FOR CRITICAL CLEANING IN THE BIOTECHNOLOGY INDUSTRY

7X™ Cleaning Solutions

MP BIOMEDICALS

www.mpbio.com

7X™ FOR CRITICAL CLEANING IN THE BIOTECHNOLOGY INDUSTRY

What is critical cleaning in biotechnology?

Critical cleaning in biotechnology refers to a very specific type of cleaning in a highly restricted environment; negative consequences arise if the cleaning is not performed adequately. The cleaning itself can affect the value of the finished product from whatever residue or contaminant is being cleaned. Critical cleaning is required by FDA, EU or cGMP in any regulated biotechnology industry to remove residues and contaminants to below a desired preset level.

What factors should I consider before selecting a detergent for critical cleaning?

- **Detergent should best match the soil to be cleaned**
- **Compatibility of the detergent to the cleaning surface**
- **Detergent should be suitable for the cleaning method**
- **Minimal impact of the detergent on the environment**

Why use 7X for your biotechnology applications?

From small glass spinner flasks to large stainless-steel fermentation tanks, from irregular shaped mixing blades to harvest tubes, our 7X series of detergents can eliminate contamination and assure critical cleaning for all your biotechnology processing requirements.

An economical 1% concentration in water is effective for various cleaning applications. Made in the USA under a cGMP compliant environment, our 7X can thoroughly clean various soils such as biomaterials, proteins, enzymes, oils, biofuels, gels, pigments, etc.

Benefits of our 7X

- Fast and effective removal of various soil types
- Rinses clean with no leftover residues
- Versatile for cleaning various surfaces
- Nontoxic for tissue and cell cultures
- Environmentally-friendly
- Concentrated liquid formulation for easy dilution
- Lot-to-lot consistency and lot number traceability
- Global availability, certification of analysis and shelf life information
- Technical and validation support
- Residue sampling techniques, ingredients disclosure and analytic methods

WHICH 7X™ SHOULD I USE?

Cat. No. 0976670

Cat. No. 0976671

Cat. No. 0976674

Cat. No. 0976675

What soil needs to be cleaned?	7X Cleaning Solution	ES 7X Cleaning Solution	7X O-Matic Cleaning Solution	ES 7X O-Matic Cleaning Solution
Bioaccumulation, proteins, oils, blood, tissue, pigments, fermentation residues, gels, starches, etc.	✓	✓	✓	✓

What surface needs to be cleaned?	7X Cleaning Solution	ES 7X Cleaning Solution	7X O-Matic Cleaning Solution	ES 7X O-Matic Cleaning Solution
Stainless steel	✓	✓	✓	✓
Glass	✓	✓	✓	✓
PTFE and other plastics	✓	✓	✓	✓
Porcelain/ceramic	✓	✓	✓	✓

Which cleaning method can be used?	7X Cleaning Solution	ES 7X Cleaning Solution	7X O-Matic Cleaning Solution	ES 7X O-Matic Cleaning Solution
Machine			✓	✓
Manual	✓	✓	✓	✓
Soak	✓	✓	✓	✓
Ultrasonic	✓	✓	✓	✓

Which detergent is phosphate free (i.e. eco-friendly)?	7X Cleaning Solution	ES 7X Cleaning Solution	7X O-Matic Cleaning Solution	ES 7X O-Matic Cleaning Solution
Phosphate free		✓		✓

What is the pH range and type of 7X?	7X Cleaning Solution	ES 7X Cleaning Solution	7X O-Matic Cleaning Solution	ES 7X O-Matic Cleaning Solution
pH range	6.0-7.5	6.5-7.5	9.0-11.0	6.5-7.5
Detergent type	Anionic	Anionic	Non-ionic	Non-ionic
Foam level	Regular	Regular	Low Foam	Low Foam

All 7X detergents are available in the following pack sizes: 1 gallon, 4 x 1 gallon, 5 gallon, 55 gallon.
Any questions? Please contact us for technical support!

7X™ PRODUCTS

7X Cleaning Solution and ES 7X Cleaning Solution, Phosphate Free are recommend to dilute to 1% with water. 7X O-Matic Cleaning Solution and ES 7X O-Matic Cleaning Solution, Phosphate Free, are recommended to dilute to 0.25-1% with water before use.

Name	Size	Cat. No.
7X™ Cleaning Solution	1 gal	097667093
	4 x 1 gal	097667094
	5 gal	097667095
	55 gal	097667098
ES 7X™ Cleaning Solution, Phosphate Free	1 gal	097667193
	4 x 1 gal	097667194
	5 gal	097667195
	55 gal	097667198
7X-O-Matic™ Cleaning Solution	1 gal	097667493
	4 x 1 gal	097667494
	5 gal	097667495
	55 gal	097667498
ES 7X-O-Matic™ Cleaning Solution, Phosphate Free	1 gal	097667593
	4 x 1 gal	097667594
	5 gal	097667595
	55 gal	097667598

We also offer ready-to use products for further ease of use.

