Evaluation of Three Different Cleaners Recommended for Ultrafiltration Systems by Direct Observations of Commercial-Scale Spiral-Wound Ultrafiltration Membranes¹

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ABSTRACT

Efficacy of cleaners designed for use with ultrafiltration systems was determined by microbiological evaluation and through visual inspection using scanning electron microscopy. The ultrafiltration system containing two commercial-scale, polysulfone membranes was soiled with sweet whey (40°C) then rinsed with water and membranes were removed. One half of each membrane was soaked for 2 h at 38°C in one of the following solutions: control (no soaking), phosphoric acid cleaner (pH 2.5), enzyme-based cleaner (pH 11.5) and chlorinated alkaline cleaner (pH 11.5). The membranes were repositioned in the ultrafiltration unit, rinsed with water, then removed and unwound for analysis. Sections of membrane, retentate spacer and permeate mesh were aseptically removed for enumeration of microorganisms remaining and for examination by scanning electron microscopy. Membranes cleaned with chlorinated alkaline cleaner averaged 2 x 10³ CFU/50 cm², enzyme-based cleaner 6 x 10⁶ CFU, acid anionic cleaner 1 x 10⁷ CFU and the control 5 x 10⁷ CFU. Scanning electron microscopy found soil and microorganisms present on all membrane materials exposed to all three cleaners.

Scanning electron microscopy (SEM) has been used to determine the possible structure of fouling material on UF membranes following circulation of whey and purified whey proteins in laboratory-bench scale units (8,9). This SEM technique also has been used to evaluate the ability of cleaning agents to remove biofilms from reverse osmosis membranes used in commercial water treatment facilities (20). In this study, SEM was used with SDS-PAGE and determinations of microbiological populations on UF membranes to directly evaluate the ability of three cleaners to remove both soil and microorganisms from a commercial-scale UF system.

MATERIALS AND METHODS

Equipment

The UF system consisted of a 380 L jacketed vat equipped with agitator and thermometer, prefilter, centrifugal pump, and valves and pressure gauges located before and after two membranes in parallel (Tri-Clover, Inc., Kenosha, WI). The spiral-wound, polysulfone UF membranes (Filmtec, Minneapolis, MN) had a 10,000 to 15,000 dalton cut-off and had not been soiled previously. A feed rate of 95 L/min/membrane with a pressure drop of 1.4 kg/cm² was selected according to recommendations of the manufacturer.

Soiling procedure

Pasteurized, separated sweet whey was obtained immediately before use from a local cheese factory. The soiling procedure consisted of recycling 380 L of whey for 2.0 h and concentrating the whey for an additional 0.5 h. Cooling water circulated in the jacket of the vat was used to maintain whey temperature at 38-43°C. Determinations of pH, percentage total solids and ash (1) and numbers of bacteria, yeasts and molds (13) were made for whey in the tank, retentate and permeate streams initially, and at the start and end of concentration.

Cleaning procedure

Following soiling, the UF system was flushed with water (209 L, 40°C) to remove loosely held soil and permeate flux was determined with water. The membranes were removed from the housings and placed in tanks containing cleaning solutions. One half of each membrane was soaked for 2 h at 38°C in one of the following solutions: control (no soaking), phosphoric acid (pH 2.5), enzyme-based cleaner (pH 11.5) and chlorinated alkaline cleaner (pH 11.5). The cleaners were obtained from three different manufacturers with their exact composition proprietary; however, manufacturer's recommended use dilutions were used.

The design of the cleaning method used for this study was based on several factors: (a) Because of membranes costs, a maximum amount of cleaning information was desired from...
each membrane. (b) It was important to have a control, that is a soiled surface, that accurately reflected the amount of soil and microorganisms present following circulation of whey. Without this information comparisons of soil and microorganism removal between cleaners would not be possible. (c) An additional design factor was the need for uniform cleaner circulation. A cleaner must contact soil to be effective. In a previous study using sanitizers, cleaners and unsoiled spiral-wound membranes prolonged soaking with the membranes removed from the retentate housings was the only effective method for sanitizing the membranes (18). The open weave of the retentate mesh provided a channel for the cleaning solutions and the design of a spiral-wound membrane gave the cleaner equal access to all sections of the membrane surface through the retentate mesh. Because the membranes were soaked in cleaning solutions problems with uneven flow throughout the membranes was eliminated.

Following 2 h of soaking in the cleaning solutions soil should have been loosened or hydrolyzed. The membranes then were replaced in the UF unit and rinsed with water (380 L, 40°C). This procedure provided the mechanical action necessary for removing loosened soil. The membranes then were removed and unwound for analysis.

Data collection

Several types of data were collected for the UF system. Permeate flux and numbers of microorganisms remaining on UF devices and on inside surfaces of stainless steel housings were determined immediately following soiling. Numbers of microorganisms remaining on membrane surfaces, retentate spacers and permeate meshes were determined and SEM photographs obtained for these same surfaces. Deposits remaining on membrane surfaces before and after cleaning were analyzed by SDS-PAGE.

Permeate flux measured in kilograms/minute was obtained initially and following soiling. Values for flux at 32, 38 and 43°C were determined and a line of best fit calculated using linear regression.

Microbiological contamination was determined for antitesseloping devices (ATDs) and inside surfaces of stainless steel housings immediately following soiling and rinsing. There were two ATDs on each end of a membrane. Following soiling, ATDs were removed, placed in sterile Whirlpak bags and 99 ml of sterile buffered rinse solution was added.

