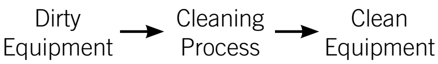
Case Studies of Enzymatic Cleaning Problems Revealed by Enzymatic Cleaning Verification Indicators

Note: *The cleaning indicator used in the case studies referenced in this article is the Pinnacle Monitor for Automated Enzymatic Cleaning from Serim Research Corp. These are dry test strips to be placed in the cleaning load to indicate the overall efficacy of an enzymatic (protease) cleaning process. The strip contains a dyed protein, which is cross-linked into an indicator pad and can only be removed by proteolytic action and rinsing. If the strip emerges from the cleaning process lighter than the internal color standard, the cleaning process is likely sufficient.*

MANY PEOPLE MAY envision the cleaning process as a black box (Figure 1). Dirty equipment goes into a washer-disinfector and then clean equipment emerges. Some requirements for cleaning are not so obvious, however, as can be seen in Figure 2. A good cleaning indicator will indicate when any of the required elements for enzymatic cleaning fail.

Figure 1

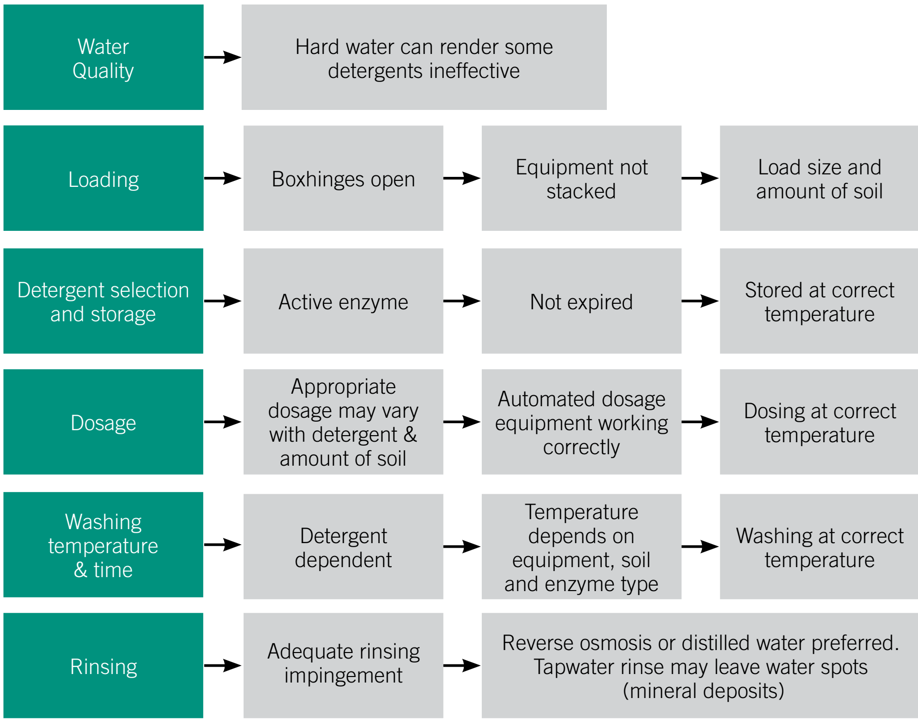
The Hospital Equipment Cleaning Process



There are some important facts to know about enzymatic cleaners. These include the following:

- Not all enzymes react the same to temperature. One enzyme may have maximum activity at 60°C, while another may lose activity.
- It is essential to follow the manufacturer's instructions (if any) on the detergent container regarding storage temperature and use.
- For diluted detergents, even minutes of exposure to high temperatures may irreversibly deactivate the enzyme. Freezing may also deactivate enzymes.
- Enzymes lose their effectiveness with age. A dosage that is effective with a fresh bottle of detergent may not be effective at the end of its shelf life.
- Differences in the way a washer-disinfector executes programmed functions, such as dilution, may result in varied cleaning outcomes among models of washer-disinfectors, even

Figure 2: Inside the cleaning process black box



though they are programmed at the same nominal settings.