Name	Size	Cat. No.
7X™ Ready-to-Use Cleaning Solution	1 gal	097668093
ES 7X™ Cleaning Solution, Ready-to-Use	1 gal	097668193
7X-O-Matic™ Cleaning Solution, Ready-to-Use	1 gal	097668493

APPLICATION NOTE

Residue Comparison of 7X™ Detergent, Contrex®AL and Liquinox® Detergents

In order to test the residue left by 7X detergent on glass and to compare its performance with the leading competition (i.e. Contrex® AL from Decon labs and Liquinox® from Alconox), three detergent test kits, UV-Vis absorbance spectroscopy and Attenuated Total Reflection FTIR spectroscopy were applied for the residue study. The experimental data demonstrated that 7X Cleaning Solution has the least residue left after cleaning.

7X™ Cleaning Solution (MP Biomedicals) ▶

CONTAINS*

- Dioctyl sulfosuccinate sodium salt
- Quadrafos (hexasodium tetraphosphate)
- Glycol ether

pH 6.0–7.5

Contrex®AL (Decon Labs) ▶

CONTAINS*

- Sodium dodecylbenzene sulfonate

pH 7.5–9.5

Liquinox® (Alconox, Inc.) ▶

CONTAINS*

- Sodium dodecylbenzene sulfonate

pH 8.5

** Chemical components and pH values are based on the manufacturers' Safety Data Sheets*

By examining the chemical structures of the three active ingredients (fatty acids) comprising the detergents, it can be determined that they are all sodium salts. Fatty acids are known to be weak acids, and the sodium comes from sodium hydroxide; the sodium salt is formed by the saponification reaction:

$$\underset{\text{fatty acid}}{R\text{–}COOH} + NaOH \longrightarrow \underset{\text{detergent}}{R\text{–}COO^{-}\,Na^{+}} + H_2O$$

When dissolved in water, the detergent salt gives the value for the pH. Because all three detergents are sodium salts of the different fatty acids shown above, all pH values should be above 7; therefore, a slightly basic solution should be formed.

The pH value of the 7X Cleaning Solution is closer to a value of pH 7 because quadrafos (hexasodium tetraphosphate) is a salt that is formed from a reaction of a strong acid (phosphoric acid) and a strong base (sodium hydroxide). According to SDS, the amount of quadrafos is 5-10%, while the dioctyl sulfosuccinate sodium salt is 1-5%. Higher concentration for the quadrafos will drive the pH more significantly compared to the sulfosuccinate salt.

1. TESTING USING DETERGENT KITS

1.1. CHEMetrics® Kit *(Cat. No. K-9400)*

The detergent CHEMets® test kit employs the methylene blue extraction method. Anionic detergents react with methylene blue to form a blue complex that is extracted into an immiscible organic solvent. The intensity of the blue color is directly related to the concentration of "methylene blue active substances (MBAS)" in the sample. Anionic detergents are one of the most prominent methylene blue active substances. Test results are expressed in ppm (mg/L) linear alkylbenzene sulfonate (equivalent weight 325). This kit has a nominal range of 0–3 ppm.

The procedure was followed as per the manufacturer's instructions:

1. Rinse the reaction tube with sample, then fill it to the 5 mL mark with sample.
2. While holding the double-tipped ampoule in a vertical position, snap the upper tip using the tip breaking tool.
3. Invert the ampoule and position the open end over the reaction tube. Snap the upper tip and allow the contents to drain into the reaction tube.
4. Cap the reaction tube and shake it vigorously for 30 seconds. Allow the tube to stand undisturbed for approximately 1 minute.
5. Make sure that the flexible tubing is firmly attached to the CHEMet® ampoule tip.
6. Insert the CHEMet assembly (tubing first) into the reaction tube, ensuring that the end of the flexible tubing is at the bottom of the tube. Break the tip of the CHEMet ampoule by gently pressing it against the side of the reaction tube. The ampoule should draw in fluid only from the organic phase (bottom layer).
7. When filling is complete, remove the CHEMet assembly from the reaction tube.
8. Remove the flexible tubing from the CHEMet ampoule and wipe all liquid from the exterior of the ampoule. Place an ampoule cap firmly onto the tip of the CHEMet ampoule. Invert the ampoule several times, allowing the bubble to travel from end to end each time.
9. Place the ampoule, flat end downward into the center tube of the comparator. Direct the top of the comparator up toward a source of light while viewing from the bottom. Rotate the comparator until the color standard below the ampoule shows the closest match. If the color of the ampoule is between two color standards, a concentration estimate can be made.