Permeate and retentate-side stainless steel surfaces were sampled with two, 50-cm² sections of permeate tube and 12, 50-cm² sections of retentate housing sampled. Retentate surfaces sampled were divided into 8 sections of sidewall and faceplates.

Following cleaning, membranes were labeled, cut in half according to treatment, placed in separate plastic bags and held at 4°C until sampled. All samples were obtained and plated within 48 h of cleaning.

Location and type of sample taken is given in Fig. 1. Each half membrane was unwound on an alcohol (60% v/v)-treated surface. The half membrane was divided into four sections and samples taken from the center of each section. Sections were numbered I, II, III and IV with I being outermost when the membrane was wound and IV being furthest inside. When unwound, the membrane consisted of several repeating layers of materials; therefore, within any section duplicate determinations were possible. A cross-section of the membrane layers is given in Fig. 2a.

Membrane surfaces were evaluated by two types of microbiological determinations. On one half of each section a 50-cm² area of membrane surface was swabbed and from the other half a 50-cm² piece was removed aseptically, placed in a sterile Whirlpak bag and 99 ml of sterile buffered rinse solution added immediately before microbiological determinations. Samples handled by the latter method were labeled modified rinse solution (MRS) sections. Retentate spacers and permeate meshes were evaluated only by the MRS method because the rough surface of both materials made swabbing unsuitable.

Sections of membrane materials (approximately 25-cm²) for evaluation by SEM were taken from the center of Sections I and IV for membrane surfaces and from Section IV for retentate spacers and permeate meshes. Soil deposits for analysis by SDS-PAGE were taken from membrane surfaces in Section IV.
TABLE 1. Composition of whey during UF.

<table>
<thead>
<tr>
<th>Composition</th>
<th>Initial</th>
<th>Start of concentration</th>
<th>End of concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T</td>
<td>R</td>
<td>P</td>
</tr>
<tr>
<td>Total Solids (%)</td>
<td>6.3</td>
<td>6.4</td>
<td>5.4</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>.52</td>
<td>.53</td>
<td>.48</td>
</tr>
<tr>
<td>pH</td>
<td>6.4</td>
<td>6.3</td>
<td>5.3</td>
</tr>
<tr>
<td>Total plate count (log10 cfu/ml)</td>
<td>4.7</td>
<td>4.7</td>
<td>2.8</td>
</tr>
<tr>
<td>Yeasts (actual number/ml)</td>
<td>&lt;1</td>
<td>2</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Mold (actual number/ml)</td>
<td>&lt;1</td>
<td>50</td>
<td>1</td>
</tr>
</tbody>
</table>

Key: T, Tank; R, Retentate; P, Permeate.

Microbiological procedures

Whey, swab samples and ATDs and UF membrane materials in dilution buffer were plated on plate count agar and potato dextrose agar (acidified with tartaric acid to pH 3.5) to determine numbers of bacteria and yeasts and mold, respectively. Bacteriological preparations and procedures were according to Standard Methods for the Examination of Dairy Products (13).

Scanning electron microscope procedures

Membrane sections for examination by SEM were placed on filter paper saturated with 1% (v/v) glutaraldehyde in a covered petri dish for 12 h. Membrane pieces then were dehydrated by placing them successively in 20, 40, 60, 80 and 100% ethanol for 30 min each. Following critical point drying, membrane sections were attached to specimen mounts with silver paint and sections coated with 5 nm of gold:palladium. A JEOL 35C scanning electron microscope (Japan Electron Optical, Ltd, Tokyo) using an acceleration voltage of 20-25 kV was used to view specimens. Magnifications to 720x and 10,000x were used with the scalebar indicating 10 and 1 µm, respectively. Photographs were on Polaroid Positive/Negative Type 55 black and white film.

Polyacrylamide gel electrophoresis

Sample preparation and SDS-PAGE were done according to the method described by Basch et al. (2). Modifications included use of a SE 600 Series Vertical Slab Electrophoresis Unit (Hoefer Scientific Instruments, San Francisco, CA) yielding gels of 1.5-mm thickness. Also, gels were run for 12 h at 15 mA/slab and 10°C, protein staining was for 4 h and slabs were destained for 1 week with four changes of destaining solution.

Statistical analysis

Treatments or methods were compared by analysis of variance. Because of an unbalanced design, significance of microbial populations found on membrane surfaces sampled by two methods and stainless steel surfaces was determined by LSD method (α=.05). The Newman-Keuls method (α=.05) was used for all other comparisons.

RESULTS AND DISCUSSION

Whey composition throughout UF is given in Table 1. Values for percentage of total solids and ash were similar initially and at the start of concentration for tank and retentate streams. Because whey was recycled for 2 h, whey composi-

Figure 3. Permeate flux for soiled and unsoiled UF membranes.
TABLE 2. Populations of microorganisms on surfaces in the UF system.