A typical customer inquiry submitted for resolution is "I followed the cleaning protocols, but the cleaning indicators don't work." After confirmation that the indicator did, in fact, work properly, investigation usually reveals a problem with the cleaning equipment or protocol. The operator is often using the manufacturer's minimum recommended dosage, minimum temperature and minimum time, which results in inadequate cleaning. Sometimes, the cause of cleaning failure is something so nuanced that the operator would be unlikely to determine it.

There are two main factors which lead to incorrect wash protocols: lack of communication among the groups involved in designing the cleaning process (system engineering) and incorrect trade-offs of wash conditions.

LACK OF COMMUNICATION (SYSTEM ENGINEERING)

Three parties are involved in designing the cleaning process: the manufacturer of the cleaning equipment; the manufacturer of chemicals and enzymes used in formulating detergents, and the manufacturers of the detergent. Usually, each party is focused on their specific area of expertise. If there is inadequate communication among these parties, the end user may not have sufficient information to effect adequate cleaning.

TRADE-OFFS IN WASH CONDITIONS

The four main parameters in a cleaning protocol include: detergent dosage, wash temperature, wash cycle time and impingement. Increases in cleaning efficacy have been demonstrated to correlate with increases in proteolytic enzyme concentration. Typically, cleaning efficacy increases as each factor is increased (temperature can be an exception); however, increasing one alone may increase efficacy. Increasing each parameter also increases costs, which can lead to trade-offs to optimize resources. Using the combination of the lowest recommended setting for each parameter is often insufficient to provide adequate cleaning and is even less likely to work for large, dirty loads, or as the detergent ages.

The following examples from actual investigations illustrate how the lack of understanding of these basic principles can lead to cleaning system failures.

Example 1: Conflicting Directions

The directions on the detergent container may instruct the user to dose "according to the equipment manufacturer's directions." The cleaning equipment manufacturer may recommend dosing "according to the detergent manufacturer's recommendation." The user may default to the lowest dosage of detergent, lowest temperature and shortest wash time, thereby, leading to ineffective cleaning.

Example 2: Titrating to the Least Amount of Detergent Possible

One savvy cleaning operator wanted to use cleaning indicators to determine the lowest effective dosage of a detergent; however, the minimum effective dose determined for a fresh bottle of detergent may be ineffective as the detergent ages or with heavily soiled loads.

Example 3: Problems Encountered in Detergent Evaluations with Enzymatic Cleaning Indicators

A washer-disinfector manufacturer was investigating potential enzymatic detergents and cleaning indicators to recommend with its new system. Most cleaning indicators showed sufficient soil removal; however, Serim's Pinnacle Monitor for Automated Enzymatic Cleaning indicator showed insufficient soil removal in all cases. Reference assays conducted on the samples of enzymatic detergent revealed most of the detergents had insufficient enzyme activity to be effective, even at the maximum recommended dosage. Unfortunately, neither the expiration date of any of the detergents nor the storage or shipping conditions were known. Samples were shipped in the winter when there was the potential for freezing. There may have been rational reasons for the lack of activity, but there was insufficient information to determine the root cause.

In addition, the cleaning indicators were removed from the washer-disinfector before the rinse cycle. After allowing the indicators to go through the rinse cycle, the Pinnacle cleaning indicators showed sufficient soil removal with the detergents containing active enzyme. These indicators performed as designed (only indicating effective cleaning when the enzyme activity was sufficient and the entire wash program, including rinse steps, had been completed).

Example 4: Malfunctioning Pumps

Some washer-disinfector users report unexpected failed results from cleaning indicators, which were traced to malfunctioning detergent dosage pumps. Peristaltic pump tubing can stretch or leak with time, resulting in incomplete detergent dosing. Pumps are typically checked monthly, but either this check is insufficiently thorough (only visually checking apparent tubing integrity versus the accuracy of dosage volume), or monthly checks are not frequent enough. This alone is enough reason to recommend the use of enzymatic cleaning indicators on a frequent basis.