RESULTS

Solutions of 1% concentrations were prepared from each of the three detergents. The glassware was allowed to soak for 3 hours before rinsing. After rinsing, ultrapure water was added to the glassware and used to test for the presence of the detergents.

The procedure presented above was applied to glassware washed using 1% solution of the detergents. The results are summarized in the table to the right.

Detergent (1%)	CHEMets® Result (ppm)
7X™ Cleaning Solution	0
Contrex®AL	0–0.25
Liquinox®	0–0.25

1.2. Hach Kit *(Cat. No. 1432-03)*

The procedure was followed as per the manufacturer's instructions:

1. Fill one of the test tubes to the upper mark (20 mL) with the water to be tested.
2. Add 12 drops of Detergent Test Solution and shake to mix.
3. Add chloroform to the lowest mark (5 mL) on the test tube. (Chloroform is heavier than water and will sink). Stopper, shake vigorously for 30 seconds and allow to stand for one minute to separate the chloroform.
4. Using the draw-off pipet, remove the water from the tube and discard.
5. Refill the test tube to the upper mark with the Wash Water Buffer and, using the draw-off pipet, remove the Wash Water Buffer and discard. This step washes away the remaining water sample.
6. Refill the test tube to the upper mark with the Wash Water Buffer, stopper and shake vigorously for 30 seconds. Allow to stand for one minute to separate the chloroform.
7. Insert the test tube containing the prepared sample in the right opening of the color comparator.
8. Fill the other test tube with demineralized water and place it in the left opening of the comparator.
9. If the color is darker than the highest reading on the color disc, the original sample may be diluted 20:1 by adding 1 mL of sample to the test tube (using the plastic dropper filled to the top, or 1-mL mark) and filling the test tube to the upper mark (20 mL) with demineralized water. Repeat Steps 2 through 9 and multiply the results by 20.

   NOTE ▸ *If the water sample is turbid, the chloroform layer must be filtered after Step 6, using the provided procedure:*

   a. Place a small ball (about the size of a large pea) of glass wool in the filter thimble.

   b. Using the draw-off pipet to remove the chloroform, filter the chloroform through the glass wool and into the extra test tube.

   c. Proceed with Step 7.

   d. Enough Wash Water Buffer is included for 32 tests. Enough Detergent Test Solution and Chloroform are included for approximately 90 tests.

RESULTS ▸

Turbidity or very intense color was not observed for any of the three detergents; therefore the procedure was followed through steps 1 to 9. The results are summarized in the table to the right.

Detergent (1%)	Hach Result (ppm)
7X™ Cleaning Solution	0–0.2
Contrex®AL	0.7
Liquinox®	0

1.3. LaMotte Kit *(Cat. No. 4507-01)*

The procedure was followed as per the manufacturer's instructions:

STEP 1: Determine if Detergent is Present

1. Use the calibrated test tube (0755) to measure 5 mL of the sample solution. Add to the screw cap tube marked Test Sample (0282).
2. Use the 0.25 g spoon (0695) to add one measure of pH Adjustment Powder (4509). Shake until dissolved.
3. Fill the pipet (0347) with *DS Indicator Reagent (4508) by squeezing the rubber bulb, then inserting the pipet into the reagent. Add this amount of *DS Indicator Reagent to the Test Sample tube. Cap and shake for one minute.
4. Allow the tube to stand until the two layers of the solution separate. The water layer will settle to the bottom and the reagent layer will rise to the top. Use chart below to determine if detergent is present.

Bottom Layer	Top Layer	Quick Reading
Colorless	Colored	No detergent in sample
Some color	Some color	Some detergent in sample
Colored	Colorless	High detergent in sample

NOTE ▸ *If the amount of detergent in the sample is to be determined, save this Test Sample and proceed to Step 2.*

STEP 2: Determine the Amount of Detergent Present

1. Use the calibrated test tube (0755) to measure 5 mL of detergent-free water. Add to the screw cap tube marked Reference Sample (0283).
2. Use the 0.25 g spoon (0698) to add one measure of pH Adjustment Powder (4509). Shake until dissolved.
3. Fill the pipet (0347) with *DS Indicator Reagent (4508) by squeezing the rubber bulb, then inserting the pipet into the reagent. Add this amount of *DS Indicator Reagent to the Reference Sample tube.
4. Add one drop of DS Reference Solution (4513). Cap and shake for one minute.
5. Allow the tube to stand until the two layers of solution separate. The color produced in the bottom (water) layer is equivalent to 1 ppm of detergent.
6. Compare the color in the bottom layer of the Test Sample Tube from Part I to the color of the bottom of the Reference Sample T.
7. Add one drop of DS Reference Solution (4513) to the Reference Sample Tube. Shake to mix. Compare the color as before: The color in the Reference Sample is now equal to 2.0 ppm. Continue this procedure, counting the number of drops of DS Reference Solution (4513) added, until the color of the bottom layer in each tube is the same. Each drop of the DS Reference Solution (4513) added to the Reference Sample Tube is equal to 1 ppm detergent in the sample.