<table>
<thead>
<tr>
<th>Surface</th>
<th>Microorganism</th>
<th>Yeast (log₁₀/50 cm²)</th>
<th>Mold (range (actual number/50 cm²))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stainless steel</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retentate - Side</td>
<td>Faceplates</td>
<td>3.2</td>
<td>&lt;10 to 1820</td>
</tr>
<tr>
<td></td>
<td>Sidewalls</td>
<td>&lt;1.5</td>
<td>&lt;10 to 10</td>
</tr>
<tr>
<td></td>
<td>Permeate - Side</td>
<td>1.0</td>
<td>&lt;10</td>
</tr>
<tr>
<td></td>
<td>ATDs</td>
<td>7.1</td>
<td>&lt;100 to 4900</td>
</tr>
<tr>
<td></td>
<td>Membrane surface</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Swabbed</td>
<td>47</td>
<td>&lt;10 to 10</td>
</tr>
<tr>
<td></td>
<td>MRS</td>
<td>77</td>
<td>&lt;100 to 40000</td>
</tr>
</tbody>
</table>

<sup>a</sup>ATDs = antitelescoping device.
<sup>b</sup>MRS = modified rinse solution.

Permeate flux initially and after soiling with whey is given in Fig. 3. Fouling with whey reduced permeate flux by 56%.

The population of microorganisms adhering to ATDs and inside surfaces of stainless steel housings is given in Table 2. Among all stainless steel surfaces sampled, the highest numbers of microorganisms were found on faceplates. Overall, ATDs had the highest populations of microorganisms except for the membrane surface. These devices are hard plastic with many joints. Greatest numbers of yeasts were found on ATDs also while molds generally were not apparent.

These values from the stainless steel surfaces and ATDs can be compared to those obtained for the membrane surfaces. Populations found on membrane surfaces by both swabbing and MRS methods were significantly higher (Table 3) than populations found on retentate surfaces in stainless steel housings. Values for populations on plastic ATDs, however, were similar to those for membrane surfaces. Microorganisms were better able to adhere to irregular polymer surfaces of membranes and ATDs than polished stainless steel housings (10).

Another difference was evident when comparing membrane surfaces which were swabbed versus membranes evaluated by the MRS method (Fig. 4 and Table 2). Significantly greater numbers of microorganisms were found with the MRS method (Table 3). Swabbing removed only those microorganisms found on the membrane surface. With the MRS method, membrane sections were placed in Whirlpak bags, dilution water was added and the contents were shaken allowing dilution water to contact all areas of the membrane. This method removed microorganisms from the surface, from backing material on the permeate side, and may have removed microorganisms loosely held within membrane pores. SEM indicated few microorganisms present in backing material on the permeate side of the membrane; however, the fibrous nature of this material (Fig. 5) made accurate surveying difficult. Because a 50-cm² section of membrane was used in the MRS method, 100-cm² of surface actually was evaluated. However, few microorganisms were found in the backing material on the permeate side; therefore, this error had less effect on the results than using a 25-cm² section of membrane for comparison with swabbed sections. These results indicated that the difference in the 2 methods probably resulted from bacteria, yeasts and mold residing within membrane pores. SEM indicated few microorganisms present in backing material on the permeate side of the membrane; however, the fibrous nature of this material (Fig. 5) made accurate surveying difficult. Because a 50-cm² section of membrane was used in the MRS method, 100-cm² of surface actually was evaluated. However, few microorganisms were found in the backing material on the permeate side; therefore, this error had less effect on the results than using a 25-cm² section of membrane for comparison with swabbed sections. These results indicated that the difference in the 2 methods probably resulted from bacteria, yeasts and mold residing within membrane pores (6). Bacteria vary in size depending on growth phase. An example is the period between exponential growth and stationary phase when cells divide faster than they grow resulting in smaller cells (7). The growth phase and therefore, size may influence ability of a microorganism to become trapped within membrane pores. Since the pores of the UF membrane were not resolve by SEM, this was unconfirmed visually.
Another indication of microorganisms residing in membrane pores was the general absence of yeasts and mold in the permeate stream. Because of their absence it is unlikely yeasts and molds were present in large numbers in the membrane material on the permeate side of the membrane. Yeasts were found in greater numbers using the MRS method than by swabbing, and if the two methods retrieved similar numbers of microorganisms from a surface then the difference would be from microorganisms loosely held in pores and released with agitation (MRS method).

The difference between the two methods was evident for all three cleaning treatments. The largest difference was for membranes cleaned with acid where swabbing indicated 70 CFU/50 cm². The low pH of the acid cleaner (pH 2.5) may have killed or injured microorganisms within soil layers uninjured.

Further comparisons between membrane materials were possible using the MRS method (Table 4). Membranes with backing contained the largest number of microorganisms followed by retentate spacers and permeate mesh for all three treatments. Retentate spacers had microbial populations similar to membranes with backing although retentate spacers had 0.4 time the surface area compared to membranes or permeate mesh. The retentate spacer has an open weave designed to increase turbulence within the retentate stream. This design resulted in less surface area in a 50-cm² section compared to other membrane materials.

Following soiling, permeate mesh contained large numbers of microorganisms compared to populations found in the permeate stream resulting from UF of whey. Permeate mesh is a finely woven material and was able to retain microorganisms during soiling (Fig. 6) and cleaning.

Although membrane with backing and retentate spacer contained similar populations of microorganisms after soiling, the retentate spacer had fewer microorganisms by comparison following cleaning treatments. This may have resulted from the retentate spacer having a smaller surface area than the membrane or it may have been easier to remove microorganisms from the retentate spacer material than membrane with backing.