Example 5: Execution of Washer-Disinfector Dilution Programming and Enzyme Temperature Sensitivity

A washer-disinfector manufacturer representative noticed that a cleaning indicator was only showing sufficient soil removal at the maximum of both the recommended concentration and temperature for his detergent. *Note: This case will be used as an example to illustrate the process of root cause determination.*

The cause turned out to be one of those hidden variables in Figure 2. The recommended temperature range of the enzymatic detergent was listed as 45°C to 65°C. The detergent was effective at cleaning at 60°C in a sonicator, but failed at 65°C. The window of temperature failure was quite narrow. When used in a Miele Model G7883 washer-disinfector, there was effective cleaning at 65°C, despite the failure reported from the washer-disinfector manufacture in their machine under the same nominal conditions.

Why then was the detergent recommended for use at 65°C? Why did it lose effectiveness in one washer-disinfector and not in a different model?

TOOLS USED IN THE INVESTIGATIONS

A modified version of the azocasein assay, was used to assess protease activity. This assay is considered a gold standard for protease activity. Trichloroacetic acid (TCA) will precipitate a large protein to which dye molecules are attached. Small dyed polypeptides produced by enzymatic digestion will not precipitate in TCA. Enzyme samples with higher protease activity produce more color in the supernatant of a TCA precipitate. A rate constant (K40) is calculated for each enzyme sample. Enzymatic detergents with K40 values between 4 and 10 at 40°C when diluted ¼ to 1 ounce per gallon are typical of protease activities found in commercial detergents.

In this investigation, a bench top sonic cleaner was used to make it easier to test factors that affect cleaning, including the details of when and at what temperature a detergent is dosed. Cycle times in sonic cleaners are also faster than in washer-disinfectors.

THE INVESTIGATION

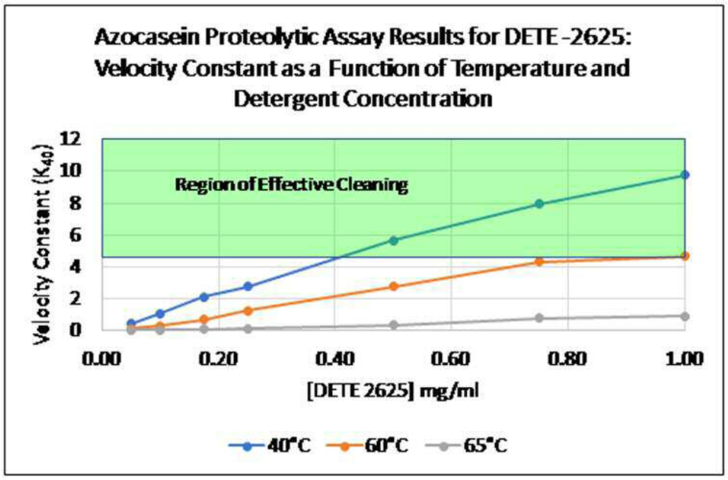
The detergent under investigation maintained good activity in the reference assay at 65°C. When the detergent was diluted in water rather than the buffer used for the reference assay, it rapidly lost activity at 65°C. The reference assay provided a false picture of the enzyme activity at 65°C in the wash situation. The high concentration of the specific buffer used in the reference assay protected the enzyme from deterioration. In the sonicator and washer-disinfector, dilution occurs into water, not the concentrated buffer.

Further investigation revealed a difference in the order of dilution and heating operations between the computer program in the washer-disinfector, in which the detergent failed, and the Miele washer-disinfector. The Miele doses detergent immediately upon filling with water and heats the diluted detergent in a pool in the bottom of the chamber. The load is sprayed with this cool water, but the timing for the wash cycle does not begin until the water temperature reaches the set temperature. Although washing occurs during the time it takes for the water to reach the set temperature, this is not counted as part of the wash hold time. The effective cleaning occurs as the diluted detergent is heating up, not during the 1-minute timed wash hold time at 65°C. The enzyme had been deactivated by the time it reached 65°C.

The washer-disinfector in which the detergent failed performs dilution only after the water reaches the set temperature. While the wash cycle settings were the same (wash for 1 minute at 65°C), different dilution protocols produced different cleaning indicator results.