NOTE ▸ *If at any time the top layer of the Test Sample or Reference Sample becomes colorless, add more DS Indicator Reagent (4508).*

If test sample color is:	Test sample contains:
Lighter than reference	Less than 1.0 ppm detergent
Same as reference	1.0 ppm detergent
Darker than reference	More than 1.0 ppm detergent

RESULTS

The amount of this reagent added is not important as long as there is some color in the top layer. The previous procedure was applied to glassware cleaned with 1% of each of the three detergents tested. The results are summarized in the table to the right.

Detergent (1%)	LaMotte Result (ppm)
7X™ Cleaning Solution	1
Contrex®AL	1
Liquinox®	1

SUMMARY OF DETERGENT KIT STUDY

Between the three kits used, the most reliable seems to be the Hach test due to its color comparator that allows matching of colors rather than comparing them individually. Also, the scale is more accurate in the case of the Hach test. Both these factors reduce the subjectivity of the individual performing the test and therefore, the results are more reliable.

Because the Hach test proved acceptable, while the CHEMetrics and LaMotte tests were prone to subjective errors, we performed UV-vis spectroscopy on the solutions tested with the CHEMets and LaMotte kits.

The principle on which the test kits work is based on the negative charge of the fatty acids that makes them soluble in water. By adding methylene blue (a positively charged dye, as shown in Figure 1) that is also soluble in water, an ion pairing occurs between the positive and the negative charge, leading to charge neutrality. The charge neutrality allows the fatty acid- methylene blue complex to be soluble in chloroform or other organic solvents. Without the charge neutrality, this would not occur.

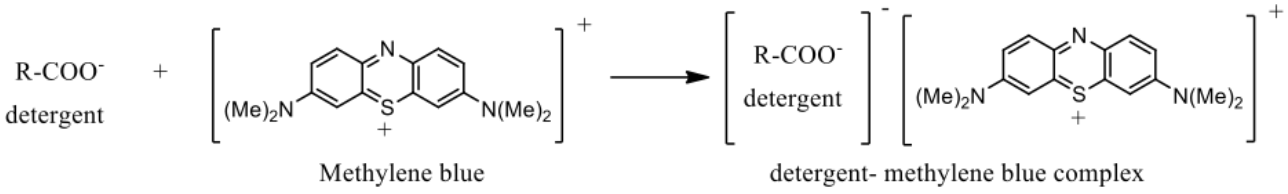

Figure 1. Formation of the charge neutral complex between detergent and methylene blue.

2. TESTING USING UV-VIS SPECTROSCOPY

2.1. UV-vis spectroscopy of the solutions tested using the CHEMetrics kit

In order to determine the presence of the detergents in the water, UV-vis spectroscopy was employed using a Lambda 900 UV-Vis-NIR instrument (Perkin Elmer) and a 1 cm optical path quartz cell.

The results are presented in Figure 2 below:

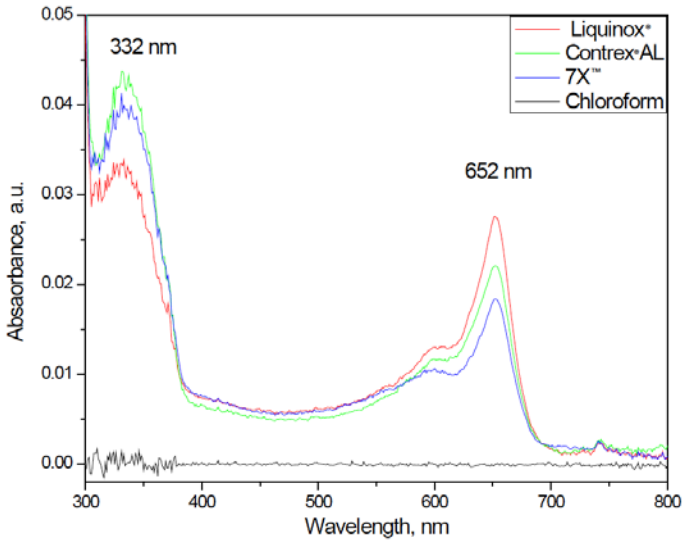

Figure 2. UV-vis absorption spectra for the three different detergent solutions tested with the CHEMetrics kit.