The three cleaners differ in ability to remove or inactivate microorganisms. Although at opposite extremes of pH, acid cleaner and enzyme-based cleaner produced similar microbiological results. Populations found on membranes following cleaning with either acid or enzyme were only slightly less than populations found after soiling with whey. Use of chlorinated alkaline cleaner resulted in lowest number of microorganisms remaining and averaged 3 x 10³ CFU/50 cm² of membrane with backing. Numbers of microorganisms on retentate spacers and permeate meshes were lowest also following use of chlorinated alkaline cleaner.

Populations of yeasts and mold on membrane materials following soiling and cleaning are given in Table 5. Yeasts frequently were found on membrane materials following soiling while mold had only an isolated occurrence on the membranes with backing and retentate spacers. Yeasts and mold were not found on membrane materials following use of chlorinated alkaline cleaner while no mold was found following acid cleaner. Only two membrane sections contained yeasts following acid cleaner; however, yeasts remained after enzyme-based cleaning. Because both chlorinated alkaline cleaner and enzyme-based cleaner were at pH.

**Figure 5. Structure of membrane backing material.**

**Figure 6. Overview of the structure of the permeate mesh.**

**TABLE 4. Comparison of numbers of microorganisms (average of log_{10}CFU/50 cm²) determined by the modified rinse solution method (MRS) for UF membrane materials following soiling and cleaning.**

<table>
<thead>
<tr>
<th>UF Membrane material</th>
<th>Treatment</th>
<th>Soiled</th>
<th>Acid</th>
<th>Enzyme</th>
<th>Chlorinated alkaline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membrane</td>
<td></td>
<td>7.7a</td>
<td>7.0</td>
<td>6.8</td>
<td>3.4</td>
</tr>
<tr>
<td>Retentate spacer</td>
<td></td>
<td>7.9b</td>
<td>6.2</td>
<td>5.9</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>Permeate mesh</td>
<td></td>
<td>5.3b</td>
<td>4.7</td>
<td>3.0</td>
<td>&lt;1.0</td>
</tr>
</tbody>
</table>

*a n=16.
*b n=8.
TABLE 5. Comparison of populations of yeasts and mold (maximum number found/50 cm^2) determined by modified rinse solution (MRS) method for UF membrane materials following soiling and cleaning.

<table>
<thead>
<tr>
<th>UF Membrane materials</th>
<th>Treatment</th>
<th>Soiled</th>
<th>Acid</th>
<th>Enzyme</th>
<th>Chlorinated alkaline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yeasts</td>
<td>40000 (13/16)^a</td>
<td>100 (2/16)</td>
<td>30800 (6/16)</td>
<td>- (0/16)</td>
</tr>
<tr>
<td></td>
<td>Mold</td>
<td>1800 (1/16)</td>
<td>- (0/16)</td>
<td>100 (1/16)</td>
<td>- (0/16)</td>
</tr>
<tr>
<td>Retentate spacer</td>
<td>Yeasts</td>
<td>36900 (5/8)</td>
<td>- (0/8)</td>
<td>- (0/8)</td>
<td>- (0/8)</td>
</tr>
<tr>
<td></td>
<td>Mold</td>
<td>400 (1/8)</td>
<td>- (0/8)</td>
<td>- (0/8)</td>
<td>- (0/8)</td>
</tr>
<tr>
<td>Permeate mesh</td>
<td>Yeasts</td>
<td>2200 (3/8)</td>
<td>- (0/8)</td>
<td>- (0/8)</td>
<td>100 (1/8)</td>
</tr>
<tr>
<td></td>
<td>Mold</td>
<td>- (0/8)</td>
<td>- (0/8)</td>
<td>- (0/8)</td>
<td>- (0/8)</td>
</tr>
</tbody>
</table>

^aSamples with counts >100/total samples.

^bNo maximum number possible since no samples were positive for yeasts/mold.

11.5, pH could not account for this difference. Chlorine in the alkaline cleaner may have rendered it more effective in inactivating yeasts.

The population of microorganisms found in each of four sections of membrane with backing following soiling and cleaning is given in Fig. 7. The ANOVA evaluation is given in Table 6. Microorganisms were deposited uniformly on membrane sections with values ranging from 3 \times 10^7 to 7 \times 10^7 CFU/50 cm^2 after soiling. Acid cleaner and enzyme-based cleaner produced comparable results. Smallest populations occurred in the outermost section (acid, 8 \times 10^7 CFU/50 cm^2; enzyme, 4 \times 10^7 CFU/50 cm^2) and largest populations in the innermost section (acid, 6 \times 10^7 CFU/50 cm^2; enzyme, 7 \times 10^7 CFU/50 cm^2). Use of chlorinated alkaline cleaner resulted in the lowest values for all four sections and unlike the other two cleaners had the highest population of microorganisms in Section III (8 \times 10^6 CFU/50 cm^2).

Since microorganisms were uniformly distributed throughout membrane sections following soiling, the pattern of fewest microorganisms in Section I indicated microorganisms in outermost sections were most easily removed. There are two possible explanations. Although membranes were soaked in cleaning solutions they were reinserted in the UF and rinsed with water. Water velocity may have been greater in outer membrane sections thereby facilitating removal of microorganisms. Additionally, microorganisms removed from outermost sections could have been redeposited in the inner sections thereby concealing removal of microorganisms from these areas.

Evidence that soaking was sufficient in allowing cleaners to reach all membrane sections is provided by the curve representing the efficacy of the chlorinated alkaline cleaner (Fig. 7). As noted previously, the population of microorganisms was greatest in Section III with all data points on this curve below those for the soiled membrane surface. If the chlorinated alkaline cleaner did not effectively contact Section IV then values should have been greatest in this area and similar to those found for a soiled membrane. This is not true for the chlorinated alkaline cleaner.

