rodina (24) plus 0.5% reprecipitated CA (Eastman CA 398-3). The BM contained the following (per liter of deionized water): KH_2PO_4, 1.0 g; $(NH_4)_2SO_4$, 0.54 g; KCl, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $CaCl_2 \cdot H_2O$, 0.13 g; $FeCl_2$, 50 mg; $MnSO_4 \cdot 4H_2O$, 50 mg; $ZnSO_4$, 44 mg; $CuSO_4 \cdot 5H_2O$, 10 mg; $NaMoO_4 \cdot 2H_2O$, 5 mg; $Na_2B_4O_7$, 1 mg; nicotinic acid, 0.3 mg; folic acid, 0.3 mg; thiamine, 0.03 mg. All media were prepared in either distilled water or facility water (FW). The FW was used to simulate the water conditions within the Roswell Test Facility and included the addition of 0.01% EDTA to minimize precipitate formation. If a solid medium was required, 2% purified agar (Difco) was added. Peptone and yeast extract, added as amendments to some enrichment cultures, were also from Difco. The enrichment flasks were incubated at 25°C with daily swirling by hand for at least 30 days, and samples from each flask were transferred to nutrient agar plates (bacteria) and cornmeal and malt agar plates (fungi) for isolation and identification.

Bacterial colonies from direct isolations, enumeration plates, and enrichment plates and flasks were subcultured until pure cultures were obtained. The isolates were screened on the basis of colony morphology on Trypticase soy agar (BBL), Gram stain, and cell morphology. Representative isolates were classified according to *Bergey's Manual of Determinative Bacteriology* (5) and the *CRC Handbook of Microbiology* (10) for a more detailed description of *Bacillus*.

Fungal colonies isolated from enrichment flasks were identified with the aid of dissecting and compound microscopes. Colonies of *Fusarium* were distinguished from *Cephalosporium*-type fungi (including *Phialophora*) by their faster growth rate (greater than 35 mm in 10 days) and the production of macroconidia (9). The frequency of occurrence of fungi in direct isolations is expressed as the number of occurrences per number of plates inoculated. Because more than one fungal species appeared on a single plate, frequencies of individual fungi do not necessarily total to the frequency of plates with fungi present. A single genus was counted only once per plate.

Specimens from CA membranes were examined for membrane degradation with dissecting and compound microscopes as well as by scanning electron microscopy at the Biology Department, New Mexico State University, and at the Dow Chemical Co. facilities.

Two cultures of *Cellulomonas* were obtained from the American Type Culture Collection (ATCC 21399 and 21681). Two isolates of bacteria from the Yuma Desalting Test Facility that were capable of filter paper degradation (S226 and S209) were provided by N. A. Sinclair, University of Arizona. N. A. Sinclair also supplied a CA membrane primary enrichment culture (C257 CNL) of waters from the Mohawk-Welton Canal, Yuma, Ariz., in which degradation of a CA membrane strip was evident.

Cellulose, CA, and CTA degradation studies. Powdered CA 398-3, CA 394-45, and CA 400-25 were obtained from Eastman. The first number of the CA type gives the acetyl content, and the second number indicates the American Society of Testing Materials viscosity rating. The acetyl contents of the CA used in this study, as determined by E. H. Hill of Tennessee Eastman Co., Kingsport, Tenn., were 40.00, 39.55, and 39.45% (wt/wt), respectively. The DSs of the CAs, as calculated with the formula of Tanghe et al. (30), were 2.49, 2.43, and 2.42, respectively. A reverse-osmosis membrane constructed from a blend of equal weights of CA 398-3 and CA 435-85S in a sheet configuration was obtained from Envirogenics Systems Co., El Monte, Calif. The membrane was peeled from the dacron backing material before use. Reverse-osmosis membrane constructed from CTA (43.6% acetyl) in a hollow-fiber configuration was obtained from Dow Chemical Co.

Several qualitative tests were used to evaluate the ability of organisms to degrade cellulose and cellulose derivatives. Fungi and bacteria were tested for the ability to clear a 0.25% (wt/vol) suspension of phosphoric acid-swollen cellulose in tubes of BM agar (21, 32). Depth of clearing was measured after 5 weeks. Fungi were also tested for the ability to clear powdered CA 398-3, phosphoric acid-treated blend membrane, and phosphoric acid-treated CTA hollow fibers with the same technique. Bacteria were also tested on plates of 0.5% (wt/vol) carboxymethyl cellulose (9M8F; Hercules, Inc., Wilmington, Del.) in BM agar (13).