The spectra presented in Figure 2 show two major absorption bands at 332 and 652 nm, with a smaller shoulder at 599 nm. The band at 332 nm is due to the presence of the benzene rings, but shifted due to the presence of the electronegative elements such as N and S present in the structure of methylene blue. The band at 652 nm is due to the actual methylene blue molecular absorbance. The shoulder at 599 nm is typically associated to a small number of methylene blue aggregates that could be formed. All measurements were performed using chloroform as blank or reference solution. As show in Figure 2 (black line), chloroform showed virtually no absorbance in the visible range of the spectrum, while a small noise signal could be observed in the UV range. The other three spectra presented all the features and the differences in terms of intensities were very small.

The changes in the absorbance values between the three detergents were very small, and the small differences could be assigned to the use of different rinsing volumes of water before testing it with the CHEMetrics kit.

Also, the diameter of the CHEMets® tubes are about 0.5 cm and the optical path of the quartz cuvette used for the UV-vis spectroscopy was 1 cm. If a direct comparison is performed, then the absorbance values should be divided by a factor of two, which brings the values even lower. This is likely the reason the human eye cannot distinguish properly between the changes in the blue intensities, as measured with the CHEMetrics kit.

Detergent	Absorbance (A)	ΔA (change in absorbance)	CHEMetrics (ppm)
7X™ Cleaning Solution	0.01837	0	0
Contrex®AL	0.02207	0.0037	0–0.25
Liquinox®	0.02757	0.0092	0–0.25

2.2. UV-vis spectroscopy of the solutions tested using the LaMotte kit

The same instrument and 1 cm optical path was used to measure the UV-vis absorption spectra of the three detergents tested previously using the LaMotte kit.

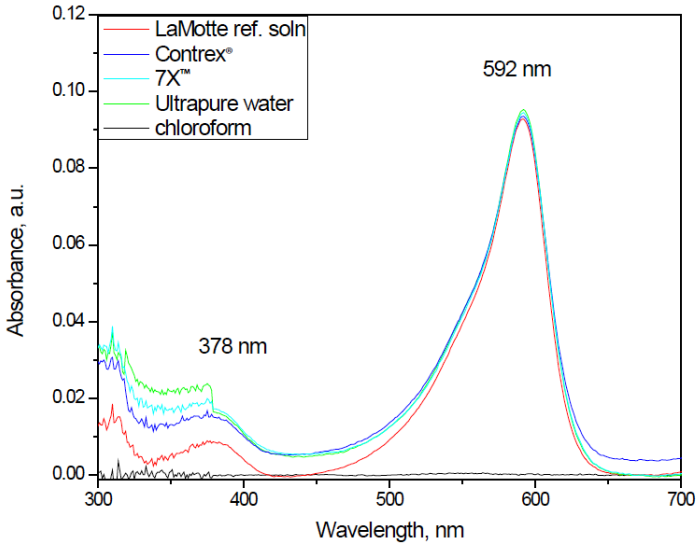

Figure 3. UV-Vis absorption spectra for the detergent solutions tested using the LaMotte kit.

The data presented in Figure 3 used a pure chloroform solution as blank or reference for the UV-vis measurements. Ultrapure water collected from a Modulab 2020 water purification system (Continental, San Antonio, TX) was used as a standard without detergent present. The water purification system has a cation exchange column, an anion exchange column and filter to assure the purity of the water. Typical use of the ultrapure water is as subphase for measurements at the air-water interface, where amphiphilic molecules (fatty acids) are spread on the water surface.

Any small amount of detergent would interfere with such measurements; therefore it is crucial to use water that is virtually detergent-free.

The main spectral features are an absorption band at 378 nm and a second one at 592 nm. The “jump” observed on top of the band centered at 378 nm is the change of the lamp from visible to the UV lamp.

The intensity of the band at 592 nm is extremely similar in all solutions tested. Whereas similar intensities were observed for the 7X and Contrex AL detergents, similar to the LaMotte’s reference solution, it is intriguing the results obtained with ultrapure water.

A similar result using ultrapure water as with the 7X and Contrex AL detergents does not imply that the ultrapure water has a similar concentration of detergent in it, but rather that the test using the LaMotte detergent kit is inconclusive. If a very small concentration of methylene blue ends up in the organic phase, the test will be positive. Also, the LaMotte kit has a lack of standardization in the measurements, and it is highly improbable that the Liquinox, Contrex AL, 7X Cleaning Solution and ultrapure water would have the same concentration of detergent at 1 ppm. In addition, it is not mentioned what is the actual dye used to assess the presence of the detergents in the solution.