Microorganisms were distributed uniformly on the retentate spacer following soiling (Fig. 8). Highest microbial populations were found on retentate spacers cleaned with acid or enzyme-based cleaner followed by chlorinated alkaline cleaner (Table 7). This was the same pattern for membrane with backing. In Section I, approximately 7 \times 10^7 microorganisms were deposited per 50 cm^2. Cleaning with acid or enzyme resulted in approximately 1 \times 10^4 CFU/50 cm^2 and following use of chlorinated alkaline cleaner there were 80 CFU/50 cm^2. In Section IV, innermost, counts were 1 \times 10^5 CFU/50 cm^2 following acid cleaner, 3 \times 10^5 CFU/50 cm^2 after enzyme-based cleaner and 5 \times 10^2 CFU/50 cm^2 using chlorinated alkaline cleaner. This pattern of increasing populations from outside to inside sections of the retentate spacers was identical to the pattern for microbial populations on membranes with backing. Population of microorganisms on retentate spacers and membranes should be similar since these...
TABLE 6. ANOVA evaluation of microbial populations found on membrane surfaces.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Sum of squares</th>
<th>Mean squares</th>
<th>F-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>3</td>
<td>132.32</td>
<td>44.11</td>
<td>80.20</td>
</tr>
<tr>
<td>Section</td>
<td>3</td>
<td>19.75</td>
<td>6.58</td>
<td>PR &lt; .001</td>
</tr>
<tr>
<td>Experimental error</td>
<td>9</td>
<td>20.82</td>
<td>2.31</td>
<td></td>
</tr>
<tr>
<td>Sampling error</td>
<td>48</td>
<td>26.51</td>
<td>.55</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>63</td>
<td>199.40</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Soiled</th>
<th>Acid</th>
<th>Enzyme</th>
<th>Chlorinated alkaline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>7.7a</td>
<td>7.0b</td>
<td>6.8b</td>
<td>3.4c</td>
</tr>
</tbody>
</table>

*aMeans followed by the same letter are not significantly different by Newman-Keuls method (α=.05).

Figure 8. Populations of microorganisms throughout the retentate spacer following soiling and cleaning.

Figure 9. Populations of microorganisms throughout the permeate mesh following soiling and cleaning.

were in direct physical contact with fouling and cleaning solutions of the same composition.

The pattern for microbial populations on membranes and retentate spacers did not hold for the permeate mesh (Fig. 9). Microorganisms were not deposited evenly throughout the permeate mesh following soiling. Fewer microorganisms were present in inner Sections III and IV than outer Sections I and II.

Flow of the permeate stream within the UF membrane differs from the retentate stream. Water and materials able to cross the UF membrane are carried in the permeate mesh which spirals down into the permeate collection tube and out of the UF system. Anywhere along this spiraling path water and other material can enter the permeate stream. The velocity of the permeate stream as it approaches the permeate tube could be greater than the velocity of the permeate stream in outer sections of the membrane and could lessen the rate of deposition of microorganisms.

Permeate mesh cleaned with acid cleaner had the greatest number of microorganisms followed by permeate mesh cleaned with enzyme-based cleaner and chlorinated alkaline cleaner (Table 8). After use of acid or enzyme-based cleaner, permeate mesh in Sections I and II had lower populations of microorganisms than mesh in Sections III and IV. Values in the latter area were similar to values for soiled permeate mesh. The concentration and type of molecules crossing into the permeate stream during soiling with whey were different than those present during cleaning. The ability of compounds in the cleaning solution to cross the UF membrane into the permeate stream influences cleaning of the permeate mesh. Additionally, permeate flux is much greater during cleaning than during UF of whey.
TABLE 7. ANOVA evaluation of microbial populations found on retentate spacers.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Sum of squares</th>
<th>Mean squares</th>
<th>F-Value</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>3</td>
<td>175.80</td>
<td>58.60</td>
<td>96.07</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Section</td>
<td>3</td>
<td>12.70</td>
<td>4.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental error</td>
<td>9</td>
<td>8.95</td>
<td>.99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sampling error</td>
<td>16</td>
<td>9.72</td>
<td>.61</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>31</td>
<td>207.17</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Treatment Mean:
- Soiled: 7.9a
- Acid: 6.2b
- Enzyme: 5.9b
- Chlorinated alkaline: <1.0c

*Means followed by the same letter are not significantly different by Newman-Keuls method (α=0.05).

Figure 10. Populations of microorganisms throughout upper and lower sections of membrane with backing following soiling and cleaning.

Further comparisons are possible between membranes with backing on either side of the retentate spacer as indicated in Fig. 2b and 10. When the membrane is assembled the upper membrane of the pair is further from the permeate tube than the lower membrane. Two pairs, each consisting of an upper and lower membrane, were sampled within each section. Populations found on the four samples of membrane were averaged for use in Fig. 7. Values used for Fig. 10 were obtained by averaging the two lower membrane samples and 2 upper membrane samples separately for each Section.

