Fungal Metabolism of Cellulose Acetate RO Membranes

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FINAL REPORT

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ABSTRACT

Based on the data presented in this study, microorganisms can degrade wet cellulose acetate (CA) reverse osmosis (RO) membranes during shutdown periods or under storage conditions. Using uniformly labeled ^{14}C cellulose as the starting compound, CA polymer was synthesized, casting solutions were made, and CA membranes were produced. After inoculating membrane samples with the microorganisms, the metabolism of the membranes was confirmed by measuring isotopically enriched carbon dioxide gas from either the Krebs cycle or anaerobic respiration . The dense zone of the asymmetric membrane, where desalting occurs, could be compromised in three months or less. Treated membrane samples, produced by sanding and hydrolyzing the membrane surface, degraded at a faster rate. Removing oxygen from the system failed to slow or stop the rate of growth. Data did not support any "special" fungus from the RO industry unique in degrading cellulose acetate membrane; instead, all organisms tested degraded these membranes, including microorganisms found in the biofilms from a field RO system and microorganisms present as laboratory contaminates.

INTRODUCTION

The original spiral-wound RO elements (see Figure 1) developed in the 1970's used CA membranes. Today, CA membranes remain industry workhorses for dirty waters that would severely foul the newer polyamide (PA) membranes, which have a rougher surface. CA membranes can operate on chlorinated feed water while PA membranes degrade rapidly in the presence of free chlorine.

Disadvantages of CA membranes relative to PA membranes include their more limited pH operating range, higher feed pressures required to produce the same flow of product water, and lower salt rejections.

The U.S. market for spiral-wound RO and nanofiltration elements is approximately $230 million a year and is growing at about 8% a year (personal communication, November 1999, Anna Cruell, editor, "Reverse Osmosis Modules and Equipment," report RLGB-147R, by Business Communications Co., Inc. 1998). Approximately 80 percent of all elements use PA membranes. About 20 percent use CA membranes because CA membranes operate better than PA membranes on dirty water and are more resistant to oxidizing disinfectants.

During storage or under shutdown conditions, molds are known to grow in the vicinity and on the membrane surface (1) that causes concern for the membrane's integrity. There are reports

that claim CA membranes cannot be degraded by microorganisms (2,3), others claim that this occurs (4-8). Others authors report microbial degradation may or may not occur depending on conditions such as the degree of substitution, whether hydrolysis occurred, and/or the type of contaminating microorganism present (9-13). This has resulted in the indefinite statement in Perry's Chemical Engineers'Handbook (14) that CA membranes may be affected by "possible biodegradation."

BACKGROUND

Cantor et al (4) reports on the biological degradation of CA RO membranes. These authors' data show that increased acetyl substitution, and not molecular weight, improves the resistance to degradation. After three months incubation, the data and pictures show that membranes placed on the surfaces of nutrient agar plates degrade to the extent that only a haze remains of the original membrane. Unfortunately, these findings cannot be extrapolated to clean membranes in storage because another carbon source may be used by the microorganisms such as the agar itself. The organic molecules produced as the result of metabolism may, with or without the collaboration of microorganisms, degrade the membranes. Another limitation is the choice of neutral pH. It is generally accepted to run and store CA membranes at a pH of 5.5 or lower. This higher pH used by these authors may result in non-representative species of microorganisms growing on the membrane and/or a faster rate of physical hydrolysis (15). Also, the conclusions are based on attributing the change in transport properties (salt rejection and water flux) to biological degradation. It is not clear how the two relate and, at best, this is an indirect measurement.

On the other hand, Ho et al.(2) reports on the inability of microorganisms to degrade CA RO membranes. This data show that operational RO units are not degraded by microorganisms. In an operating system, microorganisms would be pre-treated with chlorine and removed by filtration to prevent fouling. Thus, the desalting process is not threatened by biodegradation when operating conditions are adjusted correctly, however there is no information that can be extrapolated from this work to shutdown or storage times. Other limitations were static tests performed on the polymer and not membrane samples, colorimetric procedures subject to chemical interferences, ambiguous laboratory results such as the formation of small amounts of reducing sugar which could be interpreted to support arguments for degradation of CA. Also, no "direct attachment" by microorganisms to the membranes conflicts with the findings of others (16-18). Finally, as in Cantor et al (4), the conclusions are based on relating transport properties to biological degradation.

Leahy (5) claims a species exists that can degrade cellulose-based RO membranes but again this conclusion is based on relating transport properties to biological degradation. Sinclair (1) concludes that microorganisms exist that can degrade cellulose acetate but cannot say whether these organisms can decay RO membrane material. Nelson (19) isolated a bacterium capable of using CA with a degree of substitution (DS) of 2.5 but the material tested was a polymer, not a membrane and the tests were conducted at a pH of 7 to 8. Finally, studying the cellulase enzyme system, Song (12) at a pH of 4.5 observed degradation of CA at a DS of 0.58 to 2.19, less than the DS used in CA RO membranes.

APPROACH

If it could be shown that the carbon comprising the membrane is composed of is metabolized by microorganisms under typical RO plant operation conditions, this is a serious concern for RO modules of this type.

If the opposite is true, i.e., membrane carbon is not metabolized by microorganisms, then further studies would be required to determine if these biofilms on a membrane are detrimental to the membrane or not. Ho (2) may be inaccurate in stating that "if microorganisms are involved in membrane failure . . . a cellulolytic microflora should be present that can utilize CA as a carbon and energy source" because this should be shown experimentally. For example, if microorganisms are metabolizing adsorbed organic compounds on the membrane surface but not the membranes directly, degradation could still occur due to physical damage to the membranes or possible chemical reactions between microbial byproducts and the membranes.

The following approach and conditions were used in this study:

Fungi from the RO industry. Fungi are known cellulose degraders and would be the most probable microorganisms to demonstrate degradation. Isolated and identified fungi from the RO industry were used.

Stagnant system. Because molds and fungi usually grow under undisturbed conditions, tests were designed without any water flow across or through the membranes.

Inoculation on the membrane surface. Rather than studying biodegradation of CA polymer in suspension, inoculation of the surface of a CA membrane would more closely resemble actual conditions.

Realistic pH. Balancing hydrolysis of the CA (15) with chemical costs for acid, CA membranes operate at pH 5.5. Higher pH conditions, often used in the literature as mentioned above, may result in different microorganisms growing or abiotic hydrolysis first, and biodegradation on a more cellulose-like structure. In this study, samples were buffered at pH 5.5.

Isotopes. Assuming CA to be the sole carbon source may not be accurate. Carbon contamination can come from various sources including the agar. Analyzing for reduced sugar formation can give false positive results due to the metabolism of other carbon sources. Also, relying on colorimetric analytical procedures can result in inaccurate data due to various chemical interferences. Measuring carbon dioxide using respiration techniques may give false results. Instead, starting with an enriched ^{14}C cellulosic backbone to the CA membrane, and measuring the generation of ^{14}C carbon dioxide gas by scintillation counting, would give accurate results not subject to the above concerns.

Carbon-free medium. A carbon-free mineral salts agar was prepared and used in this study. This more closely simulates field RO conditions in which a cleaned membrane is present during shutdown or storage. Although neither in this experiment nor in the field are conditions free of other forms of available carbon, it does not seem realistic to provide abundant carbon from sources other than the membrane itself that encourages growth and may incidentally degrade CA membranes.

OBJECTIVE

The objective of this study was to include four, isolated and identified fungi from the RO industry to determine if the backbone cellulosic carbon of the polymer has gone through a biological respiration step such as the Krebs cycle (see Figure 2) or perhaps anaerobic respiration, demonstrating that the membrane's carbon can be available to these microorganisms. Included in the study will be a microbiological sample of fungal growth from an actual field RO vessel. This sample, probably more than a single microorganism, may be a more vigorous grower than the others which were grown, and repeatedly transferred, on laboratory media.

Other objectives of this study were to be determine the extent of the damage, if a breakthrough of the membrane had occurred, and if removing oxygen from the system and substituting nitrogen, would hinder the metabolism of the membrane. In practice, bagging membranes with the right polymer, and replacing the air with nitrogen, may be a means of controlling mold growth.

EXPERIMENTAL SECTION

Using labeled ^{14}C cellulose as the starting compound, CA polymer was synthesized, casting solutions were made, and CA membranes were produced. Circular samples of membranes (see Figure 3) are carefully placed into their respective test apparatuses (see Figure 4), all vials were e-beam sterilized (see Figure 5), and then inoculated with the various types of fungi. Uninoculated membranes served as controls. If the metabolism of the membrane occurs, then carbon dioxide is trapped into the small vial containing dilute sodium hydroxide. Samples of this solution (0.1mL portions) are then measured for ^{14}C using liquid scintillation counting.

Chemicals. Texas Tech University provided the ^{14}C-labeled cotton. This material was generated from cotton grown in the laboratory using tissue cultures. The carbon source for the cotton was ^{14}C-labeled glucose. Buckeye Cellulose donated the dried cotton linters.

Identification of molds. Molds were identified based on both macroscopic features and microscopic morphology. The molds B1 and B2 were both identified as *Aspergillus* spp. LY was identified as a mold resembling *Gliocladium*. LYM was the LY mold grown for six weeks, prior to the testing, on a mineral salts medium with Whatman paper as the only carbon source. MM was identified as *Aureobasidium*. The strain of MM that was tested was taken directly from a contaminated RO membrane.

Carbon-free medium. A carbon-free mineral salts agar was prepared as follows (per liter distilled water): $NaNO_3$, 2.0 g; $MgSO_4$, 0.5 g; KCl, 0.5 g; $Fe_2(SO_4)_3$ - H_2O, 0.01 g and Agar (Noble or Purified) 15 g. The final pH was buffered and adjusted to 5.5 using 0.1 M KH_2P0_4 and 0.1 M NaOH. All the chemicals were obtained from Sigma (St. Louis, MO) except for the Noble agar which was purchased from Difco (Detroit, MI).

All of the test molds were inoculated to the carbon-free agar, pH 5.5, with and without the presence of 0.1% glucose. The cultures were incubated at 30°C for two weeks. Molds grew well on the media with glucose and no growth was observed in the absence of glucose (no carbon source).