Fungi and bacteria were tested for their ability to degrade strips of filter paper (1 by 7.5 cm) in tubes of HUT-FW. After 4 weeks of incubation, the bacteria were reinoculated and enriched with yeast extract (final concentration, 0.05%) to encourage growth. Fungi were tested for the ability to degrade strips of blend membrane in tubes of BM-FW and BSY-FW. Bacteria were tested on strips of blend membrane in HUT; as in the filter paper test, after 4 weeks, bacterial tubes were reinoculated and enriched with yeast extract. The degradation of filter paper and membrane strips was assessed visually; loss of structural integrity was evaluated by vigorously swirling the tubes on a Vortex mixer. One set of blend membrane strips in HUT-FW was sterilized with propylene oxide and inoculated with fungi. Fungi were also inoculated onto deacetylated blend membrane strips in BM-FW. The membrane was deacetylated by soaking in equal volumes of ethanol and ammonium hydroxide for 24 h to the point of acetone insolubility.

More sensitive quantitative tests were performed on powdered CAs inoculated with microorganisms under several different cultural conditions. These included the following.

For CA 400-25, fungi were cultured on 3.5 g of CA 400-25 in 100 ml of BM-FW for 9 weeks. Bacteria were cultured on 2.0 g of CA 400-25 in 100 ml of HUT-FW for 8 weeks.

For CA 394-45, fungi and bacteria were cultured on 1.5 g of CA 394-45 in 100 ml of BSY for 4 weeks.

TABLE 2. Bacterial densities of membranes by direct isolation

Module	Bacterial density (CFU/cm²) at sample locations:		
	Feed end	Middle	Product end
Dow I			
Interior[a]	0	0	0
Exterior[b]	0	0	0
Dow IIb			
Interior	800	1,200	1,400
Exterior	730	1,410	1,270
Dow III			
Interior	0	770	ND[c]
Exterior	0	500	ND
Hydranautics	ND/ND[d]	0/0[d]	ND/ND[d]

[a] Interior portion of fiber bundle.
[b] Exterior portion of fiber bundle.
[c] ND, Microbial cultures not determined.
[d] Feed side/product (backing) side.

For CA 398-3, enrichment cultures from operational reverse-osmosis modules were incubated for 16 weeks in 50 ml of BM with 1% CA 398-3.

Analyses on each CA consisted of the determination of acetyl content by the solution method (2), of intrinsic viscosity by the method of Tanghe et al. (31) with an Ostwald-Fenske viscometer, and of reducing sugar content in the cultural filtrate by the ultramicro TPTZ (tripridyl-s-triazine) method of Avigad (3). The microbial biomass on CA 400-25 was deemed minimal and was carried along with the sample for acetyl determination. Before determination of intrinsic viscosity, the sample was weighed, dissolved in acetone, and filtered through a Whatman GF/D filter, a high-flow, high-loading-capacity filter with an effective retention diameter of 2.7 μm. Changes in viscosity due to acetone evaporation during filtration were regarded as minimal as samples passed through filters rapidly (less than 2 s). With CA 394-45, considerable microbial biomass accumulated because of the presence of yeast extract in the medium. Thus, weighed samples for both acetyl and viscosity determinations were dissolved in acetone and filtered through tared GF/D filters. The weight of the sample was corrected for the loss of filterable solids. Loss of sample by adsorption to the sintered glass filter support and sides of the filtration flask may have caused a small loss of accuracy in acetyl determination. With CA 398-3, samples were dissolved in acetone, filtered, and reprecipitated by directing a stream of distilled water into the acetone solution. The reprecipitated CA was dried and weighed for analysis.

The effects of microorganisms on the performance of reverse-osmosis membranes were evaluated as follows. Bundles of CTA hollow-fiber membrane 60 cm in length were disinfected in 0.5% Formalin for 1 h, rinsed in sterile distilled water for 1 h a minimum of four times, added to 100 ml of BM in 250-ml Erlenmeyer flasks, and inoculated. Flasks were gently hand swirled daily. Fiber bundles were removed at 2, 4, and 8 weeks, placed in 3% glutaraldehyde (pH adjusted to 6), and sent to Dow Chemical Co. for performance testing.

Statistical treatment of the chemical analysis data consisted of analysis of variance followed by the Student-Newman-Keuls multiple-comparisons test ($P = 0.05$) to separate significant means (29). Unreplicated values treatments were compared with 95% confidence intervals calculated from the control mean.