When these values are considered separately as in Fig. 10, additional soiling and cleaning patterns are evident. Following soiling, microorganisms were distributed evenly on upper and lower membranes. However, during cleaning microorganisms were removed from lower membranes in greater numbers than from upper membranes. When the cleaner was ineffective, as with acid and enzyme-based cleaners in Section IV, this difference was not evident. Assembled as a complete unit, the lower membrane has a convex surface exposed to the feed or retentate stream while the upper membrane has a concave surface. During cleaning, this concave or convex shape influenced the ability of a cleaner to remove microorganisms by affecting flow which resulted in greater removal of microorganisms from the lower, convex membrane surface.

Figure 11. Frequency of yeasts (Y) and mold (M) in sections of membrane with backing following cleaning.
TABLE 8. ANOVA evaluation of microbial populations found on permeate meshes.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Sum of squares</th>
<th>Mean squares</th>
<th>F-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>3</td>
<td>81.21</td>
<td>27.07</td>
<td>27.34</td>
</tr>
<tr>
<td>Experimental error</td>
<td>12</td>
<td>31.08</td>
<td>2.59</td>
<td></td>
</tr>
<tr>
<td>Sampling error</td>
<td>16</td>
<td>15.79</td>
<td>.99</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>31</td>
<td>128.08</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Treatment Mean*  
Soiled  5.3a  
Acid  4.7b  
Enzyme  3.0b  
Chlorinated alkaline  <1.0c

*Means followed by the same letter are not significantly different by Newman-Keuls method (α=.05).

Cleaning treatments therefore, were pooled for each section and did not indicate a trend in frequency of occurrence.

Cleaners must remove both soil and microorganisms from the UF system to ensure consumer safety. Microbiological methods previously discussed determined numbers of viable microorganisms on membrane components but did not indicate whether soil and inactive microorganisms remained. The presence of soil and viable microorganisms together represents an unsafe condition. If inactive microorganisms are not removed there can be added declines in permeate flux and sanitizer effectiveness (14). The SEM therefore, was used to determine if soil and microorganisms remained on membrane materials following cleaning.

Deposits on the membrane surface from Section IV formed by UF of whey are visible in Fig. 12. There was a continuous sheet of material (S) deposited on the membrane (Fig. 12a). On top of this sheet were clusters (C) of deposits which did not uniformly cover the sheet-like material and appeared thick with developed tunnel-like structures. In Figure 12b a deep crack (A) in the sheet-like material was apparent with cluster-like deposit (C) above and several bacteria (B) trapped in the deposits. Previous researchers indicated both β-lactoglobulin and bovine serum albumin were able to form sheet-like structures while α-lactalbumin and γ-globulin formed spheres or amorphous granules (8).

The appearance of the deposit following use of acid cleaner is given in Fig. 13. The sheet-like material (S) containing deep cracks (A) uniformly covered the surface (Fig. 13a). A closeup (Fig. 13b) indicated some cluster-like material (C) remaining. Notable was the presence of large numbers of coccus-shaped bacteria (B) within the sheet-like deposit. The acid cleaner removed or altered much of the cluster-like material while leaving the sheet-like material and microorganisms. These microorganisms were viable and present in large numbers (6 x 10^7 CFU/50 cm^2).

Following circulation of enzyme-based cleaner (Fig. 14) the sheet-like material found after soiling with whey was not evident. Rather, deposits had a feathery appearance with areas of membrane (M) visible (Fig. 14a). These deposits appeared aligned and may indicate the direction of retentate flow. Closer examination (Fig. 14b) indicated some small sheet-like areas (S) along with cluster-like material (C). A continuous sheet-like bottom layer as found with soiling and
Figure 13. Membrane deposits following soiling and circulation of acid cleaner.  

a. Overview (bar=10 µm) of deposit showing sheet-like deposit (S) with cracks (A).  
b. Details (bar=1 µm) of sheet-like deposit containing bacteria (B) and cluster-like material (C). A crack (A) in the sheet-like material gives an indication of deposit depth.

acid cleaning was not evident. Many coccus-shaped bacteria (B) were visible within deposits.  

The enzyme-based cleaner previously was shown to hydrolyze milk proteins (19), was referred to as enzyme A, and was most active against whey proteins of the four enzyme-based cleaners studied. The feather-like appearance of deposits in this study apparently resulted from whey protein hydrolysis by enzyme cleaner A.  

Although soil was hydrolyzed and removed, the microorganisms remained. Either sufficient soil remained to hold microorganisms or microorganisms established attachments to the soil, membrane or other microorganisms that were not broken by the enzyme-based cleaner. Since bacterial attachments can be made of lipids and polysaccharides in polymeric form (4,14,15), it is unlikely a proteolytic enzyme would hydrolyze these attachments. Previously discussed microbiological evaluations indicated $3 \times 10^7$ CFU/50 cm$^2$ following enzyme-based cleaner versus $5 \times 10^7$ CFU/cm$^2$ after soiling with whey indicating an inability to hydrolyze bacterial attachments.

Soil and microorganisms were least evident following use of chlorinated alkaline cleaner (Fig. 15). The membrane (M) was visible beneath scattered patches of soil (Fig. 15a). A fine network of cluster-like deposits was present without sheet-forming material or bacteria (Fig. 15b) indicating chlorinated alkaline cleaner hydrolyzed both protein and microorganisms and/or their attachments (17). Previously discussed microbiological data indicated $2 \times 10^4$ CFU/50 cm$^2$. The difference between microbiological data and microorganisms and soil deposits visible by SEM may result from the approximately 1-cm$^2$ field examined by SEM versus the 50-cm$^2$ section evaluated for microbiological determinations.
EVALUATION OF CLEANERS FOR UF

Figure 15. Membrane deposits following soiling and circulation of chlorinated alkaline cleaner.

a. Overview (bar=10 μm) indicating scattered patches of soil over the membrane surface (M).

b. Details (bar=1 μm) of network of cluster-like material. Bacteria and sheet-like material are not evident.