3. RESIDUE TESTING USING ATTENUATED TOTAL REFLECTION FTIR (ATR-FTIR) OF GLASS COVER SLIPS

In the research performed above, the detergent residue in the water was investigated using the detergent kits and UV-vis spectroscopy. To check the presence of residue on the glassware, we included 10 glass cover slips in the 1% detergent solution present in the glassware and let them soak for 3 hours in a similar fashion with the glassware. After rinsing of the glassware and glass cover slips, the glass cover slips were put in a clean, dry beaker and placed in an oven at 110 °C for 1 hour. After 1 hour to allow the glass cover slips to dry, the beaker was removed from the oven and cooled to room temperature. This procedure was repeated with each of the detergents used for measurements, i.e. 7X Cleaning Solution, Contrex AL and Liquinox. The ATR measurements were performed on a Bruker Optics Equinox55 FTIR instrument equipped with an ATR accessory and a DGTS detector. For measurements, a 25 reflection KRS-5 crystal was used and 100 scans for each background and sample scans.

For reference or background, a glass cover slip was cleaned using Piranha solution (20 mL H_2O_2 30% mixed with 20 mL H_2SO_4 conc). After cleaning with Piranha, the drying procedure was the same as for the detergent solutions.

For each detergent used from the total of 10 glass cover slips, 5 were taken randomly and analyzed using the ATR technique. The results are summarized in Figure 4.

Each of the three panels A, B and C contain 5 plots corresponding to the 5 glass cover slips used in the study.

The only differences between panels A, B and C are the signal intensities. As can be seen from the panels, the signal intensities are consistent within the same panel, as they were cleaned for the same time using the same detergent under identical experimental conditions. As new glass cover slips were used for each of the detergents, it makes sense for the signal intensities to be different from detergent to detergent, but not within the same batch of 5 glass cover slips.

Based on the data presented in Figure 4, very little detergent was present on the slides. In other words, the detergent did not accumulate on the surface in the place where the IR beam was hitting. It must be mentioned here that the technique is sensitive enough to detect the presence of a single monolayer of compound on the surface of a glass cover slip (data confirming this statement can be provided upon request).

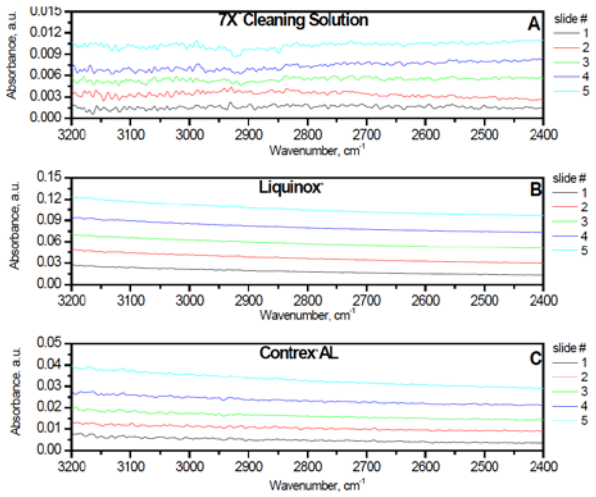

Figure 4. ATR-FTIR spectra for glass cover slips dried after washing with the three detergents.

CONCLUSION

Detergent residues left on surfaces after cleaning could compromise finished product quality in industrial manufacturing and data integrity in laboratory testing. It is one of the most important criteria in determining detergent quality. To test the residue left by 7X detergent on glass and to compare its performance with the leading competition (i.e. Contrex® AL from Decon Labs and Liquinox® from Alconox), three detergent test kits, UV-Vis absorbance spectroscopy and Attenuated Total Reflection FTIR spectroscopy were applied for the residue study. The experimental data demonstrated that 7X Cleaning Solution has the least residue remaining after cleaning.

APPLICATION NOTE

7X™ to Remove Bacteria and Microbial Debris

The purpose of this study was to use scanning electron microscopy (SEM) to investigate whether 7X can effectively remove bacterial and microbial debris adhered to glass covers.

MATERIALS & METHODS

Pseudomonas aeruginosa was inoculated on nutrient agar filled Petri dishes and allowed to proliferate. After one week of incubation, 15 mm x 15 mm glass cover slips were placed on the bacterial culture for one week. Over this period, there was almost complete bacterial coverage of the cover slip.

The three treatments chosen to compare the effectiveness of 7X detergent included a distilled water control, a diluted 1% solution, and the full strength solution. The cover slips were placed in small beakers which were half filled and therefore, half-immersed each cover slip with each treatment type. After soaking in each treatment for 3 hours at room temperature, the cover slips were removed and fixed in 2% glutaraldehyde in 0.5 M phosphate buffer and stored at 4 °C for 24 hours. The samples were prepared for SEM by rinsing in buffer, followed by a graduated series of ethanols (20, 40, 60, 70, 90 and 100%) three times for five minutes each.

The samples were then immersed in three changes of hexamethyldisulazane (HMDS) for five minutes each to dry the samples. After allowing the samples to outgas for three hours, they were coated with palladium in a sputter-coater and imaged using a FEI XL-30 ESEM FEG.