RESULTS

Performance statistics of the reverse-osmosis membranes are given in Table 1. The rates of salt rejection decline were determined by regression analysis of salt rejection over time. Regression analysis of the Purtech modules was not performed; the small membrane surface area and low fluctuating feed water pressures of the Purtech modules contributed to suboptimum and variable salt rejection data. The rates of decline of salt rejection ranged from -3.09×10^{-3} to -6.24×10^{-1}% per day for the large modules. Data for normal rates of decline are limited, but one unspecified reverse-osmosis system operated for 2 years with a salt rejection

TABLE 3. Frequency of occurrence of fungi on CA and CTA membranes as determined by direct isolation and enrichment culture

Fungus	Frequency of occurrence of fungi on membrane:						
	Hydranautics	Dow I		Dow IIb		Dow III	
		Interior[a]	Exterior[b]	Interior	Exterior	Interior	Exterior
Ascomycetes	0/0[c]	0/0	0/0	0/0	0/0	2/0	0/0
Aspergillus	0/0	0/0	0/0	0/0	2/0	0/0	0/0
Aureobasidium	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Cladosporium	0/0	0/0	0/0	2/0	2/0	0/0	0/0
Fusarium	0/0	0/0	0/0	96/67	96/100	0/0	0/0
Penicillium	2/0	0/0	0/0	2/0	4/0	0/0	0/0
Trichoderma	0/0	0/0	0/0	86/0	88/0	35/50	0/0
Yeast	0/5	2/0	0/17	0/0	0/0	0/0	0/0

[a] Interior portion of the fiber bundle.
[b] Exterior portion of the fiber bundle.
[c] Percentage of plates which had the fungus by direct isolation/percentage of flasks which had the fungus by enrichment culture.

decline of -3.65×10^{-5} to -1.94×10^{-4}% per day (23). It is unclear what sort of performance declines are characteristic of biological degradation, because alleged episodes of biological degradation have been poorly documented. However, the Dow I, Hydronautics, and Fluid Systems modules exhibited sharp declines in salt rejection capacity that were consistent with reports of alleged membrane failure (11, 12, 23), and engineers at the Roswell Test Facility attributed these rejection declines to biological degradation (B. Gibbons, personal communication).

None of the membranes showed signs of microbial degradation when viewed under light or scanning electron microscopes. The lack of visible degradation of the CA membrane contrasts with other reports (26) which show considerable microbial growth and considerable degradation of cellulose films submerged in marine environments. Scanning electron microscopy of membrane surfaces revealed only scattered bacterial cells with no apparent etching, indentation of the membrane surface, or method of direct attachment. Only scattered hyphal filaments were observed, indicating that the fungi were not colonizing the membrane surface. Except for the Fluid Systems membranes, the spiral wound and hollow-fiber membranes had no discoloration or slime indicative of microbial growth. The Fluid Systems membrane had a slime on the rejection surface which contained motile, rod-shaped bacteria. However, this membrane was stored in a plastic bag at 25 to 30°C for 90 days, a period of time that exceeded the operational time. The slime formation was believed to have occurred during storage rather than during operation.

The fungal genera isolated from reverse-osmosis modules and waters within the Roswell Test Facility and the numbers of different species or biovariants of the genus or species identified were: *Acremonium*, 1; *Alternaria*, 1; *Aspergillus*, 2; *Aureobasidium*, 1; *Candida*, 1; *Cladosporium*, 1; Cleistothecial ascomycetes, 1; *Fusarium*, 6; *Geotrichum*, 1; Mucorales, 1; Mycelia Sterilia, 1; *Penicillium*, 4; *Phialophora*, 3; *Rhodotorula*, 1; and *Trichoderma*, 1. The bacterial genera isolated and the number of different species or biovariants of the genus or species that were identified were: *Acinetobacter*, 1; *Arthrobacter*, 3; *Bacillus*, 5; *Flavobacterium*, 2; *Kurthia*, 1; *Lactobacillus*, 11; *Micrococcus*, 1; *Micromonospora*, 1; and *Pseudomonas*, 7. Organisms appear to be common soil and water inhabitants (1, 4). Although many of the fungi in these genera are known to be cellulolytic, particularly species of *Trichoderma* (1, 28, 20), cellulose bacteria such as *Cellulomonas, Cytophaga*, or *Sporocytophaga* were not isolated in either direct or enrichment cultures. Some species of *Bacillus, Micrococcus, Micromonospora*, and *Pseudomonas* have been reported to be cellulolytic (1, 28), but subsequent testing of these isolates proved negative.

The densities of bacteria as determined by plate counts in Dow and Hydranautics modules are given in Table 2. The frequencies of occurrence of fungi as determined by direct isolation and enrichment techniques for the same modules are given in Table 3.

The ability to degrade cellulose was indicated by clearing of suspensions of acid-swollen cellulose and growth on filter paper as a sole carbon source. Cellulolytic ability was widespread among the fungi isolated from the Roswell Test Facility (Table 4). None of the fungi cleared CA 398-3, phosphoric acid-treated blend membrane, or CTA hollow fibers after an incubation in excess of 4 months (data not shown). Fungal growth was sparse on blend membrane strips in BM-FW medium (Table 4) and was only slightly better growth on gas-sterilized blend membrane in HUT-FW medium (data not shown). Growth on blend membrane in BSY medium (Table 4) was good, but this growth was undoubtedly supported by yeast extract. None of the membrane strips exhibited any visual signs of degradation or loss of structural integrity after an incubation in excess of four months. Fungi grew vigorously on deacetylated blend membrane in BM-FW, and many strips were degraded to the point of fragmentation when the tubes were agitated (Table 4). The data suggest that although many of the fungi were strongly cellulolytic, they were unable to degrade intact CA reverse-osmosis membranes or their constituent CA. The vigorous growth on deacetylated membrane suggested that deacetylation was prerequisite for degradation of CA.