Preparation of inocula to radiolabeled membranes. Each test mold was inoculated to the surface of a Gelman GN6 0.45 um filter (Gelman, Ann Arbor, MI). The inoculated filter was then placed, face up, onto carbon-free agar amended with 0.1% glucose and incubated for one to two weeks at 30°C until sufficient fungal growth was achieved. Mold was then scraped off the surface of the Gelman filter with a sterile blade and the mycelium and spores were suspended in 2.0 mL sterile saline with sterile glass beads. Each solution was thoroughly mixed by vortexing

In the case of LYM, the LY mold was incubated for six weeks, prior to the testing, on carbon-free agar with Whatman paper as the only carbon source. The mold was scraped off the Whatman paper surface with a sterile blade and suspended in saline as described above. The mold was grown in this manner to promote the production of cellulase prior to testing on the labeled membranes.

In the case of MM, the mold colonies were scraped off of the surface of a contaminated RO membrane and suspended in saline. The contaminated membrane was a cellulose acetate membrane (Fluid Systems, San Diego, CA) provided by Myriam Fabien of the Bureau of Reclamation. Previous tests of the RO membrane showed that all of the mold colonies were the same *Aureobasidium*.

Inoculation of the labeled membranes. Each test membrane was placed on the surface of the carbon-free agar in a sterile screw-capped vial. In each case, 0.20 mL of a saline suspension of mycelium and spores were inoculated unto each test membrane surface.

Sterility checks were conducted on two uninoculated vials by adding 2.0 mL Tryptic Soy Broth (Difco) followed by incubation at 35°C for 48 h with further incubation at room temperature for 5 days. No bacterial of fungal growth was observed.

^{14}C Cellulose. Although uniformly labeled ^{14}C cellulose for Nicotiana Tabacum L. was a possible candidate, concerns existed over the purity of the product resulting in choosing secondary wall cotton, a very pure form of cellulose, produced from cotton grown in tissue cultures (20). This cellulose has the purity of the commercial cotton linters used industrially as the starting material for cellulose acetate for RO membranes. Figure 6 shows the location of the ^{14}C labeled carbon.

Synthesis of ^{14}C Cellulose Acetate Polymer. The procedure (21) for the synthesis of cellulose acetate is as follows:

- Prepare a catalyst stock solution by adding 9g concentrated sulfuric acid to a volumetric flask and diluting to 100 mL with glacial acetic acid.

- Add 2.7g ^{14}C cellulose and rinse with 3, 100 mL portions of chloroform followed with 100 mL of acetone and allow to air dry.

- Add above and 24 mL glacial acetic acid to a 1-L beaker, followed by 0.5 mL of the catalyst stock solution and stir thoroughly, then let the slurry stand at room temperature for 45 minutes.

- Add 0.5 mL of the catalyst stock solution to 14.5 mL acetic anhydride which is chilled to 15.5 ^{o}C.

- Place the beaker containing the cellulose slurry in an ice bath and quantitatively add the acetic anhydride solution while stirring. Take the beaker out of the ice bath when the temperature starts to decrease and allow to stand for 30 minutes with some stirring. Then leave the beaker in a water bath overnight at 62^{o}C.

- Add a solution of 1g $MgCO_3$, 120 mL glacial acetic acid, and 86 mL water slowly and filter it through a fast cellulose paper. Add, with fast agitation, 2 L of 15% glacial acetic acid. Then wash the product thoroughly with deionized water.

- Vacuum dry the product at 50^{o}C for several hours.

- The final yield was 3.24g.

- The percent acetyl (22) was 40.2% and the calculated (23) degree of substitution (DS) was 2.5. Gel permeation chromatography gave a number averaged molecular weight (M_N) of 50,000.

Membrane Casting Solution - 500g. The procedure for preparing the membrane casting solution consisted of:

- Make CA solution by mixing 252.0 g CAE 2479-4C CA, 447.8 g acetone, and 834.0 g 1,4-dioxane and in a brown glass bottle. CAE 2479-4C was a special synthetic lot made by Eastman for Separations Systems specifically for RO membranes. The acetyl content was 42.0%, and a M_N of 50,000.

- Tumble under a heat lamp (500 watts) overnight.

- Weigh 426.2 g of the above solution, 2.5 g of ^{14}C labeled CA, 10g of Eastman grade E 398-10 CA and tumble under a heat lamp overnight.

- Add 45.85 g of methanol and continue tumbling until the solution appears to be homogeneous.

- Next, add 15.45g of maleic acid (in fine powder form) and continue tumbling until the solution appears to be homogeneous.

- One day before casting membranes, pressure filter the solution using a 1.0 micron polypropylene filter cartridge under nitrogen.

Membrane Casting. The membranes, formed by a phase inversion process, were prepared by casting the aforementioned solution at 25°C onto the surface of a glass plate at the rate of 5 feet per minute. After 12 seconds air contact, the metered layer of casting solution was immersed into a gelation bath maintained at 5°C. The process conditions selected duplicate those used in the production of commercial cellulose acetate blend membranes. Also, as expected, the reverse osmosis performance of the membranes was similar to that of commercial cellulose acetate blend membranes.

Annealing of Membranes. After gelation, the newly formed membranes were then wrapped around a 4 inch PVC pipe and annealed for 5 minutes at 80°C in distilled water. The outside or annealed surface was opposite the agar and the surface where the microorganisms were transferred. The final thickness of the dense zone (see Figure 7) was approximately 2000 Angstroms with a total membrane thickness of 105 microns.

Simulating Used Membranes. New membrane samples were modified by lightly buffing the surface with 500A sandpaper for a few seconds and then placing samples into 0.01 N KOH and for one minute per sample. These samples were then quenched in 0.01N HCl, rinsed in deionized water several times, and placed in the test vials. These samples were then included with the other vials for e-beam sterilization.

Analytical Methods.

After three months all vials were opened and the dilute sodium hydroxide solutions in the small vial suspended above the membrane (see Figure 4) were determined for ^{14}C using a Packard Tri-Carb 300C liquid scintillation counter. The region for ^{14}C was preset in the instrument and was selected in the program that measured the ^{14}C in the samples. An average of three, 10-minute runs per sample were used to compute disintegrations per minute (dpm).

RESULTS AND DISCUSSION

After three months, Figure 8 showed that under aerobic conditions, fungi isolated from the RO industry and "MM", the biofilm sampled and inoculated from an actual field RO unit, metabolically used the new membrane carbon as an energy source. Each data point was based on nine averages, the standard deviation too small to be seen in the figures.

After two days into the experiment, air was replaced by nitrogen gas to determine if anaerobic conditions would stop or slow the rate of fungal attack. Figure 8 shows that this made little to no difference. From a practical standpoint, these results suggest that storing a RO element in tight polymer bags and replacing the air with nitrogen will not significantly change the rate of biodegradation of membranes during shutdown or storage times.

Included in the experiment were simulated "used" membranes, produced in the laboratory by altering new membranes by sanding and hydrolyzing the membrane surface. These samples represent field membranes that have hydrolyzed over time or perhaps were damaged by a pH upset. The results show that under aerobic conditions, more of the carbon in the "used" membranes was metabolized by these microorganisms than that of the "new" membrane samples (see Figure 9) and the same was true for anaerobic conditions (see Figure 10). These findings concur with the abundant literature reports citing increased protection and a decrease in the rate of attack of CA with increasing DS. Thus, the more glucose-like structures have more readily available carbon for microorganisms. Figures 11-12 show fungal growth on these membranes.

"MM" had the highest rate of new membrane metabolism. Although this may be due to inoculation size, the data also suggest that the field sample, unlike the others that were transferred through different media in the laboratory several times, may have had established enzyme systems for cellulosic degradation.

Several hundred vials were e-beam sterilized at the same time and left in a dark room for one month prior to inoculation. Careful inspection of the vials showed five samples with signs of microbiological growth and these vials were included in the experiment to see if these organisms (airborne or wherever they came from!) could degrade the CA samples. These five samples were "used" membrane and the results (see Figure 13) show biodegradation.

The data show metabolism of membrane carbon for every isolated and identified fungus that was included in the experiment. Also, the inoculated sample of the biofilm from the field RO unit gave similar results and all five contaminated vials did the same. This data suggests not merely a "unique" species (5) but that these membranes, and especially "used" membranes, are easily degraded by microorganisms capable of contaminating the polymer.

The controls in the experiment, assumed to be sterile samples, showed almost no ^{14}C data above the natural background. However, although very low, there are statistically significant higher ^{14}C data for the used vs. new membranes. Two explanations are possible: increased vapor pressure caused by small amounts of glucose, cellobiose, etc. formed during the hydrolysis process (not acetate because only the backbone carbons were enriched with ^{14}C) which dissolved

in the dilute sodium hydroxide and produced above background counts and/or some kind of contamination by microorganisms that find the carbon from the more "glucose-like" carbohydrate structures on the compromised membrane more bioavailable compared to the ester structures on the new membranes. Because the ^{14}C data counts are very low (only statistically above background), microorganisms are unlikely and the most obvious explanation is increase vapor pressure of ^{14}C compounds.

Field studies have shown that after cleaning off membranes under fungal attack, dye uptake suggests deterioration of the dense zone (see Figure 14). The membrane had failed prior to this photograph and suggests that fungi were the cause.

The mass of the dense zone (see Figure 7) for one vial can be calculated using a density of 1.3 g/cm^3 for dense zone CA polymer:

$M_{ds} = 1.3\pi r^2 h$

where M_{ds} is mass of the dense zone in grams, or

$M_{ds} = 1.31 \times 10^{-4}$ g

A typical surface of new membrane under fungal attack can be seen in Figure 15. By digitally scanning this photo (see Figure 16) using VisPro software, only 6% of the surface is covered by mycelia. If all the mass under the mycelia were "mineralized" to CO_2, the total mass would be:

$M_{um} = 0.06 \times 1.31 \times 10^{-4}$ g $= 7.86 \times 10^{-6}$ g

where M_{um} is the dense zone mass under the mycelia.

By multiplying this mass by the experimentally determined activity of 1.96×10^4 cpm/mL for a 1.96×10^{-2} g dry membrane sample or:

7.86×10^{-6} g $\times$ 1.96×10^4 cpm/mL / 1.96×10^{-2} g = 7.86 cpm/mL

and if the entire dense zone were mineralized to CO_2, scintillation counts would be:

1.31×10^{-4} g $\times$ 1.96×10^4 cpm/mL / 1.96×10^{-2} g = 131 cpm/mL.

