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**Use of Alkali/Acid Cleaning Agents in Biotech**

The most common equipment cleaning process in biotechnology manufacture involves cleaning first with an alkaline cleaning solution, followed by use of an acidic cleaning solution. Of course, the cleaning process usually involves a pre-rinse with ambient temperature water, a rinse after the alkaline solution and a more stringent rinse following the acidic solution. However, the focus of this Cleaning Memo is “why use both an alkaline solution and then an acidic solution?” Also note that the alkaline/acid regimen may involve use of caustic followed by phosphoric acid, an alkaline detergent followed by an acidic detergent, or an alkaline detergent followed by phosphoric acid. Also note that those concerned about disposal of phosphates may use an organic acid, such as citric acid.

One answer to that question is “we’ve always used that combination, and it works”. But my question is not whether it works (clearly done right, it works). Rather my question is “how did this regimen get adopted in the first place?” A reasonable explanation is that this alkaline/acid regimen was adopted for biotech because that was regimen commonly used in the dairy industry for CIP cleaning processes. And of course, CIP got its start in the dairy industry, particularly in milk processing. So, as CIP made its way into biotech manufacture, it made sense to use the same cleaning regimen used successfully for milk processing. After all, milk manufacture is just the first example of biotech manufacture of a protein product (I have to give credit to Dale Seiberling, the father of CIP, for this observation).

Now, what is the rationale for use of the alkaline/acid regimen in CIP cleaning for milk manufacture? That is much clearer. The caustic solution was for removal of proteins and the like in the milk. But, calcium ions and phosphate ions are also present in milk. What happens when the pH is increased (to pH 12) for an aqueous solution of calcium ions in the presence of phosphate ions? Basic chemistry (as well as real life experience) tells us the calcium ions will precipitate, primarily as calcium phosphate (that deposit in milk manufacture is called “milkstone”). Note that the presence of carbonate ions will also precipitate calcium carbonate; however the solubility product of calcium phosphate is such that the phosphate salt is precipitated well before the carbonate salt. So, when milk is cleaned with just caustic, the combination of calcium and phosphate ions at that high pH results in the precipitation of calcium phosphate. And the precipitation is made worse by the fact that it is at elevated temperatures, since calcium salts are less soluble at higher temperatures (contrary to the general rule for solubility of salts).

So, if caustic alone is used for cleaning of milk, the result is a nice white film of calcium phosphate on the process equipment surfaces. Clearly this is undesirable, so the next step is to dissolve that freshly deposited “film” of calcium phosphate by use of a phosphoric acid solution. Once the pH is lowered to the region of around 3, the calcium phosphate deposit is redissolved and rinsed away in the post-rinse.

I believe this is a fairly accurate explanation of why the alkaline/acid regimen is used for CIP cleaning of milk. And I think it is a fairly reasonable assumption that the reason the same alkaline/acid regimen was adopted in biotech is that it was a proven technology. However, it is fair to ask whether the *rationale* for its use for milk production *applies to biopharmaceutical manufacture*. The main question to ask is whether there are significant amounts of calcium and phosphate present in the “soil” when the biotech equipment is cleaned. For comparison, milk itself contains about 0.1% calcium. Since it reasonable that a biotech soil might have

phosphate ions, it can be expected that any biotech soil containing this amount of calcium would probably precipitate calcium phosphate (as in cleaning in milk production) in the alkaline wash step.

So the first question is whether there is a significant amount of calcium in the soil at the time of cleaning. If there are no sources of calcium, then it is likely that calcium phosphate does not form, and perhaps an acidic wash step is not required. It should be noted that if calcium is present and there is *no source* of phosphate ions, there still might be a problem with precipitation of calcium *carbonate* (due to carbonate salts in the soil or the presence of carbon dioxide in the water; note that this includes pick-up of carbon dioxide from the air in a spray ball operation). My personal experience has been that some biotech soils are effectively cleaned with an alkaline cleaning solution alone. There is also a recent *Biopharm International* publication (N. Rathore et al, "Bench-Scale Characterization of Cleaning Process Design Space for Biopharmaceuticals"), where caustic and a formulated detergent were utilized to evaluate the cleaning of four biotech soils in a laboratory study. In the reported results, both the caustic and the formulated detergent were found to be effective (as evidenced by visual examination) *without* the use of an acidic wash step (although I should make it clear that it was not the stated intent of the authors to demonstrate that an acidic wash was not necessary). If precipitation of insoluble calcium salts had occurred, I presume this would be picked up by visual examination (note that TOC would be of no help in detecting the presence of calcium phosphate). This suggests that at least some biotech soils could be effectively removed by use of alkali alone or an alkaline detergent alone.

Note that I am *not saying* that the acidic wash step is *not needed*. It *may be needed* if there is calcium in the soil. There also been other reasons given for use of the acidic wash step.

One reason given is that it more effectively assists in the neutralization and removal of the caustic. While it certainly assists in neutralizing the caustic from the first wash step, I'm not entirely sure that replacing just WFI for the acidic wash step might not result in just as effective removal of the caustic solution. This is something that could be determined experimentally. Wash with a typical alkaline/acidic wash regimen, and measure the conductivity *during* the final rinse step. Perform the same cleaning process *without addition of the acid* (that is, still have the wash time and flow of the acid step, but with no acid present). A comparison of conductivity during the final rinse should indicate any advantage (or lack thereof) of the acidic step.

Another reason put forward for the use of an acidic step is that it assists in either passivating or maintaining the passivation of a stainless steel surfaces. This may or may not be valid. Certainly there is no indication that relatively dilute solutions of phosphoric acid for relatively short times as used in biotech cleaning *will passivate* a stainless steel surface. However, one way to *maintain an already passivated surface* is to keep that surface clean. The acidic wash step may help in this, particularly if there are calcium salt deposits on the surface.

Am I suggesting that biotech manufacturers change their cleaning processes to omit the acidic wash step? Yes and no. My guess is that there are other higher priority items to address in biotech manufacture. However, it always helps to understand what is happening in a cleaning process so that we can more effectively design cleaning processes. I believe that this is one area where a better understanding might help simplify cleaning processes, resulting in both water and time savings. I further believe that the studies to determine whether cleaning processes can be simplified are not burdensome. However, it should be recognized that the conclusions of those studies might depend on the nature of the soil cleaned, and particularly whether calcium ions are present in that soil.