# ISOLATION AND CONTROL OF MEMBRANE FILTER DEGRADING MICROORGANISMS

by

William C. Lindemann
Department of Agronomy
New Mexico State University

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ABSTRACT

The Roswell Test Facility reverse osmosis testing program was plagued with premature failure of cellulose acetate reverse osmosis membranes. Microbial degradation of the membranes was thought to be the cause. This study was conducted to identify the mocroorganisms responsible for the problems, locate the source of contamination, and prevent future contamination.

Microorganisms were isolated from various membranes and waters and tested for their ability to degrade cellulose acetate preparations and membrane strips. None of the microorganisms isolated or enrichment cultures from facility membranes were found to degrade cellulose acetate. The microorganisms isolated were common soil and water inhabitants. Those membranes with high microbial populations generally had good salt rejection capabilities while those membranes with low microbial populations had the greatest decline in salt rejection capabilities. No evidence or microorganisms were found to support the microbial degradation hypothesis during the course of this study. The degradation of cellulose acetate membranes and other possible causes for membrane failure were discussed.

Key words: cellulose, cellulose acetate, cellulose triacetate, membranes, fibers, filters, spiral wound, water purification, water treatment, reverse osmosis, microorganisms, bacteria, fungi, decomposition, degradation, saprophytes, cellulolytic, microbes, isolations

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# INTRODUCTION AND JUSTIFICATION

The demand for fresh water is dramatically increasing in New Mexico as well as in the rest of the world. However, the supply of fresh water is finite and is quickly being exhausted. Desalinization of ocean and saline ground waters is one alternative to meet the demand.

Reverse osmosis (RO) filtration is one of the most widely used methods of desalinization and cellulose acetate (CA) is the most common membrane material used in RO filters. However, CA membrane filters have failed before their predicted life expectancy. The Roswell Test Facility (RTF), Roswell, New Mexico, of the Office of Water Research and Technology, United States Department of the Interior, has acquired notoriety for premature failuers of CA-RO membranes. The CA membrane failures of the RTF, as manifested by a loss of salt rejection capacity and increase in water flux, have often been attributed to microbial degradation (Guy, 1980; Kremen, 1980; Leahy, 1980). Indeed, the failure of CA membranes at the RTF was much greater than at other facilities using CA membranes. A microbial etiology was proposed, even to the extent of postulating the existance of a strain of cellulolytic organism endemic to the RTF.

The literature contains no direct evidence linking CA-RO membrane failure with microbial degradation. Yet RTF engineering reports concerning visual microbial degradation of CA-RO membrne filters and the reports of Cantor and Mechalis (1969) and Reese (1957) concerning microbial degradation of CA 'in vitro' prompted this investigation. At the request of Mr. John Newton, former manager at the RTF, a proposal was submitted to and approved by the Department of Interior to investigate CA-RO

membrane filter degradation at Roswell. The specific objectives were to:

(1) Identify the microorganisms(s) responsible for CA-RO membrane filter degradation.

(2) Determine the source and cause of microbial contamination within the facility.

(3) Eliminate the microbial contaminant to prevent further membrane failures.

## MATERIALS AND METHODS

### Facilities and Personnel

The research was conducted at the RTF. However, Dow Chemical Company provided fiber performance testing and scanning election microscopy tests. Additional election microscopic observations were performed at the Biology Department, New Mexico State University.

Dr. Leighton Ho (Research Associate, New Mexico State University) and Mr. David Martin (Bacteriologist, Planning Research Corporation) were responsible for the actual laboratory experiments. Mr. Martin isolated, characterized, and tested bacterial isolates. Dr. Ho worked primarily with the fungal isolates but was also responsible for the qualitative and quantitative tests during the assay for CA decomposition.

### Reverse Osmosis Module Description

The specifications and description of RO modules and membranes used in the study and referred to in the text are summarized in Table 1.

Flat membranes in a spiral wound configuration consist of a rectangular envelope of two separate membranes laminated to dacron backing material and sealed around three edges. The fourth edge opens into a

Table 1. Specifications and characteristics of cellulose acetate (CA) and cellulose triacetate (CTA) reverse osmosis modules and membranes.

| Module Name and Manufacturer | Module Size Diameter x Width (cm) | Membrane Configuration | Membrane Composition with Nominal Acetyl Content |
|---|---|---|---|
| Purtech<br>Salt Lake City, UT<br>(membrane from Envirogenics Systems Co., El Monte, CA) | 6.4 x 30.5 | Spiral wound | 50:50 blend<br>CA 39.8%<br>CTA 43.2% |
| Dow<br>Dow Chemical Co.,<br>Walnut Creek, CA | 15.2 x 121.9 | Hollow Fiber | CTA 43.5 - 43.7% |
| Hydronautics<br>Hydronautic Water Systems, Santa Barbara, CA | 21.6 x 101.6<br>(1 element) | Spiral wound | 50:50 blend<br>CA 39.8%<br>CTA 43.5% |
| Fluid Systems<br>Fluid Systems Div.<br>Universal Oil Products<br>San Diego, CA | 30.5 x 152.4<br>(1 element) | Spiral wound | Thin film composite<br>CA 40.0% |

product tube. The envelope is rolled around the product tube to form a cylinder. The rejection surface or feed side of the membrane is on the outside of the envelope; the product side of the membrane is on the inside of the envelope, laminated to the backing material. Both sides of the membrane are in contact with a plastic spacer material. Feedwater enters the module from one end, with product and brine waters exiting from the opposite end.

Membranes in a hollow fiber configuration consist of a bundle of parallel hollow fibers. Feedwater enters the module from a perforated tube situated in the interior of the fiber bundle. Product water collects in the lumen of the hollow fibers and exits from the opposite end of the module. In contrast to spiral wound modules, brine exits from the same end that the feedwater enters.

The Purtech modules were small, sacrificial modules placed in 10 locations throughout the RTF to sample different plant points for contaminating microorganisms. The Hydronautics and Fluid Systems modules consisted of several elements connected in series.

Feedwater to most of the modules was a 3000 ppm total dissolved solids (TDS) blend of Roswell city water (approximately 1000 ppm TDS) and brackish well water (approximately 13000 ppm TDS). However, one Purtech module received only Roswell city water. The feedwater pH was usually adjusted to a target pH of 5.5 - 5.8 by the addition of $H_2SO_4$. However, pH values from January 1980 to April 1981 showed a range of values from 5.6-6.9, with an average of 6.1. The pH of unacidified feedwater was around 7.5-7.6, too high for the optimal functioning of CA membranes. Other feed water pretreatments are summarized in Table 2.

Table 2. Feedwater pretreatment before reverse osmosis filtration.

| Module | Filtration | | | Chemical Treatment | | |
|---|---|---|---|---|---|---|
| | MNGS | C | Mixed | SHMP | $H_2SO_4$ | NaOCl |
| Dow I | X[1/] | X | 0[2/] | X | X | X |
| Dow II | X | X | 0 | X | X | 0 |
| Dow III | X | 0 | 0 | 0 | 0 | X |
| Hydronautics | 0 | 0 | X | X | X | X |
| Fluid Systems | 0 | 0 | X | X | X | X |
| Purtech | | | | | | |
| (Location) | | | | | | |
| 1. Pad 6 | X | X | 0 | X | X | 0 |
| 2. Pad 6 + $Na_2S_2O_3$ | X | X | 0 | X | X | 0 |
| 3. HPR | X | X | 0 | X | X | 0 |
| 4. HPR + NaOCl | X | X | 0 | X | X | X |
| 5. City | 0 | 0 | 0 | 0 | 0 | X |
| 6. Mix 3 | X | X | 0 | X | X | 0 |
| 7. MNGS | X | 0 | 0 | 0 | 0 | X |
| 8. C | X | X | 0 | 0 | 0 | 0 |
| 9. Pad 5 | 0 | 0 | X | X | X | X |
| 10. Mixed | 0 | 0 | X | 0 | 0 | X |

[1/] X = Filtration or treatment received

[2/] 0 = Filtration or treatment not received

MNGS = manganese green sand filter; C = activitated carbon filter; Mixed = mixed bed filter of MNGS and C; SHMP = sodium hexametaphosphate; HPR = high product recover pad; City = city water; Mix 3 = mixing tank 3

Operational parameters and performance statistice of RO modules are summarized in Tables 3 and 4. Performances for the Hydronautics and Fluid systems modules were obtained from the Planning Research Corporation report to the Office of Water Research and Technology (1980). Performance data for Dow modules were obtained from the RTF files. The Purtech modules (Table 4) were probably too small and were operated under too low of a pressure for accurate salt rejection measurements. Although salt rejection of Purtech modules was monitored daily, salt rejection fluctuated widely as a function of feedwater pressure and unknown factors. Consequently, performance data of Purtech modules is limited to starting and ending salt rejections. However, the Purtech modules were primarily used as traps for CA decomposing microorganisms and not as functioning units as were the larger modules.

Most of the large modules (Table 3) experienced some salt rejection declines over the period of operation. Some of the declines were rather sharp as in the case of Dow I. This sharp decline in salt rejection of the Dow I unit was described as being characteristic of microbial degradation by RTF personnel. All of the modules were operated over periods of time that were sufficient to allow exposure to the resident microflora of the RTF.