The experimental results show that scintillation counts on new membranes are near this 131 cpm/mL and, provided that only the dense zone was the actual surface inoculated, break-through of the dense zone of the membrane by these microorganisms has occurred in three months or less.

CONCLUSIONS

For the first time, definitive experimental data have demonstrated that fungi isolated from the RO industry can use CA membrane as a carbon source. The dense zone of the membrane, may have been compromised in three months or less. All fungi samples demonstrated this ability and metabolism occurred under either aerobic or anaerobic conditions.

To simulate a used membrane, samples were hydrolyzed for a short time in dilute sodium hydroxide and treated to a quick sanding with 500-A sandpaper. These membranes termed "used," had a higher rate of carbon metabolism compared with new membrane samples.

Because data show that removing O_2 from the system does not immediately slow down the rate of the metabolism by the fungi, bagging membranes in oxygen impermeable polymer bags in which the air is replaced by nitrogen will not be successful in stopping the growth and deterioration caused by these molds.

Data did not support any "special" fungus unique in degrading CA membrane. Instead, data show that not only these specific fungi identified and isolated from the RO industry, but biofilms associated with field RO systems, and microorganisms introduced through contamination were all capable of degrading these membranes.

These membranes must be protected from biological degradation during shutdown periods or storage.

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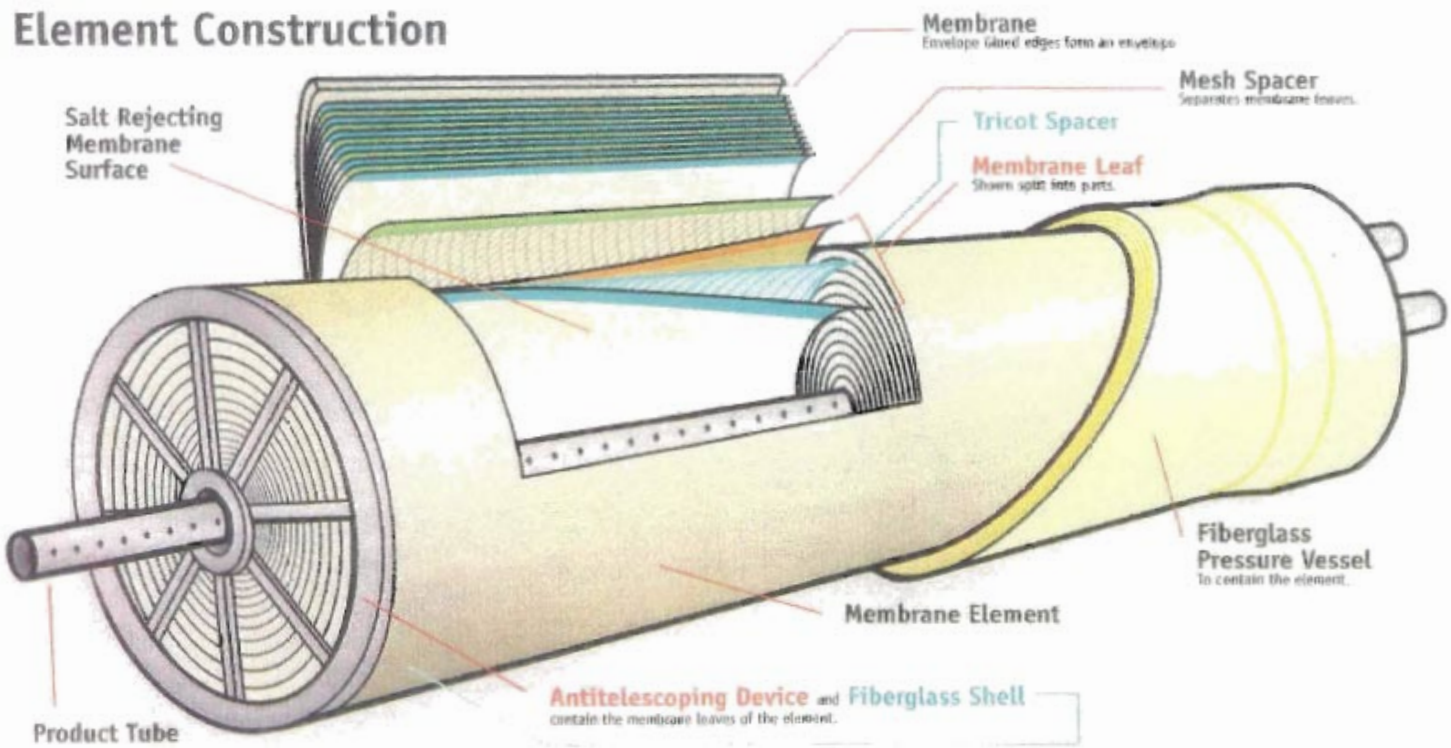


Figure 1. Typical Spiral-WoundReverse Osmosis (RO) Module.

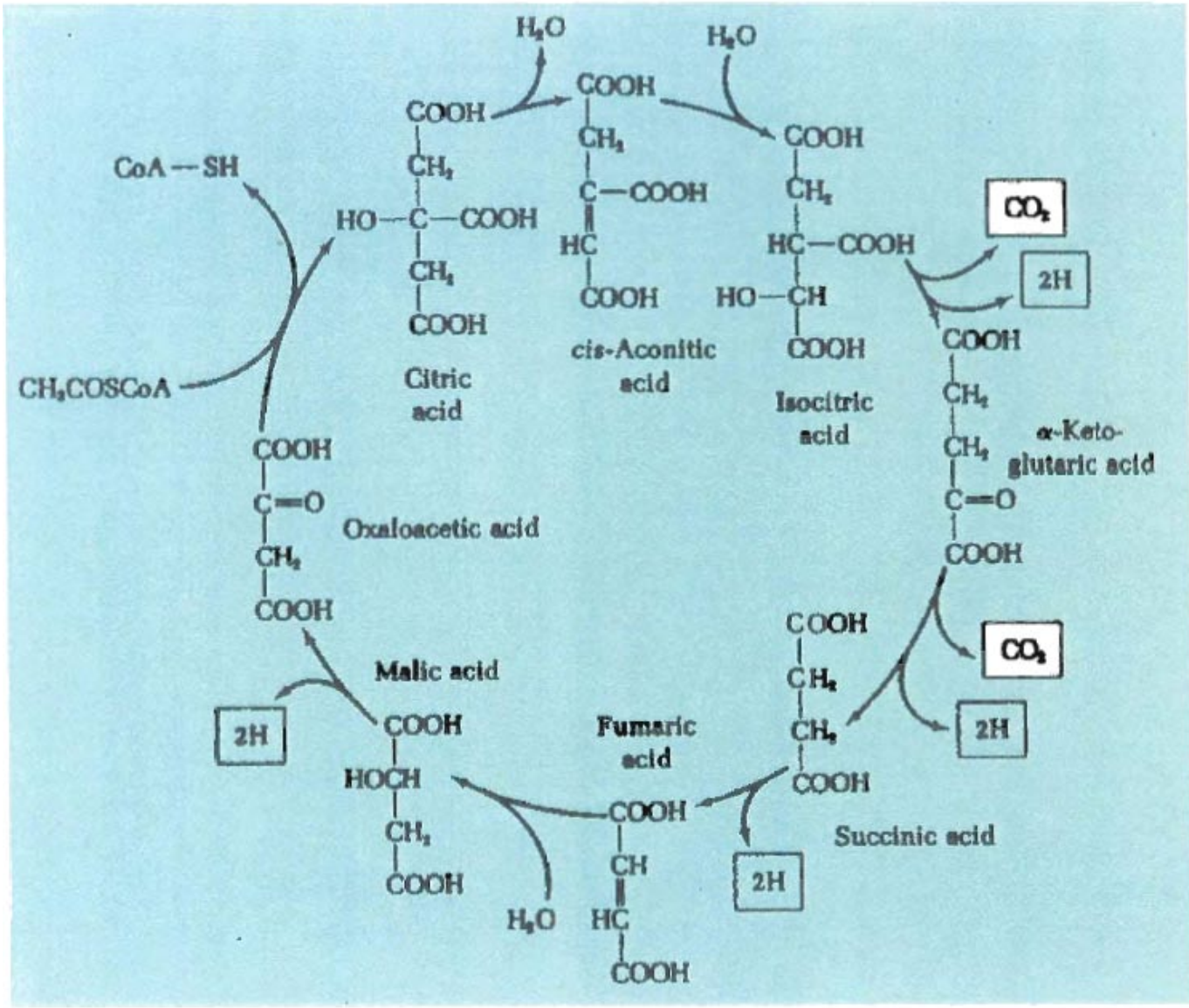


Figure 2. Carbon Dioxide Generation by the Krebs Cycle.