If microorganisms were present in clusters then SEM easily could miss remaining microorganisms.

Structure of soil deposited on the retentate spacer following circulation of whey is visible in Fig. 16. Soil and microorganisms were not deposited uniformly over the retentate spacer and clean areas (R) were visible (Fig. 16a). Heavy deposits of bacteria were covered with a thin film of soil (Fig. 16b). Many bacteria appeared to be in the process of cell division. This was in contrast to the membrane after whey circulation which had heavy soil deposits with bacteria scattered throughout.

The retentate spacer (R) following use of acid cleaner is visible in Fig. 17. Again, deposits consisted largely of coccus-shaped bacteria located in surface depressions (Fig. 17a). Bacteria were covered by a film not present after soiling (Fig. 17b). This film also was apparent on the membrane cleaned with acid and may have protected bacteria beneath from the low pH of the acid cleaner. Following soiling, 2 x 10^8 CFU/50 cm² were found on retentate spacers and 1 x 10^7 CFU/50 cm² after acid cleaning which showed that many of these microorganisms were viable and survived pH 2.5 for 2.0 h.

Less extensive deposits of soil and microorganisms were found following use of enzyme-based cleaner (Fig. 18a). A granular-like material (G) was present along with a thin film which covered clusters of bacteria (Fig. 18b). It is unlikely this material was lactose or minerals but could have been hydrolyzed protein. This material also resembled deposits of hydrolyzed bacterial cells found by SEM (17).

Few deposits were found on the retentate spacer after cleaning with chlorinated alkaline cleaner (Fig. 19a). Of the few bacteria that were evident, several appeared damaged (upper left corner) (Fig. 19b). Additional scanning indicated soil and microorganisms generally were found together. Soil was similar in appearance to the cluster-like material (C) found on soiled membranes.

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Figure 17. Deposits on retentate spacer following soiling and circulation of acid cleaner.

a. Overview (bar=10 μm) of cocci and soil generally located in surface depressions with retentate spacer (R) visible between deposits.
b. Details (bar=1 μm) of deposited cocci covered by a thin soil layer.

Unlike surfaces on the retentate-side of the UF system the permeate mesh had little soil and few microorganisms after soiling (Fig. 20a). A closeup of one deposit (Fig. 20b) showed that soil appeared in clusters and was similar to clusters found on membranes after soiling. A lactose crystal (L) appears in the upper section.

When comparing permeate mesh following use of three cleaners, soil was found only after acid cleaner. Permeate mesh cleaned by either enzyme-based cleaner or chlorinated alkaline cleaner, however, contained clusters of rod-shaped bacteria (Fig. 21). These rod-shaped bacteria were approximately 0.3 μm by 1 μm compared to cocci with about a 1 μm diameter. Large numbers of cocci and no rods were found on UF materials from the retentate-side of the membrane. Conversely, only rod-shaped bacteria were found on the permeate side. At 1 μm coccus-shaped bacteria were too large to cross the membrane while the rod-shaped bacteria at 0.3 μm were able to slip through larger pores in the UF membrane into the permeate stream and mesh. Further, large numbers of coccus-shaped bacteria and thick soil deposits obscured the presence of any rod-shaped bacteria on the retentate-side. Presence of viable microorganisms on the permeate-side of the membrane after cleaning was confirmed by microbiological evaluations. Rod-shaped microorganisms with similar dimensions were located in the permeate mesh of RO membranes used in a water treatment facility (14).

Because populations of microorganisms varied between the four sections following cleaning, SEM was used to compare membrane pieces from Section I and IV to determine if soil deposits also varied.
Deposits on the outermost sections of UF membranes following soiling with whey are visible in Fig. 22. Much less soil was apparent on membranes from the outermost section (Fig. 22a) compared to those from the innermost section (Fig. 12a). Soil was neither as extensive nor as thick and cluster-like deposits (C) with tunnels largely were absent (Fig. 22b). Bacteria were more evident, however, in outermost sections. Populations of microorganisms were uniform throughout the four membrane sections following soiling with whey (Fig. 7). The SEM photographs for innermost sections (Fig. 12), however, indicated fewer microorganisms than in outermost sections (Fig. 22).

Microorganisms may be deposited uniformly over the membrane during formation of the dynamic membrane. As UF continued, soil would be deposited over the microorganisms with the most extensive soil in the innermost sections of the membrane. This would explain the appearance of larger numbers of microorganisms in the outermost sections of membrane compared to innermost sections when populations actually were similar.

Deposits on the outermost sections of membrane cleaned with phosphoric acid are visible in Fig. 23. Deposits appeared similar to those found on inside sections of membrane cleaned with acid (Fig. 13) although deposits were neither as extensive nor thick in the outermost section. Bacteria (B) were very evident in remaining soil. Comparing outermost membrane sections cleaned with acid to outermost areas with whey soiling only (Fig. 22) acid cleaner removed little soil.