Bacteria were more limited than the fungi in the capacity to degrade cellulose. None of the 33 bacterial groups isolated from Roswell Test Facility was able to degrade acid-swollen cellulose, carboxymethyl cellulose, or filter paper (with or without yeast extract amendments) after 30 days of incubation. *Cellulomonas* cultures exhibited a pulping or macerative effect on filter paper after 10 days. However, none of the bacteria produced visible effects on strips of blend membrane in HUT-FW (with or without yeast extract amendment) after an incubation in excess of 2 months (data not shown).

Because none of the qualitative tests indicated that the bacterial and fungal isolates from the Roswell Test Facility or acquired cultures could degrade CA membranes or suspensions of CA, more sensitive quantitative tests were performed to insure that visually undetected degradation was not occurring. If CA degradation occurred, either the acetates or the cellulose polymer (or both) would be cleaved, resulting in

TABLE 4. Qualitative tests for cellulose and CA degradative ability of fungal isolates

Culture no.	Fungus	Cellulose clearing depth (mm)	Growth[a] on:			
			Filter paper	Blend membrane		Deacetylated blend membrane
				BM-FW	BSY	
RS 18	*Acremonium*	12	2	1	ND	2
RS 26	*Alternaria*	0	2	1	ND	2
RS 7	*Aspergillus*	12	2	1	2	2[b]
RS 9	*Aspergillus*	15	2	1	2	2
RS 10	*Aspergillus*	16	2	1	2	2
RS 1	*Aureobasidium*	0	0	0	ND	0
RS 11	*Aureobasidium*	0	0	1	ND	1
RS 33	*Aureobasidium*	19	2	1	2	2
RS 42	*Cladosporium*	12	2	1	2	2[b]
RS 21	Cleistothecial ascomycete	12	2	1	ND	2
RS 22	*Fusarium*	23	2	0	ND	2
RS 27	*Fusarium*	12	2	1	ND	2
RS 23	*Fusarium*	15	1	1	ND	2
RS 2	*Fusarium*	11	2	0	ND	2
RS 35	*Fusarium*	16	2	1	2	2[b]
RS 12	*Fusarium*	16	2	1	2	2[b]
RS 31	*Fusarium*	19	2	1	2	2[b]
RS 41	*Fusarium*	ND	2	1	ND	2
RS 4	*Geotrichum*	0	0	0	ND	1
RS 25	*Penicillium*	15	2	0	ND	2[b]
RS 30	*Penicillium*	24	2	1	2	2
RS 39	*Penicillium*	21	2	1	ND	2[b]
RS 40	*Penicillium*	20	ND	1	ND	2[b]
RS 3	*Phialophora*	22	2	0	2	2[b]
RS 13	*Phialophora*	21	2	1	ND	2
RS 5	*Phialophora*	17	2	1	2	2[b]
RS 24	*Phialophora*	16	2	1	2	1[b]
RS 32	*Phialophora*	19	2	1	ND	2
RS 36	*Phialophora*	20	2	1	ND	1
RS 16	*Phialophora*	16	2	1	2	2
RS 17	*Phialophora*	11	2	1	ND	2
RS 14	*Trichoderma*	ND	2	ND	ND	ND
RS 15	*Trichoderma*	27	2	1	2	2[b]
RS 28	*Trichoderma*	26	2	0	2	2[b]
RS 29	*Trichoderma*	21	2	1	ND	2[b]
RS 37	*Trichoderma*	18	2	1	2	2[b]
RS 38	*Trichoderma*	26	2	0	2	2[b]
RS 6	Yeast	12	2	1	ND	2
RS 19	Yeast	0	0	0	ND	0
RS 20	Yeast	0	0	0	0	0

[a] 0, No growth; 1, slight or questionable growth; 2, significant growth; ND, not determined.
[b] Breakage of membrane.

a lower acetyl content of the CA or a decrease in the viscosity of the cellulose polymer (or both). In addition, the reducing sugar content of the media would increase with the cleaving of the cellulose polymer.