Microorganism Sources and Media

Microorganisms were isolated from CA-RO membranes or from various waters within the facility. Two cultures of cellulose degrading bacteria (Cellulomonas) were obtained from the American Type Culture Collection (ATCC #21399 and #21681). Two bacterial isolates (S226 and S209) from the Yuma Desalting Facility, capable of filter paper degradation, were

Table 3. Operational parameters and performance statistics of reverse osmosis modules.

| Module | Period of Operation | Operating Pressure | Starting[1] Rejection | Ending Rejection | Regression[2] Coefficients | | Correlation Coefficient | Feedwater[3] Chlorine | Culture[4] Time |
|---|---|---|---|---|---|---|---|---|---|
| | | --(psi)-- | ---(%)--- | -(%)- | ---(a)----- | -(b)- | ----(r)---- | --(mg/l)-- | -(days)- |
| Dow I. vess. B | 13 Feb 1980 2 Jul 80 | 250 | 96.10 | 57.62 | $-1.74 \times 10^{-1}$ | 102.4 | 0.68 | 0.67 | 1 |
| Dow II vess. B | 13 Feb 80 16 Sept 80 | 250 | 96.49 | 95.71 | $-4.01 \times 10^{-3}$ | 96.5 | 0.81 | 0 | 1 |
| Dow II[5] vess. A | 13 Feb 80 6 Jan 81 | 250 | 95.38 | 94.63 | $-3.09 \times 10^{-3}$ | 95.5 | 0.95 | 0 | ND |
| Dow II[5] vess. A | 7 Jan 81 17 Mar 81 | 250 | 95.53 | 92.34 | $-4.26 \times 10^{-2}$ | 109.9 | 0.95 | 0.65 | ND |
| Dow III vess. A | 6 Sept 79 6 June 80 | 250 | 93.75 | 90.82 | $-1.15 \times 10^{-2}$ | 94.1 | 0.97 | 0.62 | 11 |
| Hydronaut lead element | 25 June 80 5 Aug 80 | 440 | 95.05 | 77.93 | $-3.30 \times 10^{-1}$ | 98.4 | 0.82 | 0.86 | 14 |
| Fluid Syst lead element | 24 Ap 80 14 May 80 | 430 | 92.79 | 88.52 | $-6.24 \times 10^{-1}$ | 101.3 | 0.99 | 1.12 | 90 |

[1] Sampled after 7 days from start up except for Dow III and Fluid Systems which were sampled 13 days from start up.

[2] Salt rejection decline versus time.

[3] Feedwater residual chlorine mean values at start up and shut down.

[4] Number of days from unit shut down until cultures were taken.

[5] No microbial cultures taken from these 2 modules. All other modules had microbial cultures taken after shutdown.

ND = not determined.

Table 4. Operational parameters and performance statistics of Purtech reverse osmosis modules.

| Module Location | 1980 Period of Operation | Operating Pressure | June 9 Starting Rejection | Aug 1 Ending Rejection | Feedwater Residual Chlorine | Culture[1/] Time |
|---|---|---|---|---|---|---|
| | | ---psi--- | ----%---- | ----%---- | -(mg/l)- | -(days)- |
| 1. Pad 6 | 3 June<br>5 Aug | 90 | 78 | 87 | 0 | 0 |
| 2. Pad 6 + $Na_2SO_3$ | 3 June<br>11 Aug | 90 | 79 | 87 | 0 | 0 |
| 3. HPR | 3 June<br>12 Aug | 60 | 76 | 89 | 0 | 0 |
| 4. HPR + NaOCl | 5 June<br>2 July | 60 | 78 | 71 | 0.7 | 36 |
| 5. City | 3 June<br>31 July | 50 | 91 | 84 | 0.1 | 6 |
| 6. Mix 3 | 3 June<br>5 Aug | 50 | 79 | 86 | 0 | 1 |
| 7. MnGS | 3 June<br>11 Aug | 30 | 59 | 41 | 0.6 | 0 |
| 8. C | 3 June<br>5 Aug | 30 | 59 | 57 | 0 | 0 |
| 9. Pad 5 | 3 June<br>12 Aug | 40 | 73 | 71 | 0.8 | 7 |
| 10. Mixed | 3 June<br>6 Aug | 30 | 67 | 49 | 0.5 | 6 |

[1/] Number of days from unit shutdown until cultures were taken.
HPR = high produce recovery pad
City = city water
Mix 3 = mixing tank 3
MNGS = manganese green sand filter
C = activated carbon filter
Mixed = mixed bed filter of MNGS and C

generously provided by Dr. N. A. Sinclair, University of Arizona. Dr. Sinclair also supplied a CA strip primary enrichment culture (C-257 CNL) in which degradation of the membrane strip was evident. The primary enrichment culture was used as a mixed culture inoculum in some tests.

Several media were used throughout the experiments including the basal salts yeast extract (BSY) medium of Sinclair (1981), the Hutchinson (HUT) medium of Rodina (1972), and a basal medium (BM) containing the following (per liter): $KH_2PO_4$, 1.0g; $(NH_4)_2SO_4$, 0.54g; KCl, 0.5g; $MgSO_4.7H_2O$, 0.2g; $CaCl_2.H_2O$, 0.13g; $FeCl_3$, 50mg; $MnSO_4.4H_2O$, 50 mg; $2nSO_4$, 44mg; $CuSO_4.5H_2O$, 10mg; $NaMoO_4.2H_2O$, 5mg; $Na_2B_4O_7$, 1mg; nicotinic acid, 0.3mg; folic acid, 0.3mg; thiamine, 0.03mg. Various prepared media used included cornmeal agar (Difco), malt extract agar (Difco), yeast extract agar (Difco), trypticase soy agar (BBL), and the CA agar of Cantor and Mechalas (1969). All media were prepared in either distilled water or facility water (FW). The FW was used to simulate the water conditions within the RTF and included the addition of 0.01% EDTA to alleviate precipitate formation. If a solid medium was required, 2% purified agar (Difco) was added. Peptone and yeast extract amendments added to some enrichment cultures were also from Difco.

Powdered cellulose acetates CA398-3, CA394-45, and CA400-25 were obtained from Eastmen Chemical Company. The first three numbers in the CA type designation are the nominal acetyl contents; e.g., the nominal acetyl content of CA398-3 is 39.8%. The actual acetyl contents of the cellulose acetates used (as determined by Mr. E. H. Hill of Tennessee Eastman Company) were 40.00%, 39.55% and 39.45% for CA398-3, CA394-45, and CA400-25, respectively. The number to the right of the hyphen in the CA type designation is the American Society for Testing Materials

(ASTM) viscosity. The degree of substitution (DS), e.g., the average number of acetyl groups per anhydroglucose unit of cellulose, was calculated from Tanghe et al. (1963a) and was 2.47, 2.43, and 2.42 for CA398-3, CA394-45, and CA400-25, respectively.

Two membranes were used for strip and performance tests in liquid culture. A blend membrane of equal weights of CA398-3 and CA435-85 in a sheet configuration (as in spiral wound modules) was obtained from Envirogenics Systems Company. The membrane was peeled from the dacron backing before use in membrane strip tests. A cellulose triacetate membrane in a hollow fiber configuration was obtained from Dow Chemical Company and was used in performance tests with Dow.

Isolation of Microorganisms

Microorganisms were isolated from module membranes by direct, swabbing, and enrichment culture techniques. The membranes were exposed, excised, and transferred under asesptic conditions. The Purtech modules were first cut open with a band saw before isolation. The Fluid Systems module was stored for 90 days at room temperature after shutdown before sampling. The other modules were sampled as soon as possible after shutdown (Tables 3 and 4).

Spiral wound membranes were cut open to expose both the brine and product sides of the membrane. Fungi were isolated directly by placing 100 pieces of $1cm^2$ membrane feed side down on 50 petri dishes of half strength cornmeal agar and 50 petri dishes of 2% malt extract agar. The fungal media contained 25mg/l each of penicillin and streptomycin to inhibit bacterial growth. Bacteria isolations were made by swabbing a $2cm^2$ area of membrane, agitating (vortex mixer) the swab in 2ml of pH 7 phosphate buffer, and plating on one of the following; 0.5% malt extract

agar, 0.5% yeast extract agar, half strength cornmeal agar, nutrient agar, BM-CA agar (BM plus 0.5% reprecipitated Eastman CA398-3), or BMY agar (BM plus 0.05% yeast extract). Three swabs were taken from the feed side of the membrane and 3 from the backing material on the product side. For enrichment cultures, the membranes were cut into ten, 4-6cm$^2$ pieces and placed in 125ml Erlenmeyer flasks containing 50ml of BM-CA or BMY media. After 4 weeks of incubation on a reciprocating shaker at 25°C, samples from the enrichment flask were serially diluted and plated on previously described media.

Isolation procedures for hollow fiber membranes (Dow) were similar to that for the spiral wound membranes except that small bundles of fibers were cut (1-2cm long) and separated before direct isolation, swabbing, and enrichment. However, due to the fineness of the fibers, the microorganisms isolated represented only the feed side of the membrane.

Fungal plates from enrichment flasks and direct isolation were incubated at 25°C for 10 to 15 days and identified to genus with the aid of dissecting and compound microscopes. The frequence of occurrence of fungi in direct isolations is expressed as the number of occurrences/ number of plates inoculated. Because more than one fungus could appear on a single plate, frequencies of individual fungi do not necessarily total to the frequency of plates with fungi present, but a single genus was counted only once on a single plate. Enrichment flasks were each streaked on one plate of half strength cornmeal agar and one plate of 2% malt agar. A fungus had to be present on both plates to be counted as present in the enrichment flask.

Bacterial plates from enrichment cultures and swabs were counted on a Quebec dark field colony counter and expressed as the number of colony forming units (CFU)/$cm^2$. Bacterial colonies from fibers placed on the surface of nutrient agar, swab plates, and enrichment plates were picked and repeatedly plated until pure colonies were obtained. The isolates were screened on the basis of colony morphology on trypticase soy agar, Gram stain, and cell size. Duplicate isolates were then discarded and representative isolates classified to genus and/or species according to Bergy's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974). The CRC Handbook of Microbiology (Gordon, 1973) was also used to classify Bacillus to species.

Qualitative Tests For Cellulose and Cellulose Acetate Degradation

Fungi and bacteria were tested for the ability to clear a 0.25% (w/w) suspension of phosphoric acid swollen cellulose in tubes of BS agar (Rautela and Cowling, 1966; Tansey, 1971). The depth of clearing was measured after 20 and 36 days. Blend membrane (Envirogenics) and cellulose triacetate hollow fibers (Dow) were similarly treated with phosphoric acid and incorporated into BS agar tubes with the thought that this procedure might be useful as a screen for CA degradation ability. However, none of the bacteria produced clearing in the cellulose tubes, even with the addition of 0.5% tryptone. Thus the bacteria were also tested on agar plates of 0.5% (W/V) carboxymethyl cellulose according to Hanking and Anagnostakis (1976). Cleared zones around bacterial colonies (as indicated by carbohydrate precipitation with 1% hexadecyltrimethylammonium bromide) was an indicator of carboxymethyl cellulose degradation.

