Oregon State University Research Office

Fact Sheet
Disinfection Using Chlorine Bleach

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Definitions:

1. **Free chlorine** – combined forms of HOCl (hypochlorous acid), OCl^- (hypochlorite anion) and Cl_2 (dissolved chlorine gas) in aqueous solution.

2. **Combined chlorine** – chlorine in water in chemical combination with ammonia to form inorganic amines, or with organic compounds to form organic amines.

3. **Total chlorine** – the sum of free and combined chlorine. For clean water, the total chlorine is essentially equal to free chlorine. When chloramines are present the total chlorine will be higher than the free chlorine.

4. **Disinfection** – the killing, inhibition or removal of microorganisms that can cause disease.

Liquid Bleach

Laundry bleach is a solution of sodium hypochlorite, at a pH of about 11. Once dissolved, the sodium hypochlorite interconverts with the other chemical forms of free chlorine, mainly HOCl. Sodium hypochlorite and hypochlorous acid are strong oxidizing agents and react with flammable compounds and reducing agents.

There are many different brands of laundry bleach available, and the concentration of sodium hypochlorite can vary significantly. Clorox brand is 5.25% sodium hypochlorite, but many bargain brands have lower concentrations. The only laundry bleach solution registered with the EPA as a tuberculocidal disinfectant is Clorox brand; investigators are encouraged to use Clorox for consistency in disinfection efficacy.

pH Effect

When sodium hypochlorite is dissolved in water, the resulting solution is quite basic due to the production of OH^- ions by the following reaction:

$$NaOCl\ (s) + H_20(l) \rightarrow HOCl\ (aq) + NaOH\ (aq)$$

The various free chlorine components exist in equilibrium in water; the predominant form depends on the pH. When the pH is between 2 – 7, the equilibrium favors HOCL. As the pH falls below 2, the main form is Cl_2. At a pH of 7.4, HOCl and OCl^- are about equal, and as the pH goes above 7.4, increasing proportions of OCl^- are present. Maximum disinfecting efficacy is achieved at pH 4-5, because essentially all the chlorine is present as HOCl which is two orders of magnitude more effective than OCl^-. Below pH 4, increasing amounts of Cl_2 are present which will evolve from solution and be lost to the system at atmospheric pressure. Chlorine gas is also quite toxic, so pH below 4 should be avoided. On balance, for safety and efficacy a pH of 5-7 works best; addition of a small amount of HCl to achieve this pH should be considered when disinfecting materials that may be contaminated with bacterial endospores or other resistant forms of microorganisms. For most routine disinfection, no adjustment

of pH is necessary. ***Extreme caution should be exercised when acidifying bleach solutions; too much acid will cause toxic Cl_2 to off-gas.***

Efficacy

Chlorine kills microorganisms by oxidizing free sulfhydryl groups, disruption of cell membrane and wall components, and degradation of a variety of cellular macromolecules.

The efficacy of the various forms of chlorine in water at killing microorganisms is as follows:

HOCl > OCl^- > inorganic chloramines > organic chloramines

The above hierarchy clearly shows that free chlorine is more efficacious than combined chlorine. Also, as noted above, HOCl is 100 times more effective as a disinfectant than OCl^-. Consequently, free chlorine is most effective at a pH of 5 to 7, where HOCl is the predominant form. The efficacy declines with increased pH.

Although inorganic chloramines are weaker disinfecting agents, they are more stable than free chlorine and can provide disinfection over long exposure periods.

Stability

Sodium hypochlorite solutions are unstable. When open to the air, chlorine evaporates at a high rate from the solution, rapidly reducing the concentration of free chlorine. When heated, sodium hypochlorite disintegrates. This also happens when sodium hypochlorite comes in contact with acids, sunlight, certain metals and gasses. Because it is unstable, when used for disinfection, diluted bleach should be prepared fresh **daily**. Some companies, e. g., Current Technologies, Inc., sell stabilized bleach for disinfection purposes. Some of these products are approved for use as disinfectants by the EPA.

Recommended Dilutions and Time

For routine solid surface disinfection, a fresh 10% (v/v) solution of Clorox bleach in tap, RO, or deionized water is recommended. If significant amounts of organic materials are present, free chlorine will readily react to form less efficacious organic chloramines; this may require a higher concentration of bleach, perhaps 20% or more. When disinfecting a spill of aqueous liquid, such as a bacterial culture, addition of the stock solution of Clorox should be considered to achieve an approximate 10% final concentration.

The oxidation reactions carried out by chlorine are relatively rapid, so a 10% diluted bleach solution will generally achieve destruction of most common vegetative bacterial pathogens and viruses within 2 minutes at room temperatures. Temperature will effect kill time, and generally there is a doubling of reaction time for every increase in temperature of 10 degrees Celsius, so disinfection at low temperatures will require more time. A good rule of thumb is 10 minutes of contact time with 10% bleach achieves effective disinfection for most routine applications.

Population

In selection of appropriate dilutions and contact time, consideration should also be given to the type or form of organisms present and their physical characteristics. Bacterial endospores are produced by some gram positive bacteria under certain growth conditions. These endospores are extremely resistant to chemical degradation, as are certain protozoan oocysts like the infectious form of the genus *Cryptosporidium*. Gram negative vegetative bacteria are generally more difficult to chemically kill than most gram positive cells. Mycobacteria are typically more resilient than many other vegetative bacteria. Among the viruses, non-enveloped viruses like noroviruses are more resistant to disinfection than are enveloped viruses such as influenza, HIV or herpesviruses.