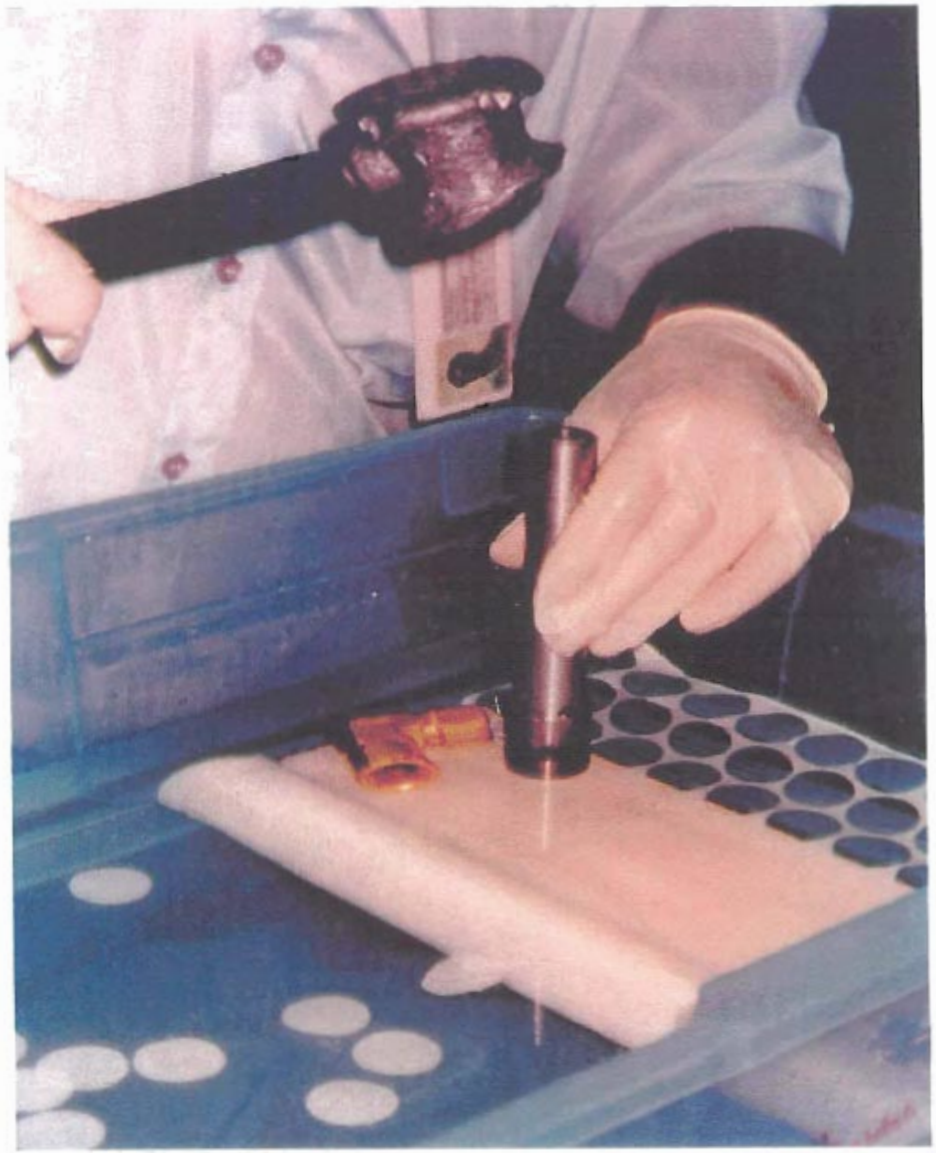


Figure 3. Punched Samples of Cellulose Acetate Membranes.

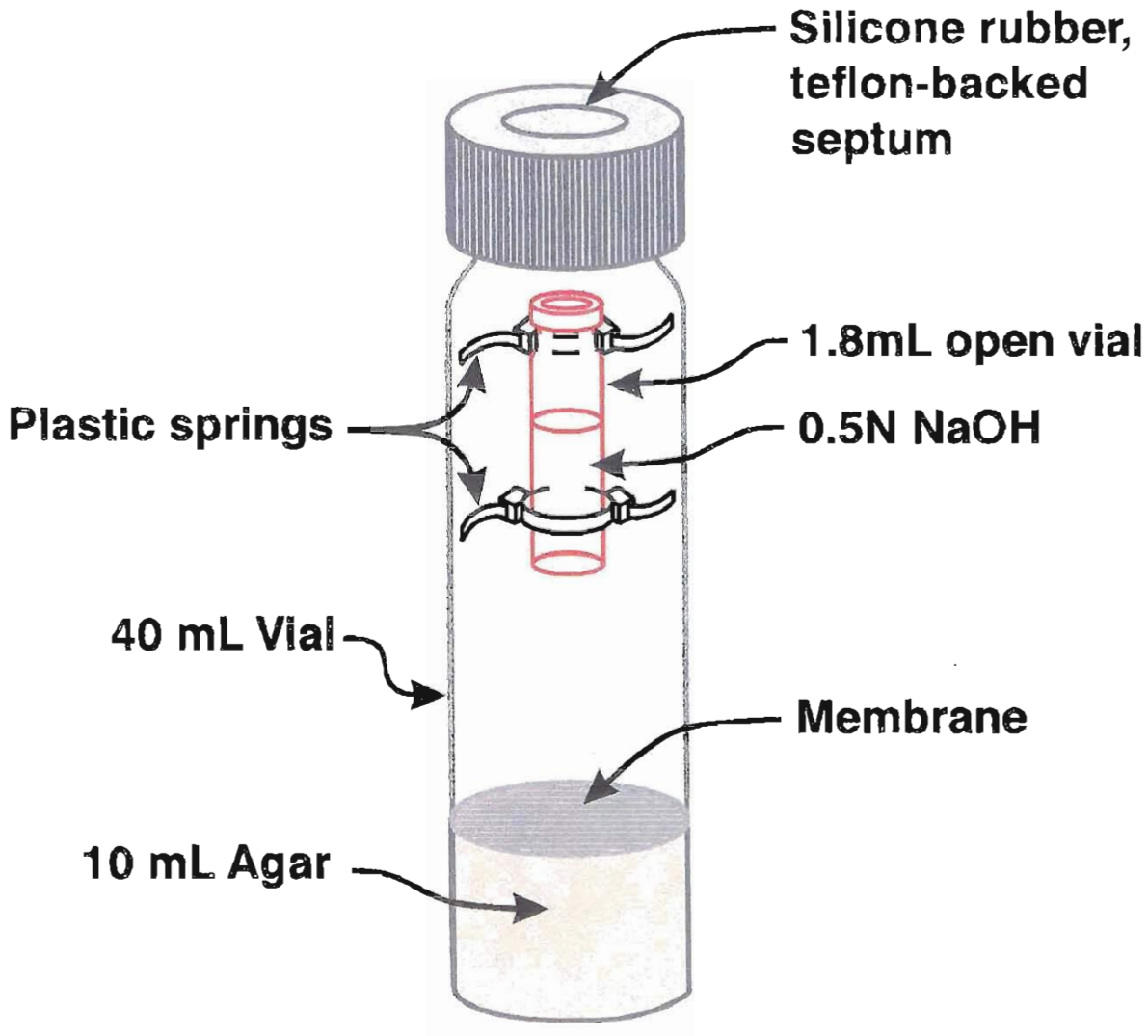
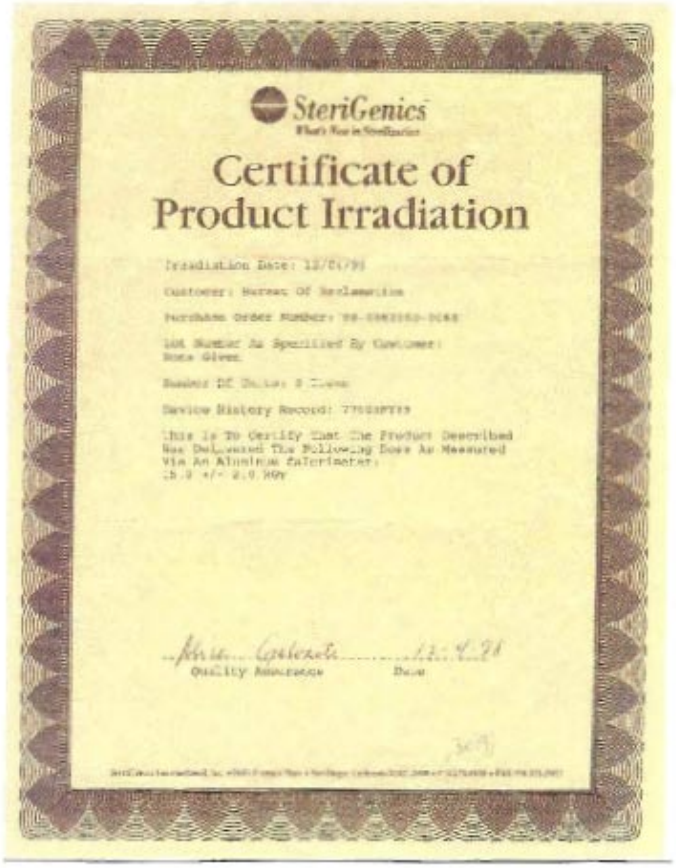


Figure 4. Diagram of the Laboratory Test Apparatus.



SteriGenics

Certificate of
Product Irradiation

Irradiation Date: 12/01/91

Customer: Bureau Of Reclamation

Purchase Order Number: 98-0982150-0048

Lot Number As Specified By Customer:
None Given

Number Of Units: 8 Trays

Device History Record: 77010FT19

This Is To Certify That The Product Described
Has Received The Following Dose As Measured
Via An Alanine Dosimeter:
15.0 +/- 2.0 kGy