Fungi and bacteria were also tested for their ability to degrade Whatman filter paper strips (1 x 7.5cm) in HUT-FW medium. When no bacterial growth was observed the tubes were reinoculated and enriched with yeast extract (final concentration 0.05%) to encourage growth. Strips of blend membrane (Envirogenics) were tested in a similar manner with BS-FW and BSY-FW media for the fungi and HUT-FW medium for the bacteria. As in the filter paper test the bacterial tubes were reinoculated and enriched with yeast extract when no growth occurred after several weeks. Both filter paper and blend membrane strips were tested for structural integrity by vigorously swirling the tubes containing the strips on a vortex mixer. Strip disintegration indicated degradation of the filter paper or membrane had occurred.

To test the possibility that autoclaving might be increasing the resistance of CA membranes to degradation, a set of membrane strips was sterilized with propylene oxide and inoculated with fungi in HUT-FW medium.

Fungi were also inoculated to deacetylated blend membrane strips in BS-FW medium. The membrane was deacetylated by soaking in equal volumes of ethanol and ammonium hydroxide to the point of acetone insolubility (overnight).

Quantitative Tests For Cellulose Acetate Degradation

Because CA degradation could not be detected visually in agar columns or on both culture strips, quantitative analyses were performed which would indicate CA degradation. Tests were performed on CA inoculated with microorganisms under several different conditions:

(1) CA398-3; Analyses were conducted on CA from enrichment cultures as previously described. Cultures consisting of 50ml of BS

medium with 1% reprecipitated CA398-3 were incubated for approximately 16 weeks. The inoculum consisted of pieces of CA membrane from experienced CA-RO modules. Approximately 5 enrichment flasks were combined for each sample.

(2) CA400-25; Fungi were cultured on 3.5g CA400-25 in 100ml BS-FW medium. Bacteria were cultured on 2.0g CA400-25 in 100ml HUT-FW medium for approximately 8 weeks.

(3) CA394-45; Fungi and bacteria were cultured on 1.5g CA394-45 in 100ml BSY medium for 4 weeks.

Analyses consisted of acetyl content by the solution method (Annual Book of ASTM Standards, 1978) intrinsic viscosity by the method of Tanghe et al. (1963), and reducing sugars by the method of Avigad (1975). Microbial biomass had to be removed from all CA samples before the intrinsic viscosity could be determined. With CA398-3, biomass was removed for both acetyl and viscosity determinations. For biomass removal, samples were dissolved in acetone, filtered through a Whatman GF/D filter (a high flow, high loading capacity filter with a retention of 2.7 um), and reprecipitated by directing a stream of distilled water into the acetone solution. The CA was then collected, dried, and weighed for the analysis. In the case of CA400-25, microbial biomass was considered minimal and carried along with the sample for acetyl determination. For determination of intrinsic viscosity, the sample was weighed, dissolved in acetone, and filtered through a Whatman GF/D filter. Changes in viscosity due to acetone evaporation were thought to be minimal as samples passed through the filters rapidly (less than 2 seconds). CA394-45 had considerable biomass accumulation because of the addition of yeast extract to stimulate growth. Thus weighed samples for

both acetyl and viscosity determinations were filtered into tared Whatman GF/D filters after being dissolved in acetone. The weight of the filterables was subtracted from the original sample weight. Loss of sample to the sintered glass filter support and sides of the filtration apparatus may have caused a small (1%) loss of accuracy in the acetyl determinations.

Tests on Cellulose Triacetate Hollow Fibers

Cooperative experiments were conducted with Dow Chemical Company to determine the effect of microorganisms on the performance of cellulose triacetate hollow fibers. Bundles of fibers were disinfected in 0.5% W/V) formalin for 1 hour, rinsed in sterile distilled water for 1 hour a minimum of 4 times, and inoculated in 250ml Erlenmeyer flasks containing 100ml of medium. The flasks were gently hand swirled daily. The fiber bundles were removed after 2, 4, and 8 weeks incubation and sent to Dow in 3% gluteraldehyde (pH 6) for performance testing.

## RESULTS

Microorganisms Isolated

Bacteria and fungi isolated from the RO modules and waters within the RTF are given in Tables 5 and 6. The organisms in both tables are, in general, ubiquitous bacteria and fungi which could be isolated from almost any soil or water source. The organisms listed could be classified physiologically as hetertrophic (saprophytic), mesophilic, and aerobic organisms which were isolated on nutritionally rich media. Undoubtedly, some microorganisms from other physiological groups were missed, the autotrophic and anaerobic bacteria for example, but such microorganisms would not be expected to be CA decomposers.

Table 5. Fungi isolated from cellulose acetate reverse osmosis membranes and Roswell Test Facility waters.

| Fungus | Isolate Number | Origin[1] |
|---|---|---|
| *Acremonium* spp. | RS18 | Dow I |
| *Alternaria* spp. | RS26 | Envirogenics blend membrane (virgin) |
| *Aspergillus terreus* Thom | RS10 | [2] MSI citric acid flush |
| *Aspergillus* sp. 1 | RS7,9 | MSI citric acid flush |
| *Aureobasidium* sp. 1 | RS1,11 | Mix tank water |
| *Aureobasidium* sp. 2 | RS33 | Purtech (City) |
| *Cladosporium* spp. | RS42 | Dow III |
| Cleistothecial ascomycete | RS21 | Dow III |
| *Fusarium* sp. 1 | RS22,27 | Purtech (HPR + NaOCl) |
| *Fusarium* sp. 2 | RS23 | Purtech (HPR + NaOCl) |
| *Fusarium* sp. 3 | RS2 | Mix tank water |
| *Fusarium* sp. 4 | RS35 | Purtech (Pad 6 + $Na_2S_2O_3$) |
| *Fusarium* sp. 5 | RS12 | Dow hollow fibers (virgin) |
| | RS31 | Purtech (mix 3) |
| *Fusarium* sp. 6 | RS41 | Dow II |
| *Geotrichum* spp. | RS4 | Brackish well water |
| *Penicillium diversum* Raper and Fennell | RS40 | Fluid Systems |
| *Penicillium janthinellum* Biourge (series) | RS39 | Fluid systems |
| *Penicillium* sp. 1 | RS25 | Envirogenics blend membrane (virgin) |
| *Penicillium* sp. 2 | RS30 | Purtech (mix 3) |
| *Phialophora* sp. 1 | RS3,13 | Dow hollow fibers (virgin) |
| | RS5,24 | Envirogenics blend membrane (virgin) |
| | RS32 | Purtech (HPR + NaOCl) |
| | RS36 | Purtech (Pad 5) |
| *Phialophora* sp. 2 | RS16 | [3] FRL citric acid flush |
| *Phialophora* sp. 3 | RS17 | FRL citric acid flush |
| *Trichoderma harzianum* Rifai aggr. | RS14,15 | Dow III |
| | RS28 | Purtech (Pad 6) |
| | RS29 | Purtech (mix 3) |
| | RS37,38 | Fluid Systems |
| yeast (unidentified) | RS6 | Dow I feed water |
| yeast (unidentified) | RS19 | Dow I |
| yeast (unidentified) | RS20 | Dow I |
| other yeasts | | |
| *Candida* spp. | ---- | Dow I |
| *Rhodotorula* spp. | ---- | Dow I & Dow II |
| *Rhodotorula* spp. | ---- | Dow III |
| *Torulopsis* spp. | ---- | Dow I |
| *Torulopsis* spp. | ---- | Dow II and Fluid Systems |

[1] For description and specifications of filters see Tables 1-4.

[2] Membrane Systems Inc., San Diego, Ca.; polysurfone membrane.

[3] Fabric Research Labs, Dedham, Mass.; polysulfone hollow fiber membrane.

Table 6. Bacteria isolated from cellulose acetate reverse osmosis membranes and Roswell Test Facility waters.

| Bacterium | Bacterium |
|---|---|
| *Acinetobacter* spp. | *Lactobacillus coryniformis* var. 2 |
| *Arthrobacter globiformis* | *Lactobacillus* sp. 1 |
| *Arthorobacter* sp. 1 | *Lactobacillus* sp. 2 |
| *Arthrobacter* sp. 2 | *Lactobacillus* sp. 3 |
| *Bacillus brevis* | *Lactobacillus* sp. 4 |
| *Bacillus circulans* | *Lactobacillus* sp. 5 |
| *Bacillus firmus* | *Lactobacillus* sp. 6 |
| *Bacillus spearicus* | *Micrococcus luteus* |
| *Bacillus subtilis* | *Micrococcus rosues* |
| *Flavobacterium aquatale* var. 1 | *Micromonospora* spp. |
| *Flavobacterium aquatale* var. 2 | *Pseudomenas alcaligenes* |
| *Flavobacterium breve* | *Pseudomonas aeruginosa* |
| *Kurthia zophii* | *Pseudomonas flourescens* var 1 |
| *Lactobacillus casei* var. 1 | *Pseudomonas flourescens* var 2 |
| *Lactobacillus casei* var. 2 | *Pseudomonas putida* |
| *Lactobacillus casei* var. 3 | *Pseudomonas* sp. 1 |
| *Lactobacillus coryniformis* var. 1 | *Pseudomonas* sp. 2 |

In general, the fungi are more known for their cellulotytic abilities than are the bacteria. The fungi in Table 5 represent a typical cross section of fungi which might be found in many soils and waters. Acremonium, Aspergillus, Aureobasidium, Phialophora, and Trichoderma have been shown (Domsch and Gams, 1970; Alexander, 1977) to be cellulolytic. Trichoderma is particularly known for its cellulase production. The other fungi in Table 5 are common saprophytes which may be weakly cellulotytic. Several of the genera (Alternaria, Cladosporium, Fusarium, Geotrichum, Candida) are pathogens on plants or animals. The cellulolytic basiodiomycetes are more known for their cellulolytic activities than those fungi in Table 5 but basidomycete fungi are rarely isolated on conventional laboratory media and should not be common in the waters of the RTF or on the membranes of CA-RO modules. The fungi were easily isolated from most RTF waters and membranes. However, due to the sporulating nature of the fungi, their appearance on petri dishes only indicates the presence of 'propagules' which may develop into actively functioning organisms and does not indicate if the organism is growing, much less if it is decomposing CA membranes.