Eastman CA 400-25 was not degraded by any of the fungi tested (Table 5). Acetyl contents were not significantly reduced in any of the samples. The range of mean acetyl contents of the inoculated samples was narrow (0.5%), and the mean acetyl content of the control (39.47%) compared favorably with determinations by Tennessee Eastman (39.45%). Although the intrinsic viscosity analysis of variance was statistically significant, only one value (1.58 dl/g, for *Fusarium* RS2) was significantly different from the mean. Because degradation of CA would lower, rather than raise, the viscosity, depolymerization of the cellulose polymer did not occur. The amounts of reducing sugars released into the medium from inoculated samples tended to be greater than control values, but only three values (32.0, 29.7, and 28.5 dl/g) were outside the ranges of non-significantly different means that included the control. The fungi producing these values (*Fusarium* RS 31, *Cladosporium* RS 42, and *Penicillium* RS 30, respectively) were all cellulolytic (Table 4); therefore, the

TABLE 5. Assays for fungal degradation of Eastman CA 400-25[a]

Fungus	Acetyl content (%, wt/wt)	Intrinsic viscosity (dl/g)	Reducing sugar content (nmol/ml)
Control	39.47a	1.51b	11.8c
Cladosporium RS 42	38.96a	1.53b	29.7ab
Fusarium RS 22	39.00a	1.49b	15.1bc
Fusarium RS 2	39.10a	1.58a	ND
Fusarium RS 12	39.21a	1.50b	17.1abc
Fusarium RS 31	39.30a	1.48b	32.0a
Penicillium RS 30	38.96a	1.50b	28.5ab
Phialophora RS 13	39.47a	1.48b	7.6c
Phialophora RS 24	39.21a	1.48b	14.0bc
Trichoderma RS 15	39.27a	1.46b	18.7abc
Trichoderma RS 28	39.98a	1.48b	22.7abc
Yeast RS 20	39.24a	1.51b	21.5abc
Aurobasidium RS 1	39.41	1.52	24.0

[a] Means within the same column followed by the same letter are not significantly different at the 5% probability level by the Student-Newman-Keuls multiple comparison test. *Aureobasidium* was not included in the analysis. ND, Not determined.

TABLE 6. Assays for fungal, bacterial, and mixed culture degradation of CA 394-45

Organism	Acetyl content (%, wt/wt)	Intrinsic viscosity (dl/g)	Reducing sugar content (nmol/ml)
Control	38.05	1.38	146.6
Fungi			
Cladosporium RS 42	ND[a]	1.43	59.5
Fusarium RS 35	37.82	1.38	85.5
Fusarium RS 12	37.93	1.41	92.7
Fusarium RS 31	38.24	1.40	90.5
Penicillium RS 39	37.78	1.41	144.0
Phialophora RS 24	37.63	1.39	117.1
Phialophora RS 16	37.95	1.36	118.5
Trichoderma RS 15	37.98	1.39	115.5
Trichoderma RS 28	38.08	1.41	140.0
Bacteria			
Sinclair 209	38.28	1.38	147.9
Sinclair 226	38.17	1.36	127.3
Sinclair C257	38.06	1.40	61.0
ATCC 21681	38.07	1.41	140.8

[a] ND, Not determined

production of these relatively small amounts of reducing sugars may have been the result of cellulase activity on contaminating cellulose in the sample (17). Other tests showed that these fungi were not able to produce significant deacetylation or depolymerization of the CA. Thus, we conclude that the production of small amounts of reducing sugars was not regarded as sufficient evidence for CA degradation.

Eastman CA 400-25 was not degraded by any of the 33 bacteria isolates tested from the Roswell Test Facility (data not shown). No significant differences were found between the bacterial isolates and the control values for acetyl content, intrinsic viscosity, and reducing sugar content. Again the reducing sugar content of the bacterial cultures was generally higher than the control, but this was attributed to factors other than CA degradation.

Yeast extract was used in the CA 394-45 medium to determine whether CA degradation required an alternate carbon source or a more nutritionally complete environment (Table 6). Growth of the microorganisms was vigorous, especially for the fungi, where the mycelia often sequestered the bulk of the powdered CA. The analysis of variance of the acetyl contents and intrinsic viscosities was not significant. The analysis of variance for reducing sugar content was significant, but treatment values were lower than the control and were attributed to the uptake of reducing sugars during growth. The high reducing sugar content in the control was due to the reducing sugar present in the yeast extract. The vigorous growth of the organisms was attributed to the yeast extract and not to growth on CA since significant reductions in CA acetyl content and intrinsic viscosity did not occur. Bacterial cultures from N. A. Sinclair, including a primary enrichment culture containing a degraded CA strip and one *Cellulomonas* culture, did not demonstrate CA degrading ability.

Primary enrichment cultures of CA membranes were analyzed for CA degradation to explore the possibility that CA degradation might require the concerted efforts of several organisms. The enrichment cultures included flasks of BM medium containing 1% (wt/wt) CA 398-3, inoculated with membrane pieces from each module and incubated for 100 days. Sufficient material from each sample was not available to determine both acetyl content and intrinsic viscosity or to perform analyses in replicate. However, analyses on control CA were done in triplicate, and 95% confidence intervals were calculated for the control. No large declines in either acetyl content or intrinsic viscosity were found (Table 7). All values of acetyl content fell within the 95% confidence intervals of the control. Values for intrinsic viscosity fell with the 95% confidence intervals of the control, except for two values that were higher and therefore not indicative of depolymerization. The amounts of reducing sugars in the medium were small and in most cases not detectable. Thus, no significant deacetylation or depolymerization of CA occurred in these enrichment cultures.