Maria Gabrecki 12-4-91
Quality Assurance Date

Bureau of Reclamation
Orientation of Vial in the Electron Beam

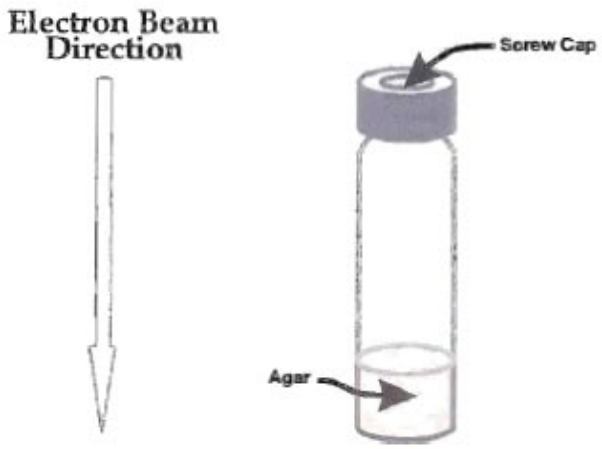


Figure 5. Certificate of E-Beam Sterilization and Vial Orientation

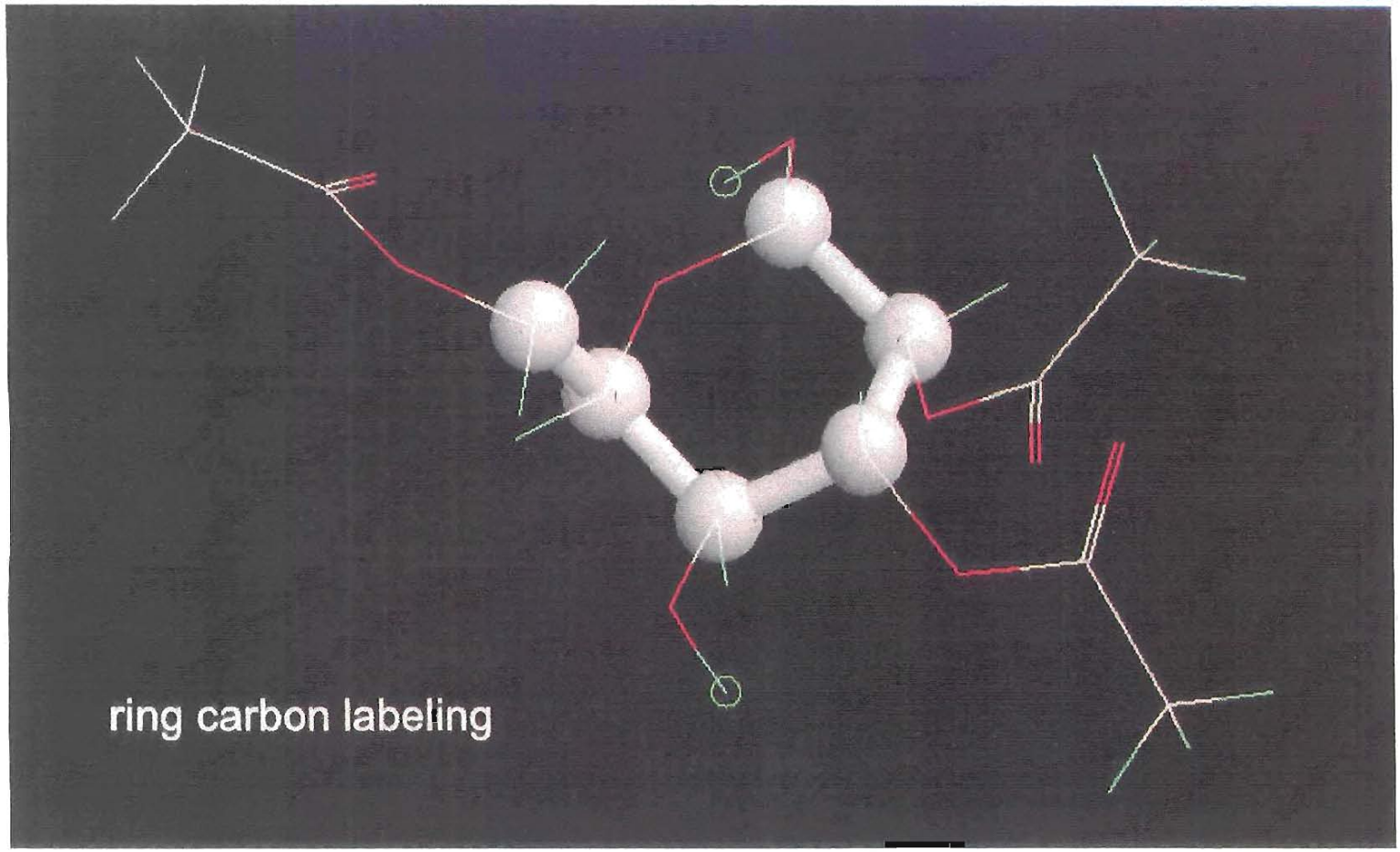


Figure 6. Cellulose Acetate Model Showing ^{14}C Label Location.
(Courtesy Dr. Harry Ridgway)

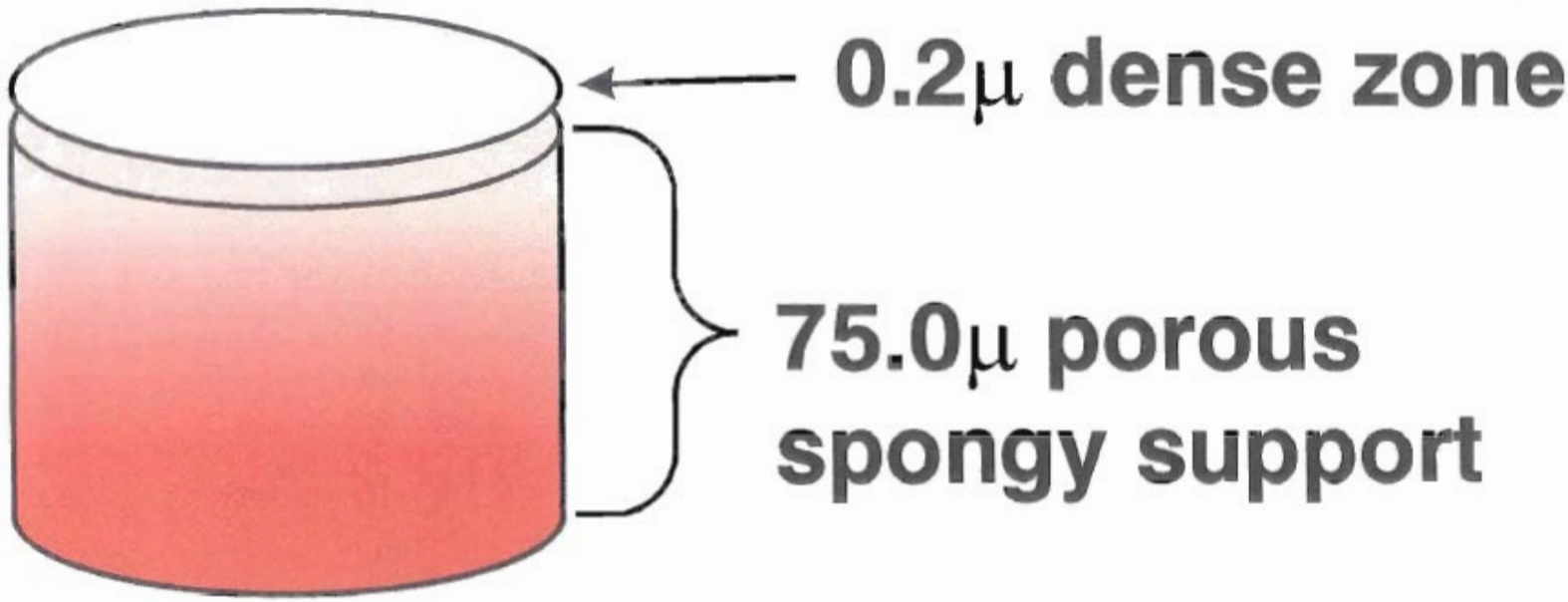


Figure 7. Diagram Showing Actual Dense Zone of Cellulose Acetate Membrane.

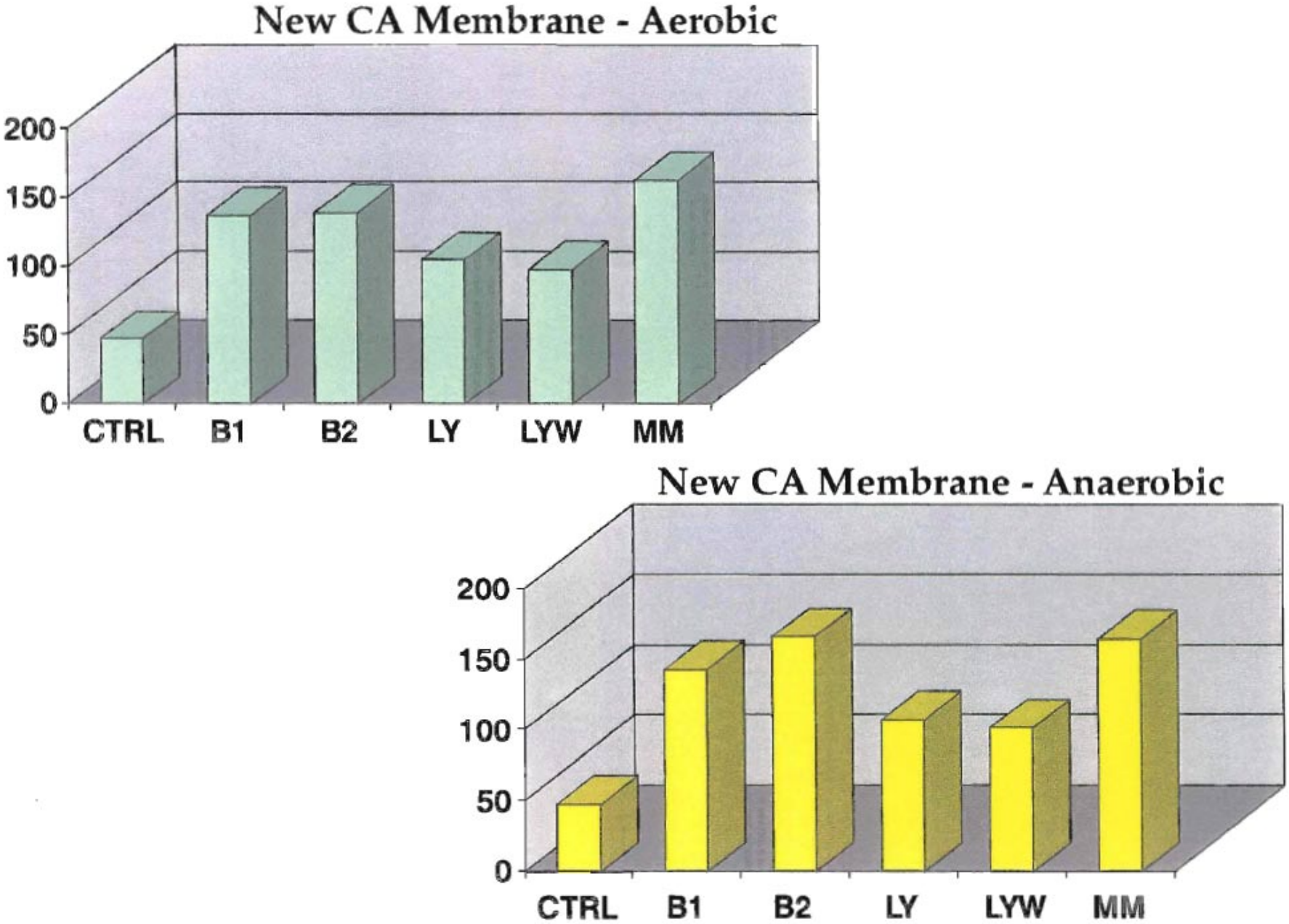


Figure 8. Plot of New Cellulose Acetate Membrane Under Aerobic and Anaerobic Conditions. Each data point consisted of an average of nine. Standard deviation was too small to plot on figure.