Similar conclusions can be drawn for the outermost membrane pieces cleaned with enzyme-based cleaner (Fig. 24). Only moderate soil removal was apparent with a film-like material surrounding bacteria. These photographs did not...
Rod-shaped bacteria located on the permeate mesh following soiling and circulation of chlorinated alkaline cleaner (bar = 1 μm).

Deposits on outermost sections of membrane following soiling and circulation of chlorinated alkaline cleaner.

a. Overview (bar = 10 μm) of deposits scattered on the membrane surface (M).

b. Details (bar = 1 μm) of a deposit containing cocci (B) embedded in a sheet-like layer.

The results of SDS-PAGE of whey and material remaining on membrane surfaces is given in Fig. 26. There were no changes for proteins found in whey initially or after concentration and no large molecular weight proteins were apparent in the permeate. Few distinct protein bands were evident for the soil deposit. Bands for lactoferrin, bovine serum albumin,
immunoglobulin and k-casein were detected. There were no distinct bands for β-lactoglobulin or α-lactalbumin. Protein was apparent through the entire molecular weight range of the gel; however, bands were smeared and indistinct. A heavy band appeared beneath the α-lactalbumin area indicating a large amount of small molecular weight proteins in the deposit. Forces in the commercial UF membrane may have resulted in damage to many of the proteins deposited on the membrane surface.

Proteins found on membranes following use of acid cleaner were similar to proteins in soil deposits. Apparently the change in appearance of deposits detected with SEM was not from removal of proteins or a change in the molecular weight of proteins.

Only a faint band below the α-lactalbumin section was found after use of enzyme-based cleaner. This was consistent with the ability of this cleaner to hydrolyze whey proteins into small molecular weight proteins not detectable with this SDS-PAGE method.

No bands were evident for deposits remaining following use of chlorinated alkaline cleaner. No proteins large enough for detection with this method were apparent.

CONCLUSIONS

None of the three cleaners evaluated (acid, enzyme-based and chlorinated alkaline) were adequate for cleaning commercial polysulfone UF membranes soiled with sweet whey; however, some general conclusions were possible.

Soiling

Microorganisms were deposited on UF membrane materials and plastic devices in greater numbers than on polished stainless steel. Visual inspection of the inside surfaces of
stainless steel housings therefore would not be sufficient to guarantee adequate cleaning.

Soil deposits became progressively heavier toward the inside of the membrane. Microorganisms by contrast were deposited uniformly throughout the membrane.

Similar populations of microorganisms were deposited on the membrane and retentate spacer; however, the membrane surface had thicker soil deposits.

Permeate mesh had small amounts of soil and fewer microorganisms deposited than retentate side surfaces.

Cleaning

Swabbing of the membrane surface was insufficient to remove microorganisms retained within the membrane and backing. Membrane materials varied in ease of removing microorganisms. In order from least to most difficult removal were: permeate mesh, retentate spacer, membrane with backing.

Microorganisms were removed from outermost to innermost sections; however, no similar pattern was seen for soil removal from the membrane surface.

The three cleaners varied in ability to remove both soil and microorganisms. Acid cleaner removed neither soil nor microorganisms. The enzyme-based cleaner hydrolyzed soil but was unable to remove microorganisms. Microorganisms and soil both were hydrolyzed by the chlorinated alkaline cleaner. Since the chlorinated alkaline cleaner and enzyme-based cleaner had the same pH the difference was probably from the chlorine content of the chlorinated cleaner (17).

Viable, rod-shaped bacteria were found in the permeate mesh following cleaning.

The inability of these cleaners to remove both soil and microorganisms from a commercial-scale UF system indicates potential safety problems. The acid cleaner removed neither soil nor microorganisms and because both exist together in the system problems readily can occur. Although the enzyme-based cleaner removed some soil, the cleaner could not dislodge microorganisms. Use of this type of cleaner could conceal the development of safety problems. When both soil and microorganisms remain in the UF system, the buildup of soil over time may become visible. Additionally, as more material covers the membrane surface, flux should decline and problems maintaining sanitizer strength or effectiveness should occur. When a cleaner removes only the soil, microorganisms are allowed to concentrate in the UF system with each successive use. The buildup of microorganisms only would be less visible on inspection and the loss of significant amounts of permeate flux and sanitizer strength less likely (11). A processor, therefore, could assume the UF system was adequately cleaned while the microorganisms actually were increasing in concentration.

Without effective cleaning sanitizers will be unable to maintain the UF system in a safe condition. Cleaners therefore must be able to remove both soil and microorganisms. Because microorganisms apparently can attach to the polymer surfaces in UF membranes (14,15) a cleaner must remove attachments to be effective. Results with Mycobacterium sp. able to adhere to cellulose acetate membranes indicated cell surface polypeptides, α-1,4 or α-1,6-linked glucan polymers and carboxyl ester bond containing substances involved in microbial attachment (15). Carboxylic ester hydrolase and crude preparations of papain and pancreatin detached the Mycobacterium. The latter two enzymes have a broad spectrum of activity in addition to other enzyme activities within the preparations. Other work also has indicated proteolytic and glycolytic activity was required, therefore, cleaning formulations containing broad spectrum enzymes may be able to remove microorganisms and biofilms (20). Regardless of the method of removal if a cleaner only inactivates microorganisms without removing them a biofilm can form from the dead microorganisms (14,20). This film would reduce sanitizer effectiveness and provide protection to remaining microorganisms. Further research is required in this area before effective cleaning of UF membranes is possible.

REFERENCES