None of the bacteria or fungi inoculated onto

TABLE 7. Assays for enrichment culture degradation of CA 398-3

Enrichment culture source	Acetyl content (%, wt/wt)	Intrinsic viscosity (dl/g)	Reducing sugar content (nmol/ml)
Control	39.98 ± 1.21[a]	0.80 ± 0.21	ND[b]
Hydranautics membrane	39.94	ND	0
Fluid Systems membrane	39.58	ND	ND
Purtech membranes			
1	39.62	ND	5.4
2	ND	1.11	0
3	ND	0.76	0
4	ND	0.79	0
5	39.05	ND	0
6	39.77	ND	0
7	ND	0.84	0
8	39.63	ND	ND
9	ND	0.88	2.6
10	ND	0.85	0
Dow II membrane product water	ND	0.90	3.9
Dow II membrane feed water	ND	1.02	8.2

[a] Confidence interval, 95%; $n = 3$.
[b] ND, Not determined.

CTA hollow fibers and incubated for up to 8 weeks resulted in significant declines in membrane performance (Table 8). Three additional cultures of *Trichoderma*, *Candida*, and inoculum from a primary enrichment culture of CA 398-3 were also tested in BM and BM-FW media. However, declines in the performance of the CTA fibers were not detected after 8 weeks of incubation (Table 9).

DISCUSSION

Reese (22) postulated that cellulose derivatives became more resistant to enzymatic attack with increasing degrees of substitution. Cowling (8) suggested that one substitution per anhydroglucose unit (DS of 1) was sufficient to impart protection from enzymatic attack. In the current study, organisms capable of vigorously degrading various forms of cellulose failed to degrade in vitro CA membranes unless the membrane had been previously deacetylated. These results are consistent with the suggestions of Reese and Cowling; deacetylation appears to be a prerequisite for biological degradation of CA membranes and their constituent CAs. Although most of the fungi were strongly cellulolytic, there was no evidence that the fungal or the bacterial isolates enzymatically deacetylated CA or depolymerized the cellulose backbone of CA. Microorganisms did not appear to affect membrane performance when inoculated onto CTA hollow fibers; according to Cantor and Mechalas (6), the loss of salt rejection is the most sensitive measure of membrane damages.

The occurrence of microorganisms and the rate of decline of salt rejection appear to be inversely related. If the slopes of the regression equations (Table 1) are compared with the densities of fungi and bacteria (Table 2 and 3), it is clear that high rates of decline of salt rejection are associated with low frequencies of microbial contamination (with the exception of the Fluid Systems membrane). In contrast, Dow IIb had the highest levels of contamination, yet the lowest rate of decline in salt rejection. The Kendall's coefficients of rank correlation (24) between rate of decline and occurrence of fungi and bacteria in Dow I, IIb, and III and in Hydranautics are −0.67 and −0.91, respectively. This inverse relationship between occurrence of microorganisms and rate of rejection decline does not support a microbial etiology for membrane failure.

The levels of feedwater residual chlorine (Table 1) do, however, appear to be directly related to the rate and magnitude of rejection decline exhibited by reverse-osmosis modules. The Kendall's coefficient of rank correlation calculated for the seven pairs of slopes and values of residual chlorine for the Dow, Hydranautics, and Fluid Systems modules (Table 1) is 0.98. Within the Dow modules, rates of rejection decline were 3 and 49 times greater in chlorinated modules (Dow I and III, respectively) than in the average rates of decline of the unchlorinated modules (Dow IIa and IIb). To verify this apparent relationship, chlorination was started on Dow IIa, a module which had previously been unchlorinated. After exhibiting extremely low rates of rejection decline over a period of operation in excess of 1 year while receiving unchlorinated feedwater, the rate of decline in Dow IIa increased 1 order of magnitude in the 10-week period during chlorination. Comparison of the slopes of regression equations of the 10-week period directly preceding chlorination and the 10-week period during chlorination shows a sixfold increase in rate of rejection decline; the slopes are different at $P = 0.001$. The *Reverse Osmosis Technical Manual* (23) recommends a maximum residual chlorine level of 1 mg/liter for CA and CTA membranes, and others (18) have shown the performance of CA membranes not to be affected by short exposure (10 to 23 days) to high levels of chlorine (3 to 30 mg/liter). Nevertheless, chlorination may affect membrane integrity. Hypochlorous acid may react directly with CA as in previously described nonspecific oxi-