An initial qualitative assessment was conducted of the areas exposed to the treatments (Figures 1-3). In addition, % bacterial coverage was calculated for each half of the cover slip (Figures 4-5). To estimate % coverage of the cover slip, each was imaged in the SEM at 10,000X using a working distance of 20.0 mm at 10 Kv. This resulted in enabling assessment of a uniform frame size (444 um^2). Bacteria and microbial debris were enumerated for six frames in each case, and the data extrapolated to produce a bacteria and cell debris surface area coverage calculation.

QUALITATIVE RESULTS

Pseudomonas aeruginosa Grown on Cover Slips and Immersed in Distilled Water

Distilled water immersion affected *P. aeruginosa* adhesion to cover slips. The distribution of cells on a non-immersed portion of the cover slip demonstrated how the bacterial cells essentially completely cover the slip (1a). If the immersed and non-immersed sections of the cover slip are compared, it is evident that the distilled water treatment (right portion of the micrograph) did lyse some of the cells (1b). A low magnification view of the distribution of the bacterial cells on the non-immersed portion of the cover slip covered with bacteria illustrate the extensive coverage (1c). In some cases, although much cellular debris is present, some cells were still intact after treatment in distilled water (1d).

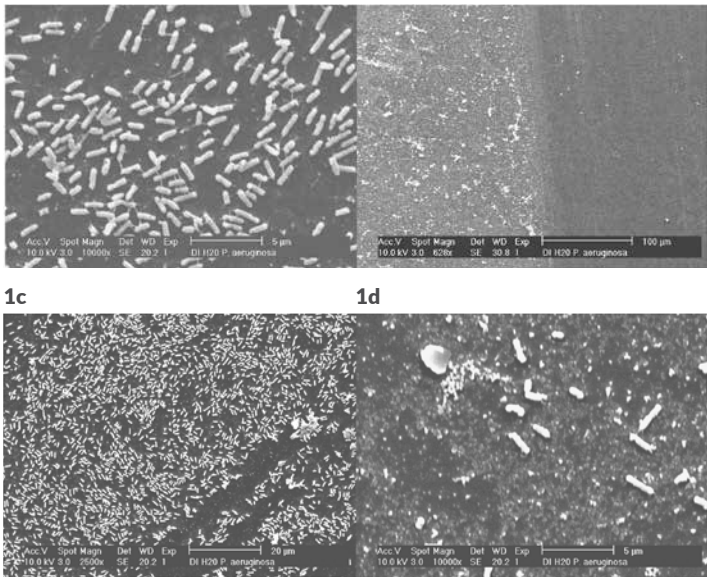

Figure 1. Examination of distilled water washout on a biofilm of *Pseudomonas aeruginosa* atheed on a glass coverslip.

Pseudomonas aeruginosa on Cover Slips Immersed in 1% Detergent 7X

1% 7X Detergent immersion affected *P. aeruginosa* adhesion to cover slips. The distribution of bacteria on the non-immersed portion of the cover slip indicates the substrate is covered with bacterial cells (2a). After treatment with 1% 7X detergent (2b), a few intact bacteria were evident; however, the organic cellular debris present was much less evident than in the distilled water treatment. The sharp demarcation between the treated and un-treated portion of the cover slip is evidenced by the lack of bacterial adhesion on the upper right portion of the cover slip (2c).

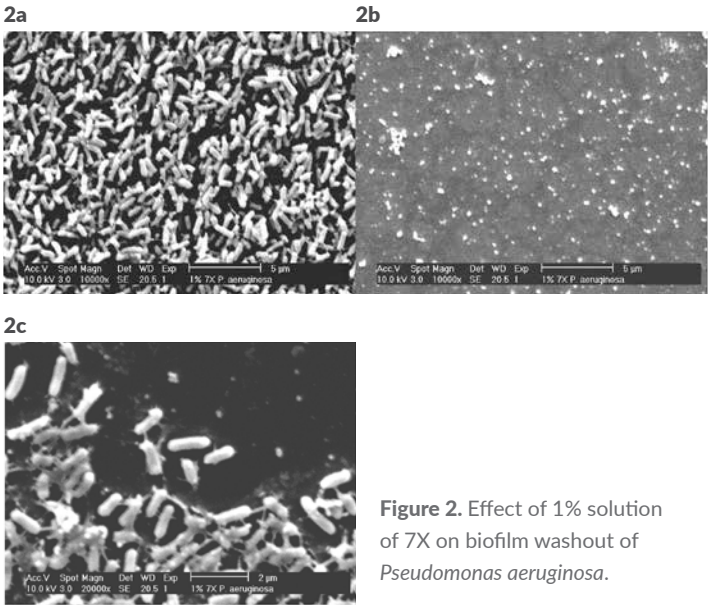

Figure 2. Effect of 1% solution of 7X on biofilm washout of *Pseudomonas aeruginosa*.