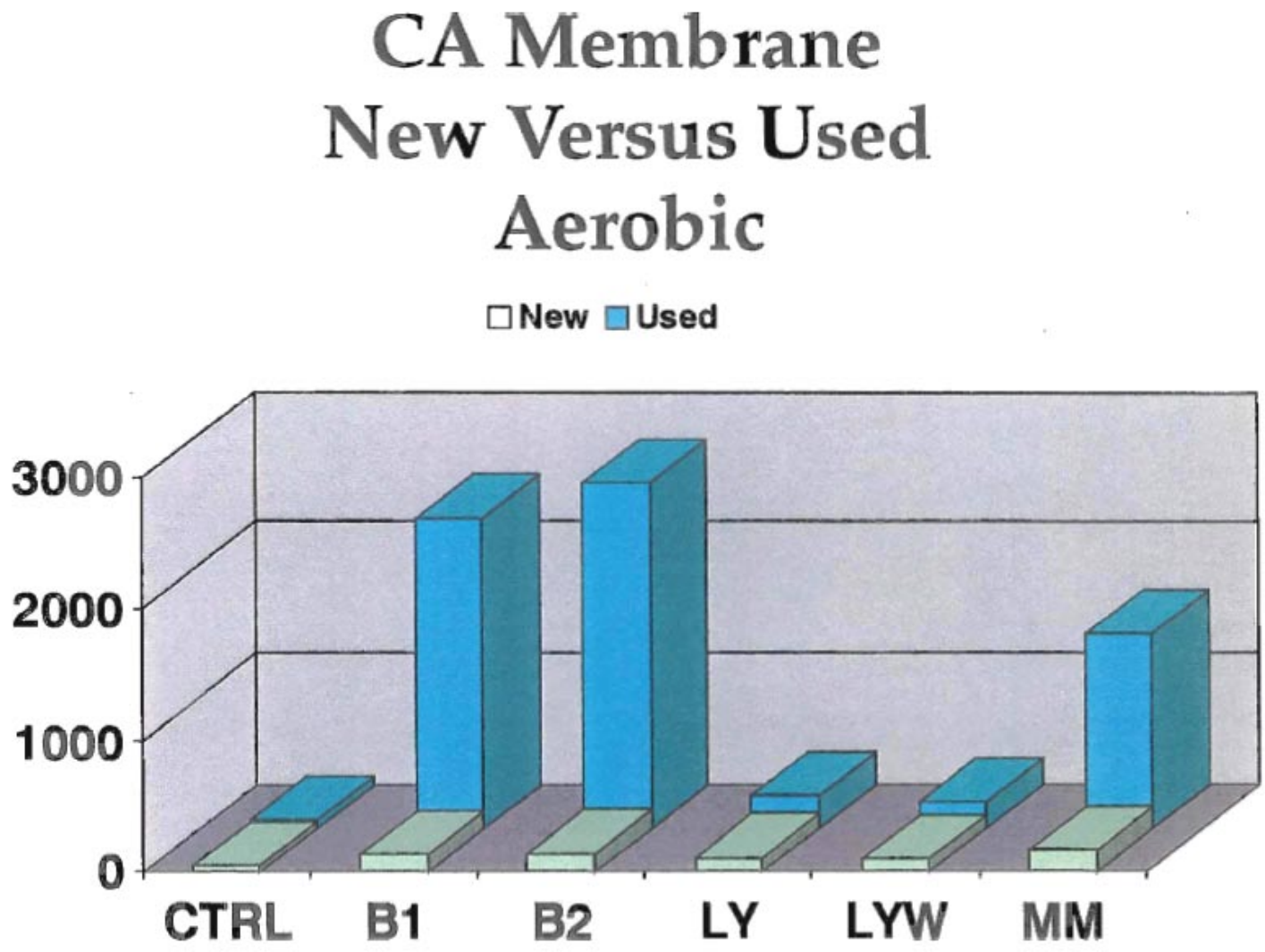


Figure 9. Plot of New Versus "Used" Membrane Under Aerobic Conditions. Each data point consisted of an average of nine. Standard deviation was too small to plot on figure. (See text for explanation of "used.")

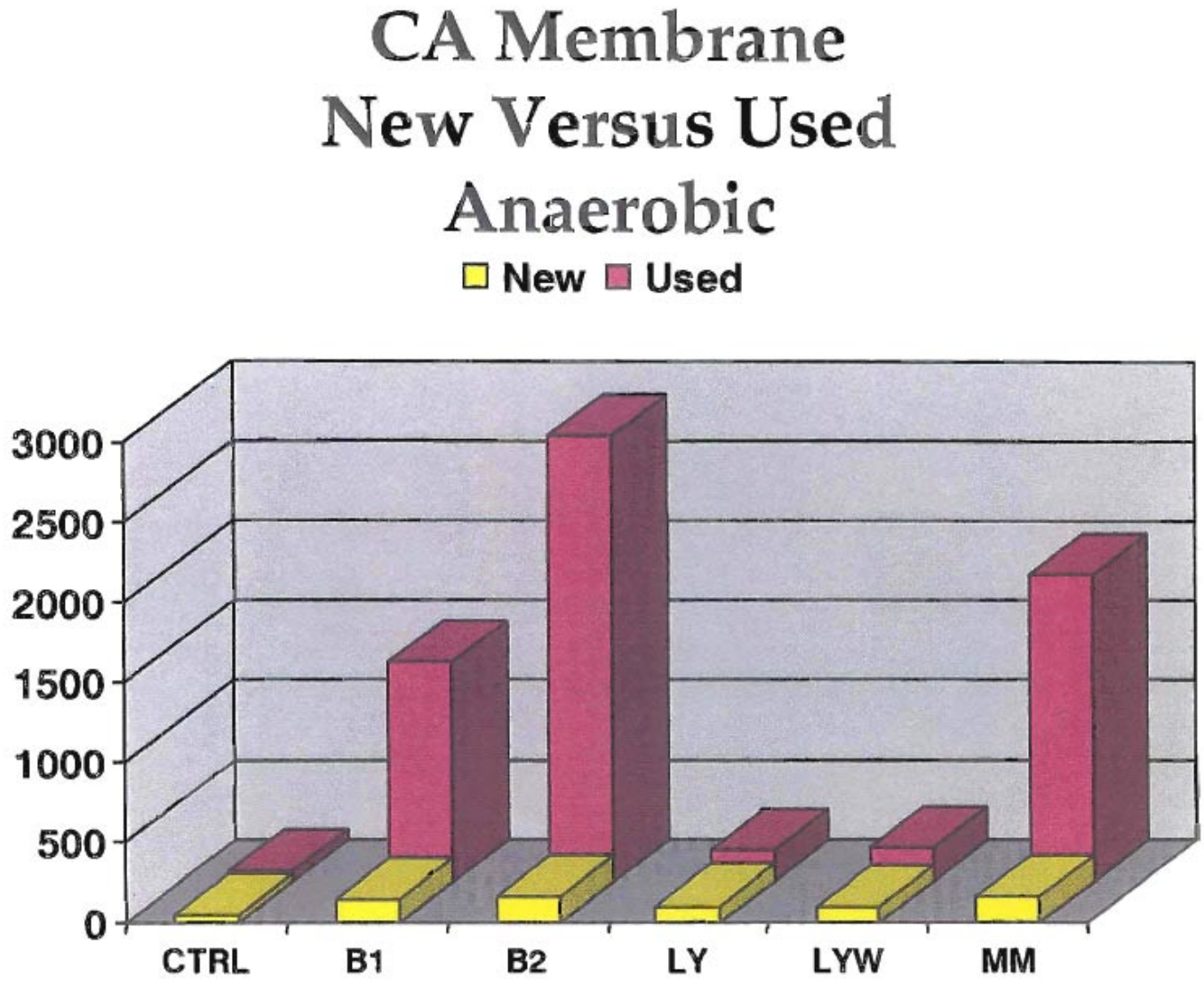


Figure 10. Plot of New Versus "Used" Membrane Under Anaerobic Conditions. Each data point consisted of an average of nine. Standard deviation was too small to plot on figure. (See text for explanation of "used.")