TABLE 8. Dow Chemical Co. performance tests of CTA hollow fibers after incubation with various microbial isolates

Organism	2-week incubation		4-week incubation		8-week incubation	
	Water flux (liters/m²/day)	Salt rejection (%)	Water flux (liters/m²/day)	Salt rejection (%)	Water flux (liters/m²/day)	Salt rejection (%)
Control	297	96.5	322	97.4	232	97.5
Fungi[a]						
Aureobasidium	310	96.8	277	97.3	285	96.4
Phialophora	269	96.3	314	96.4	297	96.9
Trichoderma	310	95.4	293	97.6	281	97.0
Bacteria[b]						
Bacillus sp. 1	306	96.3	281	97.9	293	97.1
Pseudomonas	318	95.4	293	95.5	253	97.7
Bacillus sp. 2	285	96.7	289	97.2	277	98.3

[a] Fungi were grown in BS-FW medium.
[b] Bacteria were grown in nutrient broth-FW medium.

dations of cellulose by hypochlorite (19) or react with other chemical entities such as transitional metal ions, particularly Mn, as suggested by A. R. Marsh, Dow Chemical Co., and J. H. Hageman, New Mexico State University (personal communications). In general, chlorination reduced microbial contamination of those membranes receiving chlorinated feed water and membranes cultured soon after shutdown.

Certain conditions might make CA membranes susceptible to microbial attack. Vos et al. (33) have shown that CA is deacetylated at pH conditions deviating from pH 5, with the reaction accelerating at higher temperatures. Prolonged exposure of CA membranes to neutral or slightly alkaline conditions, or a short exposure to conditions of high pH, could cause sufficient deacetylation to occur, allowing colonization by cellulolytic organisms. Rates of deacetylation of operational reverse-osmosis membranes may be greater than rates calculated by Vos et al., because their measurements were made on membranes in static as opposed to dynamic solutions. Interactions with chlorine and other metal ions may also increase rates of deacetylation. The acetyl content of a CA membrane determines the salt rejection properties in addition to protecting the CA from enzymatic attack; thus, it is unlikely that deacetylation sufficient to allow microbial colonization would occur before serious impairment of membrane performance would be apparent. If a deacetylation were to occur, the decline in membrane performance would be caused by chemical deacetylation. The effects of subsequent microbial colonization would be secondary.

These data suggest that microbial degradation of operational CA reverse-osmosis membranes at the Roswell Test Facility or other facilities is unlikely. Furthermore, it appears that as long as the CA has a high degree of acetyl substitution and the membrane is not exposed to conditions which favor deacetylation, microorganisms cannot readily degrade modern CA membranes.

TABLE 9. Dow Chemical Co. CTA hollow fibers after 8 weeks of incubation

Organism	Water flux (liter/m²/day)	Salt rejection (%)
Control	313	95.4
Trichoderma[a]	319	96.4
Candida[b]	298	97.8
Enrichment culture[c]	299	95.3

[a] Grown in BM plus yeast extract medium.
[b] Grown in BM-FW medium.
[c] Enrichment in BM-CA 398-3 medium.

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LITERATURE CITED

1. **Alexander, M.** 1977. Introduction to soil microbiology, 2nd ed. John Wiley & Sons, New York.
2. **Annual Book of ASTM Standards.** 1978. Cellulose; leather; flexible barrier materials, part 21, D871 section 10, p. 55–57. American Society for Testing and Materials, Philadelphia.
3. **Avigad, G.** 1975. Colorimetric ultramicro assay for reducing sugars. Methods Enzymol. 41:27–28.
4. **Baron, G. L.** 1968. The genera of hyphomycetes from soil. The Williams & Wilkins Co., Baltimore.
5. **Buchanan, R. E., and N. E. Gibbons (ed.).** 1974. Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Co., Baltimore.
6. **Cantor, P. A., and B. J. Mechalas.** 1969. Biological degradation of cellulose acetate reverse-osmosis membranes. J. Polymer Sci. 28:225–41.
7. **Cantor, P. A., B. J. Mechalas, O. S. Schaeffler, P. H. Allen, J. A. Hunter, W. S. Gillam, and H. E. Podall.** 1968.

Biological degradation of cellulose acetate reverse osmosis membranes. Research and development progress report no. 340. U.S. Department of the Interior, Washington, D.C.