The bacteria listed in Table 6 are also typical soil and water saprophytes. The initial isolation of bacteria resulted in 747 separate isolates that were eventually screened and placed in 34 genera. In contrast to the fungi, the possession of cellulolytic enzymes is comparatively rare. Of the bacteria listed in Table 6, Micromonospora (an actinomycete) is the only genera known to possess considerable cellulolytic activity. However, some species of Arthrobacter, Bacillus, and Pseudomonas are cellulolytic. The genera of bacteria most known for their cellulolytic activities (Cellulomonas, Cytophoga and Sporocytophaga)

were not found. The most common bacteria isolated, Lactobacillus, is generally considered a secondary decomposer and is not usually responsible for the initial attack on a substrate. A previous report (Lauer, 1972) has demonstrated the presence of coliform, iron, and sulfur bacteria at the RTF, and Guy (1975) has identified cellulose in the waters. However, the coliform, iron, and sulfur bacteria are not known cellulolytic organisms, although they may foul water systems, and the presence of cellulose alone is not evidence that CA-RO membranes are being degraded.

Microorganisms in Cellulose Acetate Reverse Osmosis Modules

Except for the Fluid Systems membrane, CA membranes from experienced RO modules showed no discoloration or slime. The Fluid Systems membrane had a slight slime on the rejection surface which contained motile rod-shaped bacteria upon microscopic examination. However, the Fluid Systems membrane was stored in a plastic bag at 25-30°C for 90 days, a period of time that exceeded the operational time (Table 3). The slime probably formed during storage rather than during operation. The Dow III hollow fiber membrane experienced scaling due to the lack of sodium hexametaphosphate or $H_2SO_4$ addition to the feedwater (Table 2). All other membranes had a clean and shiny appearance upon removal from modules and showed no evidence of microbial activity under the compound and dissecting microscopes.

A variety of bacteria and fungi were found on the membranes (Tables 7-9). Fusarium, Pencillium, Phialophora, and Trichoderma were the most frequently encountered fungi, but other genera or groups (Ascomycetes, Mucorales, Mycelia Sterilia) were sporadically found. Acinetobacter,

Arthrobacter globiformis, Bacillus brevis, Flavobacterium aquatale, and Lactobacillus were the most frequently encountered bacteria.

The Dow I, Dow III, Hydronautics, and Purtech 7 (located directly after the manganese green sand filter) had the least microbial variety and the lowest microbial population densities of the membranes examined (Tables 7-11). The Dow II membrane had the greatest microbial variety and highest microbial densities. In general, those membranes receiving chlorine had lower microbial numbers than those receiving no chlorine. The exceptions are the Fluid Systems membrane and Purtech modules 4,5,9, and 10 which received chlorinated feed water (Tables 2-4) but had large microbial diversities and high microbial numbers. However, these membranes could not be cultured directly after shutdown (Tables 3 and 4). Instead, a substantial delay occurred between shutdown of the module and membrane culture. Thus it appears that if favorable conditions and suitable inocula are present in the module at the time of shutdown, microorganisms may proliferate on, but not necessarily degrade, the membrane unless precautions are taken.

The manganese green sand filter (Purtech 7) is a prefilter designed to reduce iron and prevent iron scaling within the RO modules. However, the manganese green sand filter also reduced microbial numbers and diversity when compared to other Purtech modules (Tables 7, 9, and 10).

Although the data indicate a variety of bacteria and fungi can be found on CA-RO membranes, the data do not indicate if the microorganisms are growing on the membrane. Indeed, the membranes may simply be concentrating microbial cells or the microorganisms may be surviving on nutrients concentrated by the membrane. Microorganisms are introduced via the city and well waters, by open air storage tanks and filters, and

Table 7. Frequency of fungi occurrence on cellulose acetate spiral wound membranes as determined by direct isolation and enrichment culture.

| Fungus | Hydro-nautics | Fluid systems | Purtech (location)[1/] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Aureobasidium | 0/0[2/] | 0/7 | 1/0 | 0/0 | 0/1 | 0/0 | 23/0 | 0/0 | 0/0 | 0/0 | 0/2 | 0/0 |
| Fusarium | 0/0 | 1/0 | 100/0 | 97/9 | 0/10 | 55/10 | 30/10 | 0/10 | 3/1 | 100/10 | 1/1 | 5/6 |
| Mycelia Sterilia | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 9/0 | 0/0 | 2/0 | 1/0 |
| Penicillium | 2/0 | 98/15 | 8/0 | 36/0 | 21/1 | 56/3 | 7/1 | 93/0 | 0/0 | 45/0 | 0/0 | 1/0 |
| Phialophora | 0/0 | 0/0 | 0/0 | 2/0 | 17/2 | 85/0 | 87/0 | 0/0 | 3/5 | 0/0 | 99/1 | 10/0 |
| Trichoderma | 0/0 | 13/3 | 1/0 | 5/0 | 0/0 | 0/0 | 0/0 | 3/1 | 0/0 | 0/0 | 0/0 | 0/0 |
| Yeast | 0/1 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 |
| Isolation plates with fungi (per 100) | 2 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 15 | 100 | 99 | 22 |
| Enrichment flasks with fungi (per 10) | 0 | 10 | 10 | 9 | 10 | 10 | 10 | 10 | 6 | 10 | 2 | 6 |

1/See Table 2. for description of location for Purtech filters.

2/The first number is the number of plates (50 of 2% malt extract agar and 50 of half strength cornmeal agar) which had the fungus by direct isolation. The second number is the number of flasks (5 flasks of BMY medium and 5 flasks of BM-CA medium) which contained the fungus by enrichment culture.

Table 8. Frequency of fungi occurrence on cellulose triacetate hollow fiber (Dow) modules by direct isolation.

| | Dow I[1] | | | | | | Dow II[2] | | | | | | Dow III[3] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Feed End | | Middle | | Product End | | Feed End | | Middle | | Product End | | Feed End | | Middle | |
| | Int[4] | Ext[5] | Int | Ext | Int | Ext | Int | Ext | Int | Ext | Int | Ext | Int | Ext | Int | Ext |
| | % of agar plates with fungus | | | | | | | | | | | | | | | |
| Ascomycetes | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.8 | 0 |
| Aspergillus | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cladosporium | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| Fusarium | 0 | 0 | 0 | 0 | 0 | 0 | 98* | 94* | 96* | 96* | 64* | 90* | 0 | 0 | 0 | 0 |
| Mucorales | 0 | 0 | 0 | 0 | 0 | 1.7 | 0 | 2 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mycelia Sterilia | 0 | 1.7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| Penicillium | 0 | 0 | 0 | 0 | 0 | 0 | 8 | 0 | 2 | 4 | 0 | 2 | 0 | 0 | 0 | 0 |
| Phialophora | 0 | 1.7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2* | 0 | 0 | 0 | 0 | 0 |
| Trichaderma | 0 | 0 | 0 | 0 | 0 | 0 | 92* | 100* | 86 | 88 | 98 | 76 | 0 | 0 | 32.7* | 0 |
| Yeast | 1.7* | 18.3* | 0 | 0* | 0 | 1.7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

[1] The Dow I had 60 total plates per sample point (20 plates of 2% malt extract agar, 20 plates of half strength cornmeal agar, and 20 plates of BM-CA agar).

[2] The Dow II had 50 total plates per sample point (25 plates of 2% malt extract agar and 25 plates of half strength cornmeal agar).

[3] The Dow III had 55 total plates per sample point (15 plates of 2% malt extract agar, 15 plates of half strength cornmeal agar, and 25 plates of BM-CA agar).

[4] Interior portion of the fiber bundle.

[5] Exterior portion of the fiber bundle.

* Also found in enrichment cultures.

Table 9. Frequence of bacteria occurrence on cellulose acetate spiral wound (Hydronautics, Fluid Systems, and Purtech) and hollow fiber (Dow) membranes as determined by direct isolation and enrichment culture.

| Bacterium | Hydro[1]/ nautics | Fluid Systems | Partech (location)[2]/ 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Dow 1 | 2 | 3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Acinetobacter spp. | 5*[3]/ | 17* | 0 | 2 | 1* | 4 | 5 | 0 | 2 | 0 | 0 | 1+[5]/ | 0 | 2 | 0 |
| Arthrobacter globiformis | 9* | 3* | 2* | 4+ | 1* | 0 | 2 | 4 | 1* | 0 | 5 | 1* | 0 | 4 | 0 |
| Arthrobacter sp. 1 | 0 | 1* | 0 | 0 | 2 | 0 | 1* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Arthrobacter sp. 2 | 0 | 0 | 0 | 0 | 0 | 0 | 1+ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Bacillus brevis | 1* | 2* | 1* | 2* | 1* | 0 | 2* | 1* | 0 | 2* | 0 | 1* | 0 | 3 | |
| Bacillus circulans | 0 | 0 | 2+ | 1+ | 0 | 0 | 0 | 2+ | 0 | 1+ | 0 | 0 | 0 | 6* | 0 |
| Bacillus firmus | 0 | 0 | 0 | 3+ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 0 |
| Bacillus spearicus | 0 | 2* | 0 | 0 | 0 | 1+ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Bacillus subtilus | 4* | 4* | 0 | 0 | 6* | 0 | 0 | 0 | 0 | 0 | 0 | 1* | 0 | 4+ | 0 |
| Flavobacterium aquatale var. 1 | 1* | 0 | 1* | 1* | 0 | 1+ | 1* | 0 | 1* | 0 | 0 | 2+ | 0 | 2 | 0 |
| Flavobacterium breve | 0 | 0 | 0 | 0 | 0 | 0 | 2+ | 1+ | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Kurthia zophi | 0 | 0 | 0 | 0 | 0 | 0 | 1+ | 0 | 0 | 1+ | 0 | 0 | 0 | 1+ | 0 |
| Lactobacillus casei var. 1 | 0 | 6* | 0 | 0 | 0 | 1* | 0 | 0 | 0 | 1+ | 0 | 0 | 0 | 1+ | 0 |
| Lactobacillus casei var. 2 | 0 | 0 | 1* | 0 | 1* | 1+ | 2+ | 1+ | 0 | 0 | 0 | 0 | 0 | 1+ | 0 |
| Lactobacillus casei var. 3 | 0 | 0 | 0 | 0 | 0 | 0 | 1+ | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 |
| Lactobacillus coryniformis var. 1 | 3* | 4* | 3 | 5 | 1+ | 3 | 6 | 7 | 2 | 1* | 0 | 1* | 0 | 17 | 0 |
| Lactobacillus coryniformis var. 2 | 1* | 0 | 0 | 1* | 1+ | 0 | 0 | 3* | 0 | 0 | 2* | 0 | 0 | 0 | 0 |
| Lactobacillus sp. 1 | 0 | 1* | 0 | 0 | 3 | 0 | 0 | 0 | 1* | 0 | 0 | 0 | 0 | 5 | |
| Lactobacillus sp. 2 | 0 | 5* | 3* | 1* | 2* | 1* | 0 | 4* | 0 | 0 | 3+ | 0 | 0 | 7 | 0 |

Table 9 (Continued). Frequence of bacteria occurrence on cellulose acetate spiral wound (Hydronautics, Fluid Systems, and Purtech) and hollow fiber (Dow) membranes as determined by direct isolation and enrichment culture.