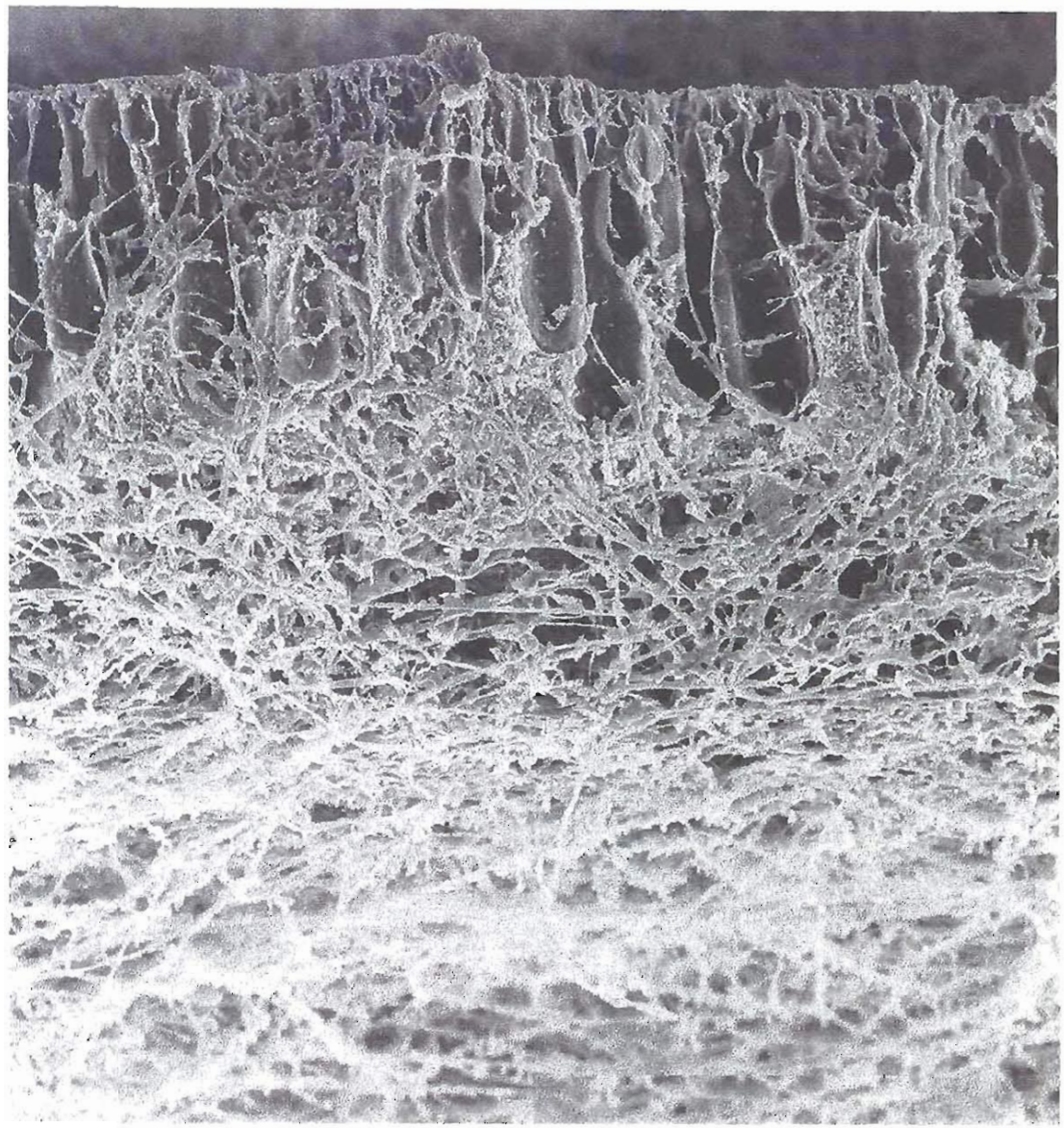


Figure 11. SEM Photo of Membrane Surface Under Fungal Attack. Annealed Surface on Top. 300X
(Courtesy Dr. Larry Blanton and Mr. Mark Grimson, Texas Tech University)

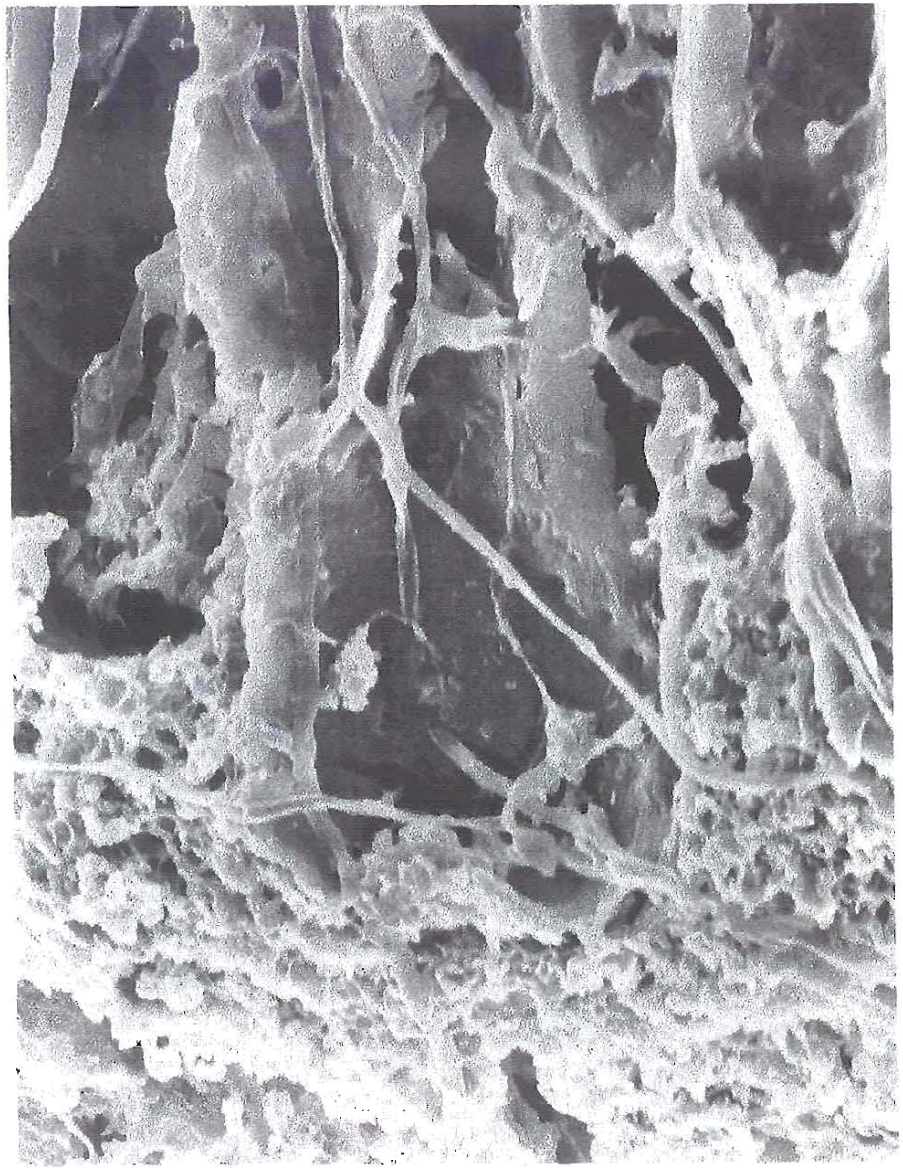


Figure 12. Additional SEM Photo of Membrane Surface Under Fungal Attack. 1000X
(Courtesy Dr. Larry Blanton and Mr. Mark Grimson, Texas Tech University)

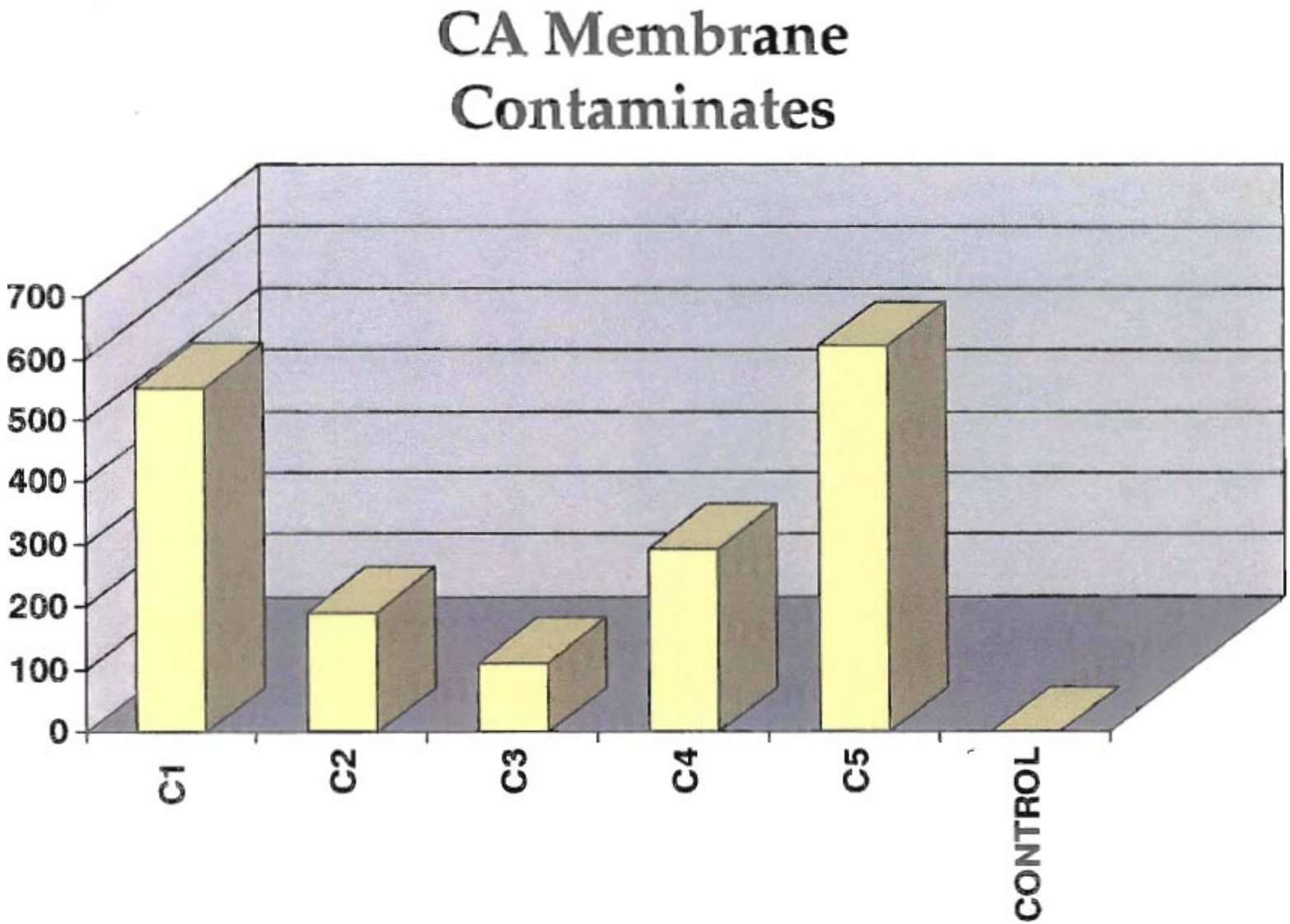


Figure 13. Carbon Metabolism of Membrane by Microbiological Contaminates.

