



## Coming Clean A New Method of Washing Yeast with Chlorine Dioxide

by Dana Johnson  
Birko Corporation

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Immersed in the hustle and bustle of a busy brewery, it is easy for one to forget that yeast is a living entity that requires special care, that it is something more than just another brewing ingredient. Yet even with that yeast-care is a very touchy subject among brewers. Ask ten brewers how they handle their slurries and you'll probably hear ten different answers. Washing yeast is one of the more controversial practices - some breweries rely on it, while others avoid it all together. In this article, we will review the most commonly used method of washing yeast, and will introduce a new procedure that is being explored at some breweries.

### **Tried and True**

The "tried and true" method of washing yeast utilizes phosphoric acid ( $H_3PO_4$ ) to acidify the yeast slurry to around pH 2, where it is held for a given amount of time, ranging from two hours to overnight. In theory, undesirable organisms are destroyed by the low pH and trub is removed from the yeast. The healthy yeast remains suspended and is used for pitching, while dead cells and trub collect at the bottom of the washing vessel.

However, there are several problems with acid-washing. It reduces the populations of most wort-spoiling bacteria, but is less successful with beer-spoilers such as lactic-acid bacteria, and is generally not effective on wild yeasts and molds(1). Further, the low pH tends to stress the yeast, and for this reason most breweries wash with acid only rarely. Recently, a few breweries have begun using an acid-free wash that allows them to wash their yeast on a regular basis.

### **An Alternative Method**

Chlorine dioxide ( $ClO_2$ ) has been used for decades to disinfect drinking water. In this scenario, chlorine dioxide kills by penetrating the hydrophobic region of the bacterial membrane and oxidizing it(2). Chlorine dioxide reacts with sulfur-containing amino acids, which form cell membranes. The proteins get destroyed, the membrane ruptures and the organism dies(3).

Chlorine dioxide is relatively new to the brewing industry. It is gaining acceptance as a post-rinse sanitizer, but is not widely-recognized as a yeast-washing agent. Given what is known about it, however, it makes sense that it might be an effective, economical and safe alternative to phosphoric acid.

Chlorine dioxide has (in theory) over 2.5 times more oxidation capacity than elemental chlorine but does not have a chlorine-like flavor profile. Chlorine dioxide does not form trihalomethanes, as does sodium hypochlorite (household bleach) and iodophors, and breaks down to innocuous compounds, namely table salt and water(4).

Breweries that use chlorine dioxide to wash yeast begin with a measured amount of sodium chlorite

solution, which is "activated" to release ClO<sub>2</sub> by pouring it into a small amount of acidified water. The activated solution is then immediately added to the yeast slurry and mixed well. In theory, the chlorine dioxide that is released "washes" the yeast in as little as five to ten minutes, depending on contaminant-levels. The yeast can then be pitched immediately or stored refrigerated until needed.

The small breweries currently using chlorine dioxide for this purpose do so without any yeast performance problems. Most, however, do not have the means to check yeast viability and test contamination levels.

Low (1-2) parts-per-million (ppm) levels of chlorine dioxide kill organisms such as wort-spoilers, but not much is known about its efficacy against the myriad contaminants that can find their way into a brewer's yeast. Our purpose here is to begin finding out.

There were two questions we wanted to answer: Does washing yeast with chlorine dioxide reduce the number of spoilage organisms? Does washing yeast with chlorine dioxide have any effect on the viability of the yeast? Testing the long-term stressing effect of chlorine dioxide, such as on the formation of petite mutants, will be the subject of subsequent studies.

### **Test Procedure**

Two different strains of pure ale yeast were propagated using unhopped malt extract and stored at 4° C (39° F) for about two weeks. Just before testing, each was dosed with a mixture of Acetobacter, Lactobacillus, Enterobacter and wild yeast. Six clean and sanitized glass growlers were divided into the following two sets:-One liter of 4° C-water was added to each growler. Each yeast slurry was distributed equally among the three growlers in its set, which were then tightly capped and inverted for good mixing. The viability and cell-counts of each was read using the methylene-blue staining method and hemacytometer. The slurries' cell densities differed by a factor of 1.5, which reflected their distinct growth characteristics in the malt extract.

Contamination levels were tested by drawing a 0.1 ml sample from each and plating onto LMDA medium. All plates were incubated aerobically for 84 hours at 30° C (86° F) and checked for colony forming units (CFUs).

The "water-wash" slurries were stored at 4° C for two hours and inverted every 15 minutes to keep the yeast suspended. After this time, each was tested in the same manner as before for viability and contaminants.

The "acid-wash" slurries were treated with 2.5 ml 85% phosphoric acid each, giving a pH 2.2, and were stored at 4° C for two hours and inverted every 15 minutes to keep the yeast suspended. After this time, each was tested in the same manner as before for viability and contaminants.

The "ClO<sub>2</sub>-wash" slurries were each treated with 50 ml activated sodium chlorite (0.5 ml of 8.3% sodium chlorite concentrate, added to 100 ml cold water which had been acidified to pH 3), which was calculated to yield 20-50 ppm in the volumes treated. The activated sodium chlorite concentration of each slurry was measured using a sodium chlorite titration test kit.

### **Conclusion**

Not surprisingly, washing the yeast with water affected neither viability nor contamination levels. Phosphoric acid did a good job of removing the gram-negative spoilers tested and reduced the Lactobacillus population by about 20 times. As impressive as this may be, it is unsatisfactory

because the levels remaining are 180 times above those generally tolerated. Three CFU's-per-1.0-ml is considered acceptable, as opposed to the 55-per-0.1-ml listed in Table 2.

While phosphoric acid did a respectable job of destroying the bacteria, the viability of the yeast suffered to varying degrees (~14.5% and ~4%). It also seems to have had no effect on the wild yeast.

Chlorine dioxide appears to have cleared both slurries of bacteria quite thoroughly, even at the 13-ppm-levels we achieved, without decreasing the viability of the yeast. This was accomplished inside of ten minutes, rather than over the two-hour period required for the acid wash. The six CFUs of Acetobacter appearing after treatment at ten minutes were gone when the slurry was tested again at 24 hours. Since chlorine dioxide is gaseous and dissipates quickly, this effect may be explained by residual "unactivated" sodium chlorite being "activated" over time by the acids produced by the remaining contaminants. As with the acid-wash, chlorine dioxide had no discernable effect on the wild yeast population.

While our test was not intended to be definitive, it shows that chlorine dioxide may be an effective alternative to phosphoric acid as a yeast-wash. The primary advantages of chlorine dioxide are that it can destroy the tougher spoilers without compromising the yeast's viability, and is safer to handle.

All this having been said, remember that there is no substitute for proper sanitation. The best way to keep undesirable organisms from infecting beer is to keep all the equipment clean, sanitized and unexposed.

**References:**

- (1) Dr. George Fix, Rocky Mountain Microbrewing Symposium, Colorado Springs, Colorado, February 20, 1998.
- (2) W.J. Masschelein, Chemical Oxidation, 1992, Technomic Publishing Company, Inc., Lancaster, PA, page 170.
- (3) Dave Miller, "The Troubleshooter," Brewing Techniques, March/April, 1998, page 28.
- (4) Dana Johnson, "Applications of Chlorine Dioxide: A Postrinse Sanitizer that Won't Leave a Bad Taste in Your Mouth," Brewing Techniques, March/April, 1997, page 76.

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*Dana Johnson has been with Birko Corporation R&D since 1979 and is Manager of the CON-TACT-IT® Bacteria Detection System. He has authored several articles on cleaning and sanitizing in the brewery.*