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Lactobacillus sp. 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1* | 0 | 0 | 0 | 0 |
| Lactobacillus sp. 4 | 2* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Lactobacillus sp. 5 | 0 | 6* | 2* | 1* | 1* | 0 | 1* | 1* | 0 | 0 | 0 | 0 | 0 | 12 | 0 |
| Lactobacillus sp. 6 | 1* | 0 | 0 | 1* | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 21 | 0 |
| Micrococcus luteus | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2+ |
| Micrococcus roseus | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1+ |
| Micromonospora spp. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2* | 0 | 0 |
| Pseudomonas alcaligenes | 0 | 0 | 1* | 0 | 1* | 0 | 0 | 0 | 0 | 0 | 2* | 0 | 0 | 0 | 0 |
| Pseudomonas aeruginosa | 3* | 0 | 0 | 0 | 1+ | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 13 | 1* |
| Pseudomonas flourescens var. 1 | 2* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Pseudomonas flourescens var. 2 | 0 | 0 | 0 | 0 | 0 | 1+ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3* | 0 |
| Pseudomonas putida | 1* | 0 | 0 | 0 | 1* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3* | 0 |
| Pseudomonas sp. 1 | 1* | 0 | 0 | 0 | 1* | 1* | 0 | 0 | 0 | 0 | 0 | 1+ | 0 | 2 | 0 |
| Pseudomonas sp. 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2+ | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

1/ No direct isolations taken from Hydronautics, Fluid Systems or Dow I membranes.

2/ See Table 2 for description of location for Purtech filters.

3/ The number indicates the number of times the organism was identified on petri plates.

*indicates organism was isolated by enrichment culture only.

+indicates organism was isolated by direct isolation only.

Table 10. Bacterial population densities of cellulose acetate spiral wound modules.

| Module | Feed Side | | | Product Side | | |
|---|---|---|---|---|---|---|
| | Feed End | Middle | Brine End | Feed End | Middle | Brine End |
| | colony founding units/$cm^2$ | | | | | |
| Hydronautics | ND | 0 | ND | ND | 0 | ND |
| Fluid Systems | ND | 3640 | ND | ND | 169 | ND |
| Purtech | | | | | | |
| 1 | 476 | 122 | 1528 | 0 | 0 | 0 |
| 2 | 83 | 253 | 456 | 20 | 12 | 70 |
| 3 | 235 | 58 | 201 | 5 | 112 | 516 |
| 4 | 277 | 10 | 82 | 339 | 1 | 35 |
| 5 | 20 | 23 | 24 | 3 | 5 | 7 |
| 6 | 91 | 34 | 16 | 69 | 190 | 149 |
| 7 | 1 | 0 | 0 | 11 | 0 | 0 |
| 8 | 68 | 25 | 332 | 3 | 0 | 0 |
| 9 | 13048 | 7112 | 12768 | 8690 | 1440 | 6664 |
| 10 | 6608 | 7504 | 7392 | 1812 | 432 | 432 |

ND = not determined

Table 11. Bacterial population densities of cellulose triacetate hollow fiber (Dow) membranes.

| Module | Feed End | | Middle | | Brine End | |
|---|---|---|---|---|---|---|
| | Int[1/] | Ext[2/] | Int | Ext | Int | Ext |
| | colony forming units/cm$^2$ | | | | | |
| Dow I | 0 | 0 | 0 | 0 | 0 | 0 |
| Dow II | 800 | 730 | 1200 | 1410 | 1400 | 1270 |
| Dow III | 0 | 0 | 770 | 500 | ND | ND |

[1/] Interior portion of fiber bundle.

[2/] Exterior portion of fiber bundle.

ND = not determined.

within the virgin membranes themselves. Many areas within the RTF are conducive to harboring microorganisms or even encouraging growth (activated carbon filters, dead end water pipes, scums on water storage tanks produced by the iron bacteria). Martin (1981) indicated that the bacteria isolated from the CA-RO membranes were similar to the organisms found in the facility waters. The membranes did not harbor different bacteria nor was a genera more dominant on the membrane than in the waters. Martin (personal communication) also indicated that a nutrient was present in the facility waters. When purified agar plates were made with distilled water and inoculated with RTF organisms, no microorganisms developed. However, when purified agar plates were made with facility waters, limited microbial growth occurred.

The fungi numbers may be particularly misleading. Again, the presence of fungal colonies on petri plates only indicates a viable 'propagule' was present at the time of plating. An actively growing fungus could develop in one area of the RTF plumbing system and release thousands of spores to eventually be trapped by RO modules.

Qualitative Tests For Cellulose and Cellulose Acetate Degradation

Before CA membranes can be biologically degraded microorganisms must not only be present but they must have the enzymatic capability to degrade CA. The data in Table 12 indicate that cellulolytic activity was common in the fungi tested. Acid swollen cellulose and filter paper cellulose were utilized by the fungi as sole carbon sources. None of the fungi cleared Eastman CA398-3, phosphoric acid treated blend membrane or cellulose triacetate hollow fibers after incubation in excess of 4 months (data not presented). The fungi grew poorly on blend membrane strips in BS-FW medium but grew quite well in BSY medium. None of

the blend membrane strips showed visual signs of degradation or fragmentation upon vortex agitation after more than 4 months of incubation. Thus, the limited growth of fungi on blend membrane in BS-FW was probably due to low levels of previously discussed nutrient in the RTF waters, while the good growth of fungi in BSY medium was undoubtedly on the yeast extract and not on the blend membrane. However, the fungi grew vigorously on the deacetylated blend membrane strips, and many strips were degraded to the point of fragmentation after several weeks. Gas sterilization (instead of heat sterilization) only slightly increased the degradation of blend membrane strips.

These results show that while most of the RTF fungi are capable of vigorous cellulose degradation, the same fungi are unable to attack intact membranes, suspensions of CA398-3, or suspensions of phosphoric acid treated membranes. The ability to degrade cellulose or the presence of cellulase (as proposed by Guy, 1975) may not be prerequisite to CA degradation. Degradation of the deacetylated membrane is consistent with the tenet (Reese, 1957) that acetylation of cellulose imparts resistance to enzymatic attack. However, cellulose enzymes would be necessary for complete degradation of a CA membrans after removal of the acetate groups.

Only 3 bacterial isolates ( Acenitobacter, Kurthia zophi, and Micrococcus luteus) weakly cleared carboxymethyl cellulose, and none of the isolates degraded filter paper cellulose, with and without yeast extract amendment, after 30 days of incubation. None of the bacterial isolates degraded blend membrane strips in HUT medium with and without yeast extract amendment. However, ATCC isolates (known to be cellulolytic) degraded filter paper but did not degrade blend membrane strips.

Table 12. Qualitative tests for cellulose and cellulose acetate degradative ability of fungal isolates.

| Fungus Number | Genus | Cellulose[1] Clearing Depth (mm) | Filter[2] Paper Growth | Blend[3] Membrane BS-FW | Blend[3] Membrane BSY | Deacetylated[4] Blend Membrane Growth | Deacetylated[4] Blend Membrane (Breakage) |
|---|---|---|---|---|---|---|---|
| RS 18 | Acremonium | 12 | 2[5] | 1 | ND | 2 | |
| RS 26 | Alternaria | 0 | 2 | 1 | ND | 2 | |
| RS 7 | Aspergillus | 12 | 2 | 1 | 2 | 2 | (32) |
| 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 | (24) |
| RS 21 | Cleist. 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 | (17) |
| RS 12 | Fusarium | 16 | 2 | 1 | 2 | 2 | (17) |
| RS 31 | Fusarium | 19 | 2 | 1 | 2 | 2 | (24) |
| RS 41 | Fusarium | ND | 2 | 1 | ND | 2 | |
| RS 4 | Geotrichum | 0 | 0 | 0 | ND | 1 | |
| RS 25 | Penicillium | 15 | 2 | 0 | ND | 2 | (17) |
| RS 30 | Penicillium | 24 | 2 | 1 | 2 | 2 | |
| RS 39 | Penicillium | 21 | 2 | 1 | ND | 2 | (17) |
| RS 40 | Penicillium | 20 | ND | 1 | ND | 2 | (17) |
| RS 3 | Phialophora | 22 | 2 | 0 | 2 | 2 | (24) |
| RS 13 | Phialophora | 21 | 2 | 1 | ND | 2 | |
| RS 5 | Phialophora | 17 | 2 | 1 | 2 | 2 | (17) |
| RS 24 | Phialophora | 16 | 2 | 1 | 2 | 1 | (24) |
| RS 32 | Phialophora | 19 | 2 | 1 | ND | 2 | |
| RS 36 | Phialophora | 20 | 2 | 1 | ND | 1 | |

Table 12 (Continued). Qualitative tests for cellulose and cellulose acetate degradative ability of fungal isolates.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 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 | (17) |
| RS 28 | Trichoderma | 26 | 2 | 0 | 2 | 2 | (17) |
| RS 29 | Trichoderma | 21 | 2 | 1 | ND | 2 | (32) |
| RS 37 | Trichoderma | 18 | 2 | 1 | 2 | 2 | (24) |
| RS 38 | Trichoderma | 26 | 2 | 0 | 2 | 2 | (24) |
| RS 6 | Yeast | 12 | 2 | 1 | ND | 2 | |
| RS 19 | Yeast | 0 | 0 | 0 | ND | 0 | |
| RS 20 | Yeast | 0 | 0 | 0 | 0 | 0 | |

1/ Depth of clearing of acid swollen cellulose in BS medium, 36 days from inoculation.

2/ Growth on filter pater in HUT medium, 11 days from inoculation.

3/ Growth on blend membrane strips in BS-FW medium (31 days from inoculation) and BSY medium (22 days from inoculation).