Pseudomonas aeruginosa on Cover Slips Immersed in Full Strength 7X

Full strength 7X detergent immersion affected adhesion of *P. aeruginosa* to cover slips. The non-immersed portion of the cover slip is covered with bacteria as well as a prominent microbial film (3a). 7X detergent at full strength appeared to be more effective at removing both intact microbes and organic debris than the 1% detergent solution or distilled water (3b).

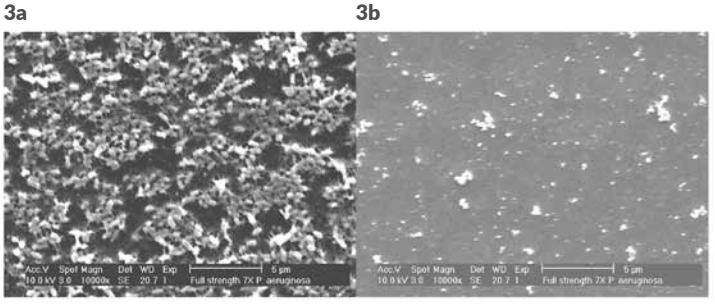

Figure 3. Effect of 1% solution of 7X on biofilm washout of *Pseudomonas aeruginosa*.

QUANTITATIVE RESULTS

The quantitative relationship between surface area of the cover slips covered with bacteria and debris with type of treatment (Figures 4-5) confirms what the qualitative visual evidence suggests. There was a significant reduction in the number of intact bacteria in all the treatments (Figure 4). However, the amount of cellular organic debris exhibited a significant trend with each treatment (Figure 5). The amount of debris was approximately the same in the distilled water treatment. In contrast, in the 1% 7X formulation, there was a reduction to less than 20% of the surface area examined. Additionally, the full strength 7X detergent exhibited less than 10% debris coverage adhering to the cover slip surface.

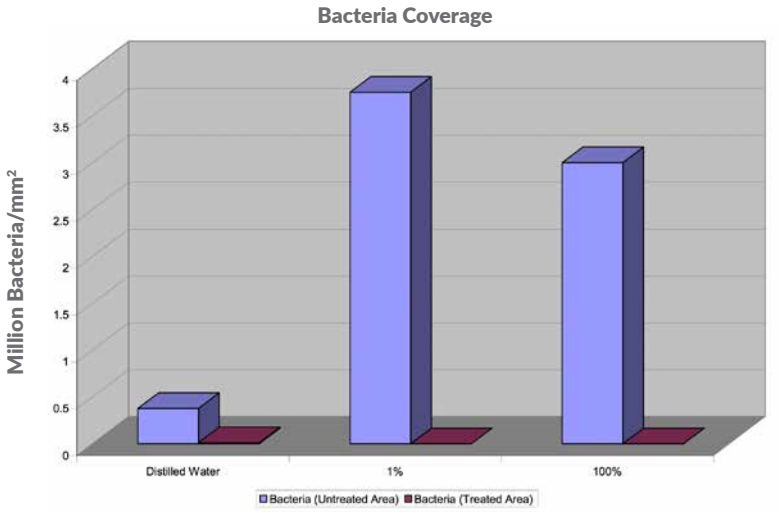

Figure 4. Bacteria coverage expressed as number/surface area of the cover slip.

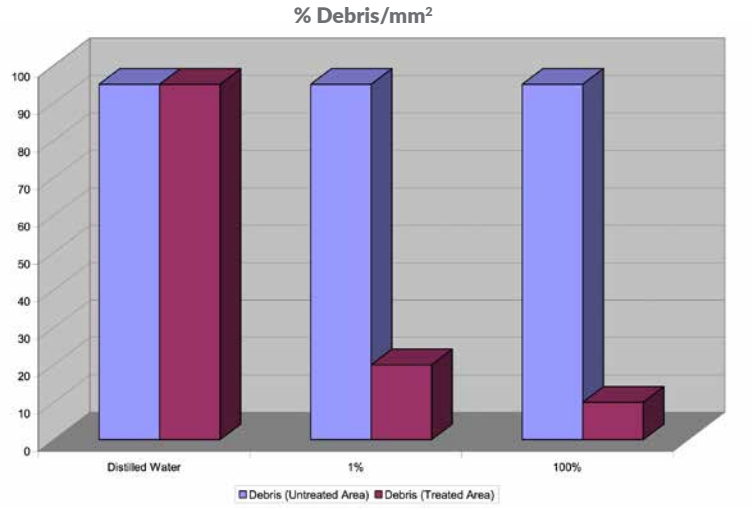

Figure 5. Bacteria cell debris/surface area of the cover slip.

CONCLUSION

Qualitative and quantitative analysis of the scanning electron microscopy (SEM) images has demonstrated that 7X can effectively remove bacterial and microbial debris adhered to glass covers.

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