Perhaps the detergent manufacturer validated the cleaning using a washer-disinfector that employed a protocol similar to the Miele, which would have suggested that cleaning would be effective to 65°C. Even had the detergent vendor used the reference enzyme assay,

Figure 3



usage at 65°C would have been apparently justifiable. The cleaning indicator correctly indicated that use of this detergent at 65°C was not appropriate for all washer-disinfector designs.

Figure 4

Cleaning Verification Indicator for DETE-2625 Following 3 Minutes of Sonication at Different Temperatures, Enzyme K_{40} = 4

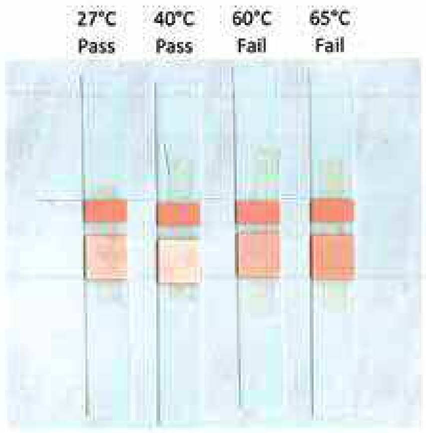
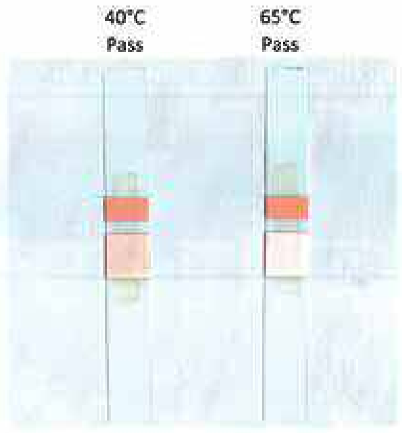


Figure 5

Cleaning Verification Indicator for DETE-2625 Following 1 Minute Nominal Wash in a Miele w/d at Different Temperatures, K40 = 4



MODELING THE BEHAVIOR OF THE DETERGENT

To validate that this unusual cleaning behavior could be triggered by an enzyme known to be commercially used for this purpose rather than some unknown fluke of detergent formulation, Creative Enzymes (Shirley, New York) recommended we investigate their enzyme used in the manufacture of detergents effective at low temperatures, DETE-2625. The temperature profile of DETE-2625 was similar to that of the commercial detergent that failed at 65°C. In the reference assay, DETE-2625 protease activity dropped to half from 45°C to 60°C (Figure 3). Virtually all activity was lost at 65°C, as indicated by the low K40 value. For DETE-2625, the activity dropped at 65°C, even when diluted in the assay buffer, whereas the comparative detergent only lost activity in the reference assay when the detergent was diluted in water, instead of the buffer.

For use in the sonicator and the Miele washer-disinfector, the DETE-2625 enzyme was dissolved into a simple low-foaming detergent formulation. The proteolytic activity of DETE-2625 was characterized in the sonicator at 27, 40, 60, and 65°C. Cleaning efficacy increased between 27°C and 40°C, but dropped substantially between 40°C and 60°C (Figure 4). One would expect enzyme activity to continue to increase with temperature, but that is not the case for DETE-2625, which was developed for low-temperature cleaning. In the Miele washer-disinfector, however, cleaning was more effective when set to 65°C than when set to 40°C (Figure 5), as in the example from the commercial detergent above. Recall that in the Miele washer-disinfector, the enzymatic cleaning actually takes place while the detergent heats from room temperature on its way to 65°C; this explains this apparent contradiction.

Thus, it is possible to develop justifiable recommendations for use of an enzymatic detergent that may be effective in one model of washer-disinfector, but not in other models.

CONCLUSION

Even when following the instruction information provided by detergent and equipment manufacturers, one may still end up with insufficiently clean equipment. Enzymatic cleaning indicators should be used frequently to assess the efficacy of enzymatic cleaning protocols on each site's equipment. Ⓒ

RESOURCES

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