4/ Growth on deacetylated membrane strips in BM-FW medium (11 days from inoculation) and the number of days until strip breakage if breakage occurred.

5/ 0 = growth; 1 = slight or questionable growth; 2 = good growth; ND = not determined.

Neither the Sinclair isolates nor the Sinclair enrichment culture degraded the blend membrane strips in HUT medium with and without yeast extract amendment (data not presented).

Apparently the RTF bacterial isolates did not have the enzymatic ability to degrade cellulose or CA, as opposed to the fungi which at least demonstrated cellulolytic abilities. As with the fungi, those bacteria demonstrating cellulolytic abilities (ATCC isolates) were not capable of degrading reprecipitated CA or CA strips.

Several CA membranes were examined under the scanning election microscope at New Mexico State University. Bacteria cells and fungi hyphae were observed on the membrane surface, and several holes or indentations were apparent. However, the organisms did not appear to be dissolving the membrane or otherwise causing the surface aberrations. The organisms appeared to be using the membrane surface as a support only. The surface aberrations were attributed to physical damage or membrane imperfections.

Several organisms were inoculated onto cellulose triacetate hollow fibers in BS (fungi) and nutrient broth (bacteria) media and incubated for 2, 4, and 8 weeks. The fibers were sent to Dow Chemical Company for performance testing and scanning electron microscopy. The data (Table 13) indicate no significant decline in percent salt rejection with any of the organisms tested. Scanning election microscopy again showed the presence of bacteria cells and fungal hyphae. Surface aberrations were attributed primarily to physical damage and not degradation. However, the microscopist (Dr. Ng, Dow Chemical Company) thought that the Trichoderma inoculated sample may have produced some damage on membrane surface.

Additional performance tests were performed on 3 cultures (2 fungi and 1 CA enrichment culture) after electron microscopy examination indicated possible damage to cellulose triacetate fibers. However, the data (Table 14) again indicate no significant decline in salt rejection or increase in flux.

Mr. Terry Marsh (Dow Chemical Company) concluded (personal communication) that microbiological degradation was not the cause of cellulose triacetate hollow fiber degradation.

Quantitative Tests for Cellulose Acetate Degradation

Several quantitative biochemical tests were performed on CA preparations. If microbial degradation of CA was occurring, several indicators should be apparent in the degradative process:

(1) the CA should lose acetyl groups resulting in a reduction of percent acetyls on the CA molecule, and/or

(2) the viscosity of the CA solution should decrease as the cellulose polymer is degraded, and/or

(3) the amount of reducing sugars should increase in solution as the glucose units are cleaved from the CA molecule.

An organism which degrades CA may not produce 3 positive tests. For example, some species of cellulose degraders do not release reducing sugars into the medium.

The data from the quantitative CA degradation tests are given in Tables 15-17. In general, no organism was found to degrade CA, although values were sporadically statistically significant.

The acetyl contents of CA400-25 (Tables 15 and 16) were generally lower than the uninoculated controls. The difference between the control

Table 13. Dow Chemical Company performance tests of cellulose triacetate hollow fibers after incubation with various microbial isolates.

| Organism | 8 Week Incubation | | 4 Week Incubation | | 2 Week Incubation | |
|---|---|---|---|---|---|---|
| | Flux | Salt Rejection | Flux | Salt Rejection | Flux | Salt Rejection |
| | gal/ft$^2$/day | ----%---- | gal/ft$^2$/day | ---%--- | gal/ft$^2$/day | ----%---- |
| Control | 5.7 | 97.5 | 7.9 | 97.4 | 7.3 | 96.5 |
| Fungi[1/] | | | | | | |
| Aureobasidium | 7.0 | 96.4 | 6.8 | 97.3 | 7.6 | 96.8 |
| Phialophora | 7.3 | 96.9 | 7.7 | 96.4 | 6.6 | 96.3 |
| Trichoderma | 6.9 | 97.0 | 7.2 | 97.6 | 7.6 | 95.4 |
| Bacteria[2/] | | | | | | |
| Bacillus | 7.2 | 97.1 | 6.9 | 97.9 | 7.5 | 96.3 |
| Pseudomonas | 6.2 | 97.7 | 7.2 | 95.5 | 7.8 | 95.4 |
| Bacillus | 6.8 | 98.3 | 7.1 | 97.2 | 7.0 | 96.7 |

[1/] Fungi grown in BS-FW medium.

[2/] Bacteria grown in nutrient broth-FW medium.

Table 14. Dow Chemical Company performance tests of cellulose triacetate hollow fibers after incubation with various microbial isolates.

| Organism | Flux | Salt Rejection |
|---|---|---|
| | -----gal/ft$^2$/day----- | --------%------- |
| Control | 7.69 | 95.4 |
| *Trichoderma*[1/] | 7.83 | 96.4 |
| *Candida*[2/] | 7.32 | 97.8 |
| Enrichment Culture[3/] | 7.34 | 95.2 |

[1/] 8 weeks growth in BM plus yeast extract medium.

[2/] 8 weeks growth in BM-FW medium.

[3/] 8 weeks enrichment in BM-CA398-3 medium.

values of Tables 15 and 16 reflects the amount of CA put into the medium and the difference between individuals running the analyses. The acetyl content of the standard as determined by Eastman was 39.45% and compares very closely with the acetyl content of the control in Table 15. The analysis of variance indicated no significant differences between acetyl contents for either of the analyses (Table 15 and 16). The ranges of acetyl content was less than 1% in each analysis.

The intrinsic viscosities for CA400-25 samples were generally higher than the control values for the bacteria (Table 16) and lower than the control values for the fungi (Table 15). However, the control for the bacteria analyses was too low and was not included in the analysis of variance. The intrinsic viscosities for bacteria (Table 16) were not significantly different, but the intrinsic viscosities for the fungi were statistically different at the $P = 0.01$ level. The range of the fungi values was very narrow (0.10 dl/g), and only 1 value (Fusarium sp. 3) was significantly different than the other values. Because the Fusarium value was higher than the control, depolymerization of the CA polymer did not occur. The high value was probably experimental error.

The reducing sugar contents for CA400-25 were statistically different for the fungi (Table 15) but not for the bacteria. Most of the reducing sugar values for the fungi and bacteria were higher than the control (Table 15 only). These higher values probably reflect very limited growth of the bacteria and fungi on the previously described but unidentified nutrient in the RTF waters, introduction of sugars upon inoculation of the flasks, or the result of B-glucosidase activity on short fragments of CA (Halliwell, 1962). The amounts of reducing sugars

Table 15. Quantitative tests for cellulose acetate degradation with fungal isolates. The flasks of BM-FW medium contained 3.5g of CA400-25 per 100ml of medium. Incubation time was 67 days at 25°C.

| Fungus | Acetyl Content | Intrinsic Viscosity | Reducing Sugar Content |
|---|---|---|---|
| | --%(w/w)-- | ---dl/g-- | --nm/ml-- |
| Control | 39.47a[1/] | 1.51b | 11.8a |
| Aurebasidium RS1 | 39.41a* | 1.52b* | 24.0ab* |
| Cladosporium RS42 | 38.96a | 1.53b | 29.7ab |
| Fusarium sp.1 RS22 | 39.00a | 1.49b+ | 15.1bc |
| Fusarium sp.3 RS2 | 39.10a | 1.58a | ND |
| Fusarium sp.5 RS12 | 39.21a | 1.50b | 17.1abc |
| Fusarium sp.5 RS31 | 39.30a | 1.48b | 32.0a |
| Penicillium RS30 | 38.96a | 1.50b | 28.5ab |
| Phialophora sp.1 RS13 | 39.47a | 1.48b | 7.6c |
| Phialophora sp.1 RS24 | 39.21a | 1.48b | 14.0bc |
| Trichoderma RS15 | 39.27a | 1.46b | 18.7abc |
| Trichoderma RS28 | 39.98a | 1.48b | 22.7abc |
| Yeast RS20 | 39.24a | 1.51b+ | 21.5abc |

[1/] Means followed by the same letter are not significantly different at the 5% probability level by the Student Newman Kuels multiple comparison test.

n = 3 unless otherwise indicated; *n = 1; +n = 2; ND = not determined.

Table 16. Quantitative tests for cellulose acetate degradation with bacterial and yeast isolates. The flasks of HUT-FW medium contained 2.0g of CA400-25 per 100ml of medium. Incubation was 60 days at 25°C.

| Bacteria | Acetyl Content | Intrinsic Viscosity | Reducing Sugar Content |
|---|---|---|---|
| | %(w/w) | --dl/g--- | --nm/ml- |
| Control | 38.62 | 1.37 | ND |
| Acinetobacter spp. | 38.16 | 1.89 | 28.8 |
| Arthrobacter globiformis | 37.92 | 1.45 | 25.6 |
| Arthrobacter sp. 1 | 38.73 | 1.53 | 23.0 |
| Arthrobacter sp. 2 | 38.37 | 1.24 | 27.8 |
| Bacillus brevis | 37.57 | 1.55 | 27.3 |
| Bacillus circulans | 38.45 | 1.44 | 26.8 |
| Bacillus firmus | 38.35 | 1.42 | 27.6 |
| Bacillus sphearicus | 38.56 | 1.35 | 32.4 |
| Bacillus subtilis | 38.74 | 1.54 | 24.8 |
| Flavobacterium aquatale var. 1 | 38.65 | 1.44 | 31.6 |
| Flavobacterium aquatale var. 2 | 38.19 | 1.73 | 32.2 |
| Flavobacterium breve | 38.51 | 1.41 | 25.0 |
| Kurthia zophii | 37.96 | 1.42 | 20.4 |
| Lactobacillus casei var. 1 | 37.98 | ND | 26.3 |
| Lactobacillus casei var. 2 | 38.31 | ND | 27.0 |
| Lactobacillus casei var. 3 | 37.96 | ND | 28.2 |
| Lactobacillus coryniformis var. 1 | 38.09 | ND | 26.9 |
| Lactobacillus coryniformis var. 2 | 37.92 | ND | 23.8 |
| Lactobacillus sp. 1 | 38.42 | ND | 22.4 |
| Lactobacillus sp. 2 | 38.10 | ND | 29.6 |
| Lactobacillus sp. 3 | 37.92 | ND | 27.9 |
| Lactobacillus sp. 4 | 38.16 | ND | 32.0 |
| Lactobacillus sp. 5 | 38.17 | ND | 33.7 |
| Lactobacillus sp. 6 | 38.03 | ND | 29.1 |
| Micrococcus luteus | 38.00 | ND | 27.8 |
| Micrococcus rosues | 38.23 | ND | ND |
| Micromonospora spp. | 38.05 | 1.62 | ND |
| Pseudomonas alcaligenes | 38.14 | 1.27 | 22.6 |
| Pseudomonas aeruginosa | 38.40 | 1.56 | 30.0 |
| Pseudomonas flourescens var. 1 | 37.95 | 1.24 | 31.5 |
| Pseudomonas flourescens var. 2 | 37.96 | 1.41 | 28.4 |
| Pseudomonas putida | 38.47 | 1.43 | 30.8 |
| Pseudomonas sp. 1 | 38.47 | 1.48 | 33.7 |
| Pseudomonas sp. 2 | 38.09 | 1.43 | 26.0 |
| Candida spp. | 38.14 | 1.58 | 46.4 |
| Rhodotorula spp. | 39.58 | 1.26 | ND |
| Rhodotorula spp. | 38.28 | 1.51 | ND |
| Torulopsis spp. | 38.77 | 1.54 | 39.2 |
| Torulopsis spp. | 38.09 | 1.73 | 36.2 |