8. **Cowling, E. B.** 1963. Structural features of cellulose that influences its susceptibility to enzymatic hydrolysis, p. 1–32. *In* E. T. Reese (ed.), Advances in enzymatic hydrolysis of cellulose and related materials. Macmillian Publishing Co., New York.
9. **Gams, W.** 1971. Cephalosporium-artige Schimmelpiltze (Hyphomycetes). Gustav Fischer Verlag, Stuttgart.
10. **Gordon, R. E.** 1973. The genus *Bacillus*. *In* A. J. Laskin and H. A. Lechevalier (ed.), CRC Handbook of microbiology, vol. 1. CRC Press, Cleveland.
11. **Guy, D. B.** 1975. Bacterial contamination of the Roswell O.W.R.T. Test Facility. A technical report to the Roswell Test Facility. Office of Water Research and Technology, U.S. Department of the Interior, Washington, D.C.
12. **Guy, D. B.** 1980. Biodegradation of cellulosic membranes at the Roswell and Yuma desalination test facilities, p. 12 and 15. *In* Pure water, vol. 2. International Desalination and Environmental Association, Teaneck, N.J.
13. **Hankin, L., and S. L. Anagnostatis.** 1977. Solid media containing carboxymethyl-cellulose to detect Cx cellulose activity of microorganisms. J. Gen. Microbiol. **98**:109–115.
14. **King, W. M., P. A. Cantor, L. W. Schoellenbach, C. R. Cannon.** 1970. High-retention, reverse-osmosis desalination membranes from cellulose acetate, p. 17–26. *In* A. F. Turbak, (ed.), Membranes from cellulose derivatives. Applied Polymer Symposium no. 13. Interscience Publishers, New York.
15. **Kremen, S. S.** 1980. An overview of the microbiological problem and some potential causative agents. Workshop on microbiological membrane degradation. Eighth Annual Conference and International Trade Fair of the National Water Supply Improvement Association, Ipswich, Mass.
16. **Leahy, T. M., III.** 1980. Recent experience with CTA hollow fiber membranes at the Roswell Test Facility. Technical Proceedings Eighth Annual Conference and International Trade Fair of the National Water Supply Improvement Association, Ipswich, Mass.
17. **Lonsdale, H. K.** 1966. Properties of cellulose acetate membranes p. 93–160. *In* U. Merton, (ed.), Desalination by reverse osmosis. MIT Press, Cambridge, Mass.
18. **McCutchan, J. W., and J. Glater.** 1980. The effect of water pretreatment chemicals on the performance and durability of reverse osmosis membranes. Final report to Membrane Processes Division. Office of Water Research and Technology, U.S. Department of the Interior, Washington, D.C.
19. **Nevell, T. P.** 1963. Oxidation, p. 164–184. *In* R. L. Whistler (ed.), Methods in carbohydrate chemistry, vol. 3, Cellulose. Academic Press, Inc., New York.
20. **Nilsson, T.** 1973. Studies on wood degradation and cellulolytic activity of microfungi. Studia Forestalia Suecica Nr. 104. Skogshogskolan, Royal College of Forestry, Stockholm.
21. **Rautela, G. S., and E. B. Cowling.** 1966. A simple cultural test for relative cellulolytic activity in fungi. Appl. Microbiol. **14**:892–898.
22. **Reese, E. T.** 1957. Biological degradation of cellulose derivatives. Indust. Eng. Chem. **49**:89–93.
23. **Reverse Osmosis Technical Manual.** 1979. Office of Water Research and Technology, U.S. Department of the Interior, Washington, D.C.
24. **Rodina, A. G.** 1972. Methods in aquatic microbiology. University Park Press, Baltimore.
25. **Saltonstall, C. W., Jr.** 1977. Progress in membrane development for reverse osmosis. Desalination **22**:229–234.
26. **Sieburth, J. M.** 1975. Microbial seascapes. University Park Press, Baltimore.
27. **Sinclair, N. A.** 1981. Microbial degradation of reverse osmosis desalting membranes. A final report to the Bureau of Reclamation. U.S. Department of the Interior, Washington, D.C.
28. **Siu, R. G. H.** 1951. Microbial decomposition of cellulose. Reinhold Publishing Co., New York.
29. **Sokal, R. R., and R. J. Rohlf.** Biometry. W. H. Freeman and Co., San Francisco.
30. **Tanghe, L. J., L. B. Genung, and J. W. Mench.** 1963. Determination of acetyl content and degree of substitution of cellulose acetate, p. 201–203. *In* R. L. Whistler, (ed.), Methods in carbohydrate chemistry, vol. 3, Cellulose. Academic Press, Inc., New York.
31. **Tanghe, L. J., L. B. Genung, and J. W. Mench.** 1963b. Intrinsic viscosity, approximate degree of polymerization and molecular weight of cellulose acetate, p. 210–212. *In* R. L. Whistler, (ed.), Methods in carbohydrate chemistry, vol. 3, Cellulose. Academic Press, Inc., New York.
32. **Tansey, M. R.** 1971. Agar-diffusion assay of cellulolytic ability of thermophilic fungi. Arch. Mikrobiol. **77**:1–11.
33. **Vos, K. D., F. O. Burris, Jr., and R. L. Riley.** 1966. Kinetic study of the hydrolysis of cellulose acetate in the pH range of 2–10. J. Appl. Polymer Sci. **10**:825–832.