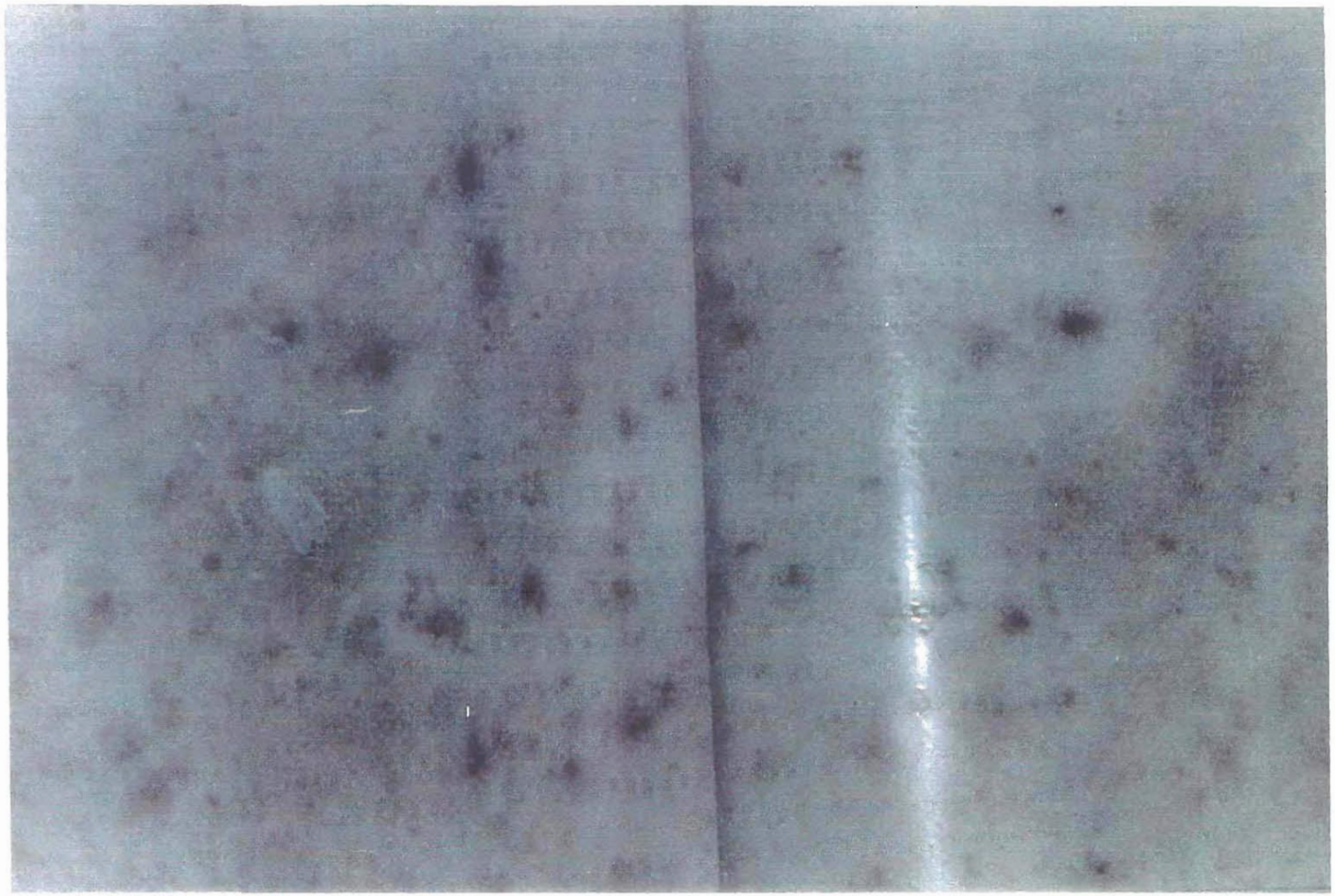


Figure 14. SEM Photo From the Field Showing Dye Uptake on Damaged Membrane Surface Believed Caused by Fungi.

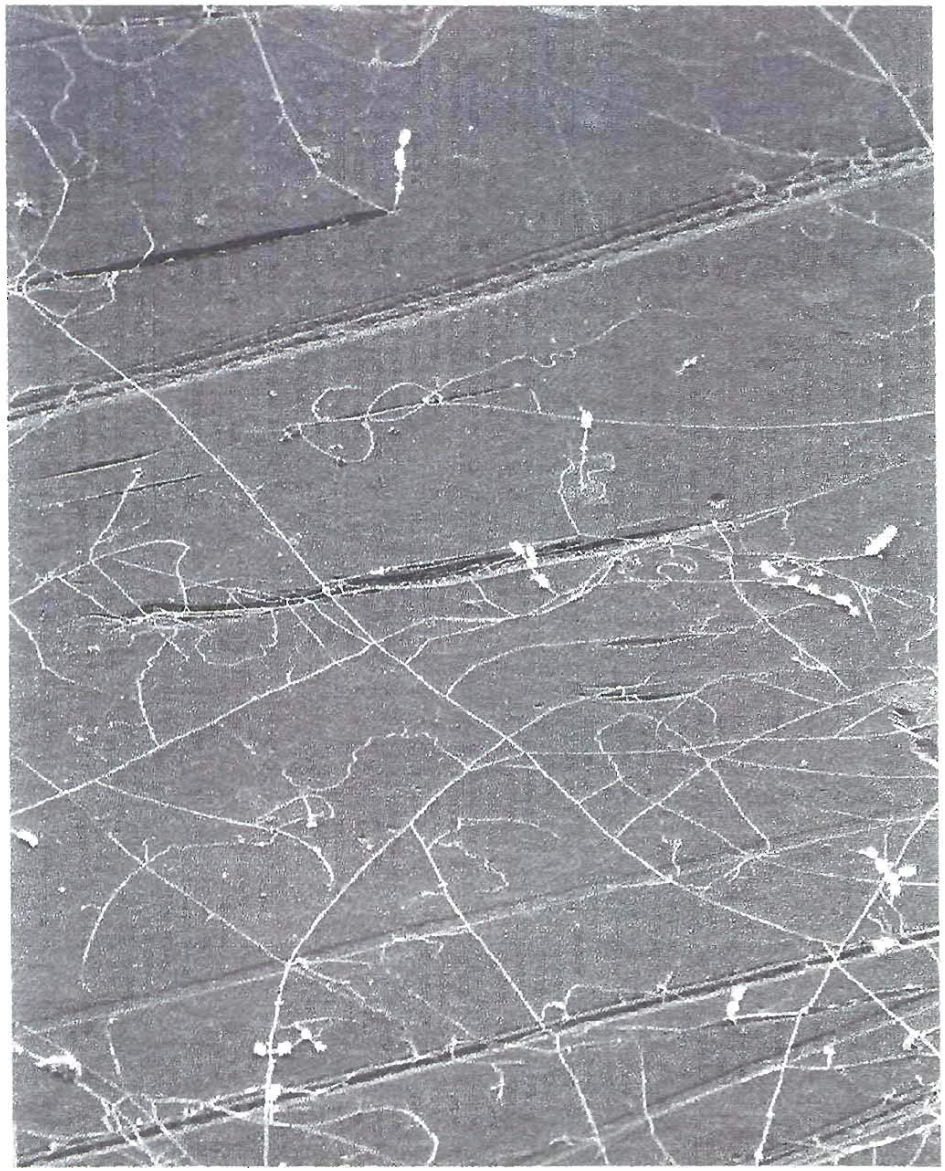


Figure 15. SEM Photo of the Annealed Membrane Surface Under Fungal Attack.

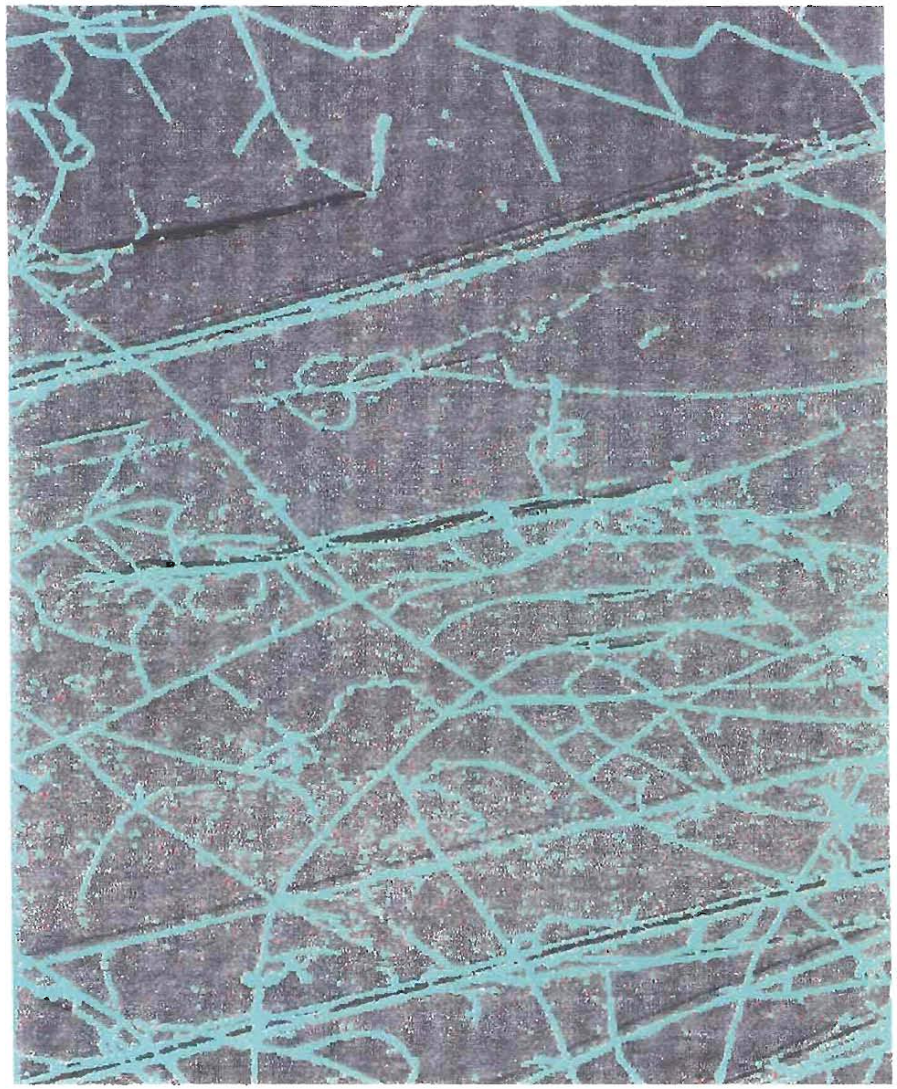


Figure 16. Digitally Scanned Image of Previous Photo Showing VisPro Software Enhanced Recognition of Mycelia.