Table 17. Quantitative tests for cellulose acetate degradation with fungal isolates, Sinclair isolates, a Sinclair enrichment culture, and a Cellulomonas (ATCC 21681) culture. The flasks of BSY medium contained 1.5g of CA394-45 per 100ml of medium. Incubation time was 30 days at 25°C except for ATCC 21681 which was incubated for 18 days.

| Organism | Acetyl Content | Intrinsic Viscosity | Reducing Sugar Content |
|---|---|---|---|
| | %(w/w) | ---dl/g--- | -nm/ml- |
| Control+ | 38.05 | 1.38 | 146.6 |
| Cladosporium RS42 | ND | 1.43 | 59.5 |
| Fusarium sp. 4 RS35 | 37.82 | 1.38 | 85.5 |
| Fusarium sp. 5 RS12 | 37.93 | 1.41 | 92.7 |
| Fusarium sp. 5 RS31 | 38.24 | 1.40 | 90.5 |
| Penicillium | 37.78 | 1.41 | 144.0 |
| Phialophora sp. 1 RS24 | 37.63 | 1.39 | 117.1 |
| Phialophora sp. 2 RS16 | 37.95 | 1.36 | 118.5 |
| Trichoderma RS15 | 37.98 | 1.39 | 115.5 |
| Trichoderma RS28 | 38.08 | 1.41 | 140.1 |
| Sinclair 209 | 38.28 | 1.38 | 147.9 |
| Sinclair 226 | 38.17 | 1.36 | 127.3 |
| Sinclair C257 (mixed culture+) | 38.06 | 1.40 | 61.0 |
| ATCC 21681* | 38.07 | 1.41 | 140.8 |

n = 2 unless otherwise indicated.

*n = 3

+n = 4

Table 18. Quantitative tests for cellulose acetate degradation of enrichment cultures. The flasks of BS medium contained 1.0g of CA398-3 per 100ml of medium. Incubation time was 110 days at 25°C except for the Dow samples which were incubated for 75 days.

| Enrichment Culture Source | Acetyl Content | Intrinsic Viscosity | Reducing Sugar Content |
|---|---|---|---|
| | %(w/w) | --dl/g-- | -nm/ml- |
| Control | 39.98±1.21* | 0.80±0.21* | ND |
| Hydronautics | 39.94 | ND | 0 |
| Fluid Systems | 39.58 | ND | ND |
| Purtech | | | |
| 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 produce | ND | 0.90 | 3.9 |
| Dow II feed side | ND | 1.02 | 8.2 |

*95% confidence interval, n = 3.

produced were so low that it is doubtful if sugar production was biologically significant. However, Penicillium RS30 and Cladosporium RS42 produced the greatest amounts of reducing sugars as well as the largest declines in acetyl contents. This correlation may be evidence for cellobiose octaacetase and B-glucosidase activity on CA oligomers, although the correlation between reduction of acetyl content and production of reducing sugars for all fungi is weak ($r = -.25$).

Yeast extract was used in the CA394-45 medium to determine if CA degradation might require an alternate carbon source or a more nutritionally complete environment (Table 16). Growth of the microorganisms was vigorous, especially fungal growth where the mycelia often sequestered the bulk of the powdered CA. The analysis of variances of the acetyl contents and intrinsic viscosities were not significant. The analysis of variance for reducing sugar content was significant, but the significance lies in the values lower than the control and was 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 because significant reductions in CA acetyl content and intrinsic viscosity did not occur. Bacterial cultures from Dr. Sinclair, including a primary enrichment culture, containing a degraded CA strip, and one Cellulomonas culture did not demonstrate CA degrading ability.

To further explore the possibility that CA decomposition might require the concerted efforts of several organisms, CA398-3 enrichment cultures were analyzed. Unfortunately, there was not enough material from each sample point to determine both acetyl content and intrinsic

viscosity or to perform analyses in replicate. However, control samples were performed in triplicate and 95% confidence intervals calculated. No large declines in either acetyl content or intrinsic viscosity were seen. All values for acetyl content fell within the 95% confidence interval. All values for intrinsic viscosity fell within the 95% confidence interval except for the Purtech 2 and Dow II feed side samples which were higher than the control confidence interval. The amounts of reducing sugars in the medium were negligible and no controls were analyzed. Degradation of CA in the enrichment cultures did not occur or was negligible.

## DISCUSSION

At the beginning of this study it was assumed that CA-RO membrane degrading microorganisms were present and actively metabolizing CA within the RTF. This assumption was based on:

(1) The report of Guy (1975) including the finding of cellulase within the RTF waters, the reporting of slimy black failed CA modules, and the premature failure of CA-RO modules when no other cause could be identified.

(2) The manuscript of Cantor and Mechalas (1969) during a literature search.

(3) The conversations with the former RTF manager, Mr. John Newton, concerning CA-RO membrane failure and the notoriety concerning the existence of a CA degrading microorganism unique to Roswell.

(4) The unexplained failure of CA-RO modules at the RTF.

However, the organisms isolated from RTF waters and from experienced and/or failed membranes were not capable of degrading CA in a variety of

qualitative and quantitative tests. Most of the isolates were common water and soil saprophytes. Although most of the fungal isolates demonstrated cellulolytic abilities, none of the bacterial isolates were strongly cellulolytic. Even the cellulolytic fungi were not capable of CA degradation.

In order to prove CA-RO membrane failure at the RTF is caused by microorganisms at least 3 criteria must be met:

(1) Organisms must be present on the membrane.

(2) Organisms on the membrane surface must have the ability to degrade the membrane under the environmental conditions of the working module.

(3) Organisms meeting the previous 2 criteria must be able to cause a performance decline in membranes when inoculated onto membranes.

The first criterion was easily demonstrated. Organisms were present on the membrane surfaces. On some membranes (Dow II, Fluid Systems, and Purtech 9 and 10) the microbial densities and population diversities were high. In general, the densities and diversities of the microorganisms were dependent upon chlorine concentration and the length of time between module shutdown and culture. The Fluid Systems membrane was so contaminated that a slight slime was evident. Yet the mere presence of microorganisms does not indicate degradation is taking place. Indeed, the growth of microorganisms on the surface of a CA-RO membrane does not indicate CA degradation. Because the CA-RO membrane is a filter, the organisms and substrate for the organisms can simply be trapped on the membrane surface.

The second criteria for proof of CA-RO membrane degradation was not met. None of the isolates, enrichment cultures, Sinclair isolates or enrichment cultures, or ATCC cultures were shown to degrade various CA preparations, membrane strips, or hollow fibers, and no microbial damage was observed on membrane samples taken for microscopic examination.

Cellulose acetate is a polymer of B(1-4) linked glucose units with varying degrees of acetate substitution of the anhydroglucose units. As the DS increases, the susceptibility to microbial degradation decreases (Reese, 1957) and the salt rejection capabilities increase (Lonsdale, 1966; Smith et al., 1970). Generally, one substitution (DS = 1.0) is thought to be sufficient to confer protection from enzymatic attack, but because the DS represents an average value, a somewhat higher DS is required to insure at least one acetate per anhydroglucose unit. Even a DS = 2.0 does not guarantee 1 acetate on each anhydroglucose unit. A CA of DS = 2.5 or greater is probably immune to microbial attack whereas membranes of less than DS = 2.5 have been shown to degraded by cellulolytic bacteria and fungi (Cantor and Michalis, 1969; Reese, 1957). The degree of polymerization of the cellulose backbone does not appear to markedly affect the performance of the membrane (Manjikian, 1965). However, degradation of the cellulose backbone in a functioning CA membrane could cause a loss of structural integrity and ultimate decline in membrane performance.

The method of degradation of CA could take several avenues, but the most likely mode of degradation is the cleavage of acetates (acetyl esterase) from anhydroglucose units and then cleavage of the cellulose polymer (cellulase enzyme complex including Cl enzyme, B(1-4) glucanase or Cs enzyme, and B glucosidase). However, if the acetate DS imparts

much of the salt rejection capabilities, only acetyl esterase would seem to be needed by an organism to cause a decline in membrane salt rejection, and cellulolytic ability would not be necessary. Degradation of the cellulose polymer without first cleavage of the acetates is unlikely.

Nevertheless, Reese (1957) demonstrated that cellulolytic fungi were able to completely degrade soluble CA (DS = 0.76). The fungi not only produced cellulose and B glucosidase but also produced acetyl esterase and cellobiose octaacetase that deacetylated oligomers of CA such as cellobiose octaacetate. The same fungi were not able to degrade cellulose triacetate. Apparently the ability of the fungi to degrade CA was contingent on the presence of adjacent unsubstituted anhydroglucose units. When such adjacent unsubstituted anhydroglucose units occur, cellulose is able to cleave the polymer into oligomers. An adequate distribution of such points results in oligomer size fragments that can be deacetylated by cellobiose octoacetate. Thus the failure of microorganisms to degrade CA with high degrees of substitution can be explained. High degrees of substitution (DS = 2.5 or greater) prevent adjacent unsubstituted anhydroglucose units and the initial attack on the CA polymer.

Early CA membranes were commonly constructed of a single CA, usually CA398-3 (DS = 2.45), whereas membranes constructed of a blend of cellulose diacetate (generally DS = 2.4-2.5) and cellulose triacetate (generally DS = 2.86) are currently in widespread use (Saltonstall, 1977). Membranes are also constructed solely from cellulose triacetate in hollow fiber configuration such as in the Dow units. Thus, the newer CA membranes should be immune from microbial attack as long as the acetates remain intact.

Yet several reports have documented the susceptibility of CA to degradation. Cantor and Mechalas (1969) had dramatic membrane failures with membranes prepared in their laboratory. Salt rejection went from 90% to 0% in 350 hours of operation using CA383-40, but the CA432-130 never showed signs of failure. Membranes placed on nutrient agar plates completely disintegrated and virtually disappeared after 3 months of incubation. However, Cantor and Mechalas prepared their own membranes rather than using commercially prepared membranes, used nutrient agar as a medium for CA membrane disintegration tests, and did not control pH (assumed to be neutral or above) as is commonly done in RO facilities. Cantor and Mechalas showed that CA membranes could be degraded under ideal conditions but failed to prove that CA degradation can occur under functioning RO conditions.

Sinclair (1981) found cellulose and CA degrading bacteria at the Yuma Test Facility. Sinclair stated "it is without question that microorganisms that have the ability to decompose cellulose and cellulose acetate do indeed occur in source water" at the Yuma Test Facility. The majority of Sinclair's work delt with cellulose degradation rather than with CA degradation, but several isolates, six of which were shown to be fluorescent Pseudomonas species, were capable of CA membrane strip deterioration. However, the degradation of the CA strips occurred in yeast extract amended media prepared with canal water (pH 7.6 or greater), and the strips always deteriorated at the media-air interface. Sinclair had no evidence that RO membrane material was degraded 'in situ' because all isolates were taken from the canal (feed) water before entering the plant and Sinclair was doubtful if CA degrading bacteria from the canal water could survive the pretreatment conditions and multiply on the

membranes. Indeed, the pH, nutrient concentration, microbial populations, and DS of functioning CA-RO modules would be quite different. As with the Cantor and Mechalas (1969) study, Sinclair showed that microorganisms could degrade CA with DS = 2.5 or less under optimum conditions, but did not prove if CA-RO membranes could be degraded within the Yuma Test Facility.

Assuming the feedwater supply to the CA-RO modules was kept at optimal conditions, none of the modules examined in this study (Dow units, Purtech units, Hydronautics unit, Fluid Systems unit) experienced performance declines due to microbial degradation of the CA membrane. This statement is based on the following:

(1) The Dow units were manufactured from cellulose triacetate membrane material which is known (and proven) to be immune from microbial attack. The Dow I unit (chlorinated) had the most drastic decline in membrane performance (salt rejection percent) but had the least microbial contaminants. The Dow II unit had the least decline in performance but had the most microbial contaminants (Dow II was not chlorinated). The Dow III unit (chlorinated) had a small decline in performance and had very few contaminating organisms. If the decline in salt rejection of the Dow units was caused by microbial degradation, some correlation between microbial numbers and membrane failure should be evident. The "competitive species" theory as presented by the Planning Research Corporation (1980) is not a viable hypothesis to explain the Dow units' performances.

(2) The Purtech units were manufactured from a 50-50 blend of cellulose diacetate and cellulose triacetate, the DS of which should be between 2.5 and 2.86. All 10 of the modules were clean and shiny with no visible microbial contamination. The salt rejection data is not stable enough to determine if any loss in performance occurred.

(3) The Hydronautics unit was also manufactured from a blend of cellulose diacetate and cellulose triacetate. No visual signs of microbial growth or microbial degradation were observed and the microbial density was low.

(4) The Fluid Systems membrane probably had the lowest DS of any of the membranes, but the exact DS was not known. No damage to the membrane was observed after microscopic examination, even though microbial numbers were high and a slight slime was evident. The high microbial numbers were attributed to growth after shutdown. The lead element of the four element module was the only element with performance problems. The Planning Research Corporation (1980) attributed the decline of the lead element, and subsequent decline of a replacement element, to excessive pressure or chlorine effects on the membrane. As with all the filters, no CA degrading microorganisms were among the organisms isolated from the Fluid Systems membranes.

However, previously CA-RO membrane failures at the RTF may have been due to microbial degradation. Older CA membranes were constructed of CA, cellulose diacetate, and blends with degrees of substitution less than 2.5. Such membranes would be more susceptible to degradation than those now in use. More investigation of previous membrane failures,

including the events and conditions leading to the failure and the DS of the membrane, needs to be performed at the RTF. Some of the reported cases of black, slimy and degraded CA modules (Guy, 1980) pertain not to the membranes themselves but to the cellulose prefilters. Many cellulolytic fungi were found at the RTF which could quickly degrade unsubstituted cellulose. Caution must also be used when reporting the presence of cellulase enzyme. Cellulase alone will not degrade substituted cellulose.

CA-RO membrane failure may be caused by other factors including microbial fouling, chemical scaling, membrane compaction, mechanical failure, etc. Deacetylation by suboptimum pH conditions or other chemical interactions could be particularly serious. Not only are the salt rejection capabilities lessened but the cellulose polymer backbone would be more prone to microbial attack as previously discussed.

The feedwater pH is particularly critical. Vos et al., (1966) showed that the hydrolysis rate of CA398-3 was approximately 30 times greater at pH 8 than at pH 6. Thus, high pH feedwater in operating RO systems could render a membrane more prone to microbial attack by removing acetates from the cellulose polymer or highly substituted CA membranes (cellulose triacetate for example) normally not susceptible to attack, may experience chemical deacetylation to the point of easy microbial degradation. The previous reports of Cantor and Mechalas (1969) and Sinclair (1981) indicated CA degradation 'in vitro'. Yet the degradation occurred in media or canal water where the pH was 7 or greater, favoring CA hydrolysis and thus bacterial growth. In the RTF (and at the Yuma Test Facility) the feedwater pH is maintained at 6 or less, not only discouraging CA hydrolysis but bacterial growth in general. However, most fungi grow quite well at low pH's. Vos et al.,

(1966) also showed that temperature accelerated CA hydrolysis which might help to explain why most of the CA-RO membrane failure at the RTF occurred during the summer months.

Chemical damage to CA membranes may also result in a direct loss of membrane performance or cause deacetylation and eventually microbial degradation. Levels of feedwater residual chlorine appeared directly related to the magnitude of rejection declines experienced by RTF CA-RO modules. The Kendall's coefficient of rank correlation (Soka and Rohlf, 1969) between b and residual chlorine for the 7 modules in Table 3 is 0.98. The relationship between rejection declines and chlorination is clearly demonstrated in the performance of Dow II vessel A. After a year of operation, the rejection decline was less than a percentage point. When feedwater chlorination was started the rejection dropped 3 percentage points in 2 months. The slope of the regression of the rejection decline after chlorination is an order of magnitude greater than the slope before chlorination (Table 3). It is not known if membrane performance declines associated with chlorination are a result of a direct reaction of hypochlorous acid with CA or involves other chemical entities such as transitional metal ions as suggested by Mr. A. R. March, Dow Chemical Company (personal communication). Cellulose is subject to a nonspecific oxidation by hypochlorite (Nevell, 1963), but McClutchen and Glater (1980) showed that performance of CA membrane was not affected by short exposures (10-23 days) to high levels of chlorine (3-30 ppm). Nevertheless, the Reverse Osmosis Technical Manual (Anon, 1979) recommends a maximum residual chlorine level of 1ppm for CA and cellulose triacetate membranes.

Dr. J. H. Hageman (Chemistry, New Mexico State University) also expressed concern for metal ions (particularly manganese ions) interfering with CA. Dr. Hageman (personal communication) suggested that manganese ions may deacetylate CA membranes and was mildly surprised when told of manganese green sand filters placed before the CA-RO modules.

Although this study failed to isolate CA decomposers at the RTF, organisms with the capability to degrade CA of less than DS=2.5 have clearly been identified elsewhere. However, the previously discussed reports of Reese, Sinclair, and Cantor and Mechalas demonstrated CA degradation generally under conditions that were very favorable to microbial growth (high nutrients), chemical deacetylation (pH7 to pH8), with membranes or CA preparations of less than DS = 2.5, and with organisms not isolated from functioning CA-RO membranes. In contrast, functioning CA-RO membranes generally operate under conditions of low pH (6 or less), with low or nil levels of organic and inorganic nutrients, and recently with membranes constructed of triacetate or diacetate and triacetate blends with high degrees of acetate substitution. In addition, the occurrence of low microbial densities with high rates of salt rejection and moderate levels of chlorine does not lend support to a microbial etiology for membrane performance. Failure to keep membrane feedwater at low pH or other conditions conducive to deacetylation of CA membranes and the use of CA membranes with less than DS = 2.5 could result in microbial degradation. Microorganisms may have caused CA-RO membrane failure at the RTF in the past, but no evidence or organisms were found to support the microbial etiology theory during this study.

## SUMMARY

No evidence or microorganisms were found to support the microbial etiology for CA-RO membrane failure at the RTF. However, microbial degradation may have occurred in the past and could occur in the future if CA membranes are used with low degrees of acetate substitution and/or the membranes are exposed to conditions which favor CA deacetylation. Feedwater pretreatment was critical in determining the number and diversity of organisms on the membrane and may be more important in influencing membrane function than is otherwise thought. Chlorine and possibly manganese ion concentration effects on membrane function should be further explored.

Most of the membrane contaminating organisms were common soil and water saprophytes, some of which were shown to be cellulolytic. Entry into the RTF plumbing system probably occurs through the city and well waters, but contamination could also occur via dust fall into storage tanks, mix tanks, and filter beds.

Prevention of future microbial problems should center on currently used sanitation methods. Chlorine was effective in reducing microbial numbers on membranes but was also correlated with membrane salt rejection decline. Prevention of microbial slime formation in storage tanks, eliminating dead water areas within the plant, and monitoring source waters for CA degrading microorganisms are also recommended. The use of CA membranes with degrees of acetate substitution of 2.5 or greater and the monitoring of feedwater pH and chemical composition should be the most effective means of prevention. Acidification of all source waters as soon as they enter the plant and maintaining a low pH of all waters within the plant would discourage bacterial growth.

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