Vinoxide-HTC

EXECUTIVE SUMMARY

Vinoxide-HTC is a solution that is easy to prepare at home, and asserted to be useful for sterilizing explants, TC clean boxes, and TC tools. It is made of sterilized vinegar and 3% hydrogen peroxide at the time of use. This document is produced multinationally, with references to the USA, Europe and Australia.

Authors: Gregorio J. Placeres, Ohio, USA Gregorio_J@msn.com

Arthur Sale, Tasmania, Australia. ahjs@ozemail.com.au

Version history: 1.0 (18 Jan 2011)

Background

Vinoxide-HTC has been promoted by Gregorio Placeres as an alternative sterilant in home TC, and especially for rescuing contaminated plants. The name (***Vinoxide-HTC***) is used to distinguish it from the chemistry vinoxide anion $H_2C{=}CHO^-$ and to distinguish it from Vin Oxide Industries (based in India, supplying lead-based products). The HTC stands for **H**ome **T**issue **C**ulture. The unqualified name *Vinoxide* can result in confusion. Since Vinoxide-HTC is a home-brew product, it is difficult to find facts regarding it and there is no official MSDS. This document draws on documentation for peracetic acid (PAA), hydrogen peroxide, and vinegar for relevant data.

Ingredients

- **Hydrogen peroxide** (H_2O_2) as a 3% solution in water. Purchase it from pharmacies. There is usually some acid in the solution to lower the pH and make the H_2O_2 stable, which it is not in alkaline solution. The bond structure of hydrogen peroxide is more like H:O–O:H. In the presence of a catalyst or spontaneously, hydrogen peroxide breaks down into water and oxygen:

  $$2\ H_2O_2 \rightarrow 2\ H_2O\ +\ O_2$$

  Catalysts include compounds of the transition metals (for example chromium, manganese, iron, cobalt, nickel, copper, zinc) and *peroxidase* and *catalase*. The *catalase* enzyme is present in almost all animal and plant cells and eliminates the poisonous effects of small amounts of peroxides produced in the organism, destroying them through the above reaction.

- **White Vinegar.** Actually, what you want for Vinoxide-HTC is acetic acid ($CH_3.COOH$) but white vinegar *made from spirits* is mostly acetic acid and water. Any other plant products and flavours are undesirable so don't use wine, rice, malt or balsamic vinegar, for example. The acetic acid concentration is between 4% and 8%, and a working average is 5%. Most of the rest is water. Vinegar is stable from room temperature to boiling.

Process

You use hydrogen peroxide : vinegar in a 4:1 ratio. Decide how much Vinoxide-HTC you want to make and divide that by 5. That's the amount of vinegar, and later you will add four times as much of 3% peroxide. You will end up with a solution which is 2.4% peroxide, 0.8 – 1.6% acetic acid, and the rest water. Example 1: to make 500mL of Vinoxide-HTC, 100mL of white vinegar and 400mL of 3% peroxide. Example 2: to make 5 cups of Vinoxide-HTC, 1 cup of vinegar and 4 cups of hydrogen peroxide.

Heat the vinegar in a microwave-safe container on full power to boiling (1 minute should be enough for 100mL). This is to achieve three purposes:

- Accelerate the reaction between acetic acid and hydrogen peroxide
- To kill any organisms in the vinegar (yes some bacteria do love vinegar and can live in it)
- To destroy any traces of catalytic enzymes (proteins) in the vinegar

Then add the hydrogen peroxide to the hot vinegar. Assuming the hydrogen peroxide is at room temperature, the end temperature of the mix will be about 36°C (97°F). You will very shortly have an equilibrium mixture of hydrogen peroxide, acetic acid, peracetic acid ($CH_3.COOOH$, aka **PAA**, aka peroxyacetic acid) formed by reaction of the previous two ingredients speeded up by the heat, water, and some impurities.

$$CH_3.COOH + H_2O_2 \leftrightarrow CH_3.COOOH + H_2O$$

The reaction is slow[1] and will not go to completion one way or the other.

The quantities in the Vinoxide-HTC recipe provides approximately equal numbers of hydrogen peroxide and acetic acid molecules. The molecular mass of H_2O_2 is approximately (2x1 + 2x16)=34 and the molecular mass of acetic acid $CH_3.COOH$ is approximately (1x12 +3x1 + 1x12 + 2x16 + 1x1) = 60, so the active ingredients (using the average concentration of acetic acid in vinegar as 5%)are initially present approximately in the molecular ratio

H_2O_2 : $CH_3.COOH$ = 34x2.4% : 60x1.0% = 81.6 : 60 = 1.36 : 1

The actual concentration of PAA in Vinoxide-HTC is estimated by Gregorio at 0.15% (1500ppm). Since the ingredients are readily available in the solution, destruction of PAA is replaced slowly by further reaction. To test this concentration, you should get a commercially available test strip, for example '*IBT Peracetic Acid Test*', from *Integrated Biomedical Technology* http://www.ibtbiomed.com/tech_corner/pdf/monitoring_peracetic.pdf .

[1] The reaction is accelerated by sulphuric acid (H_2SO_4) in commercial production of peracetic acid (PAA). The sulphuric acid provides a very high concentration of H^+ ions. Do not try this at home.

Storage

If you need to store Vinoxide-HTC, put it in a clean[2] glass bottle, preferably brown or opaque, in the dark in a cool place. Don't rely on a long shelf life, as the peroxide will break down with time and the peracetic acid reaction will shift to the left. Store out of the reach of children and label prominently.[3]

3% Hydrogen peroxide is already stabilized before sale with a low pH, and the addition of acetic acid to it is unlikely to affect its shelf-life significantly. Unopened, it ought to be stable for a year, but this drops to about a month or two after opening and creating Vinoxide-HTC. Peracetic acid itself is quite stable.

Corrosion

PAA is corrosive to aluminum, stainless steel, copper, chromium and many other metals. Stainless steel instruments like forceps may be corroded after frequent use, so wash with water as soon as possible after use. Glass is a good storage or stirring material.

Contact with aluminum might cause the generation of hydrogen, with fire and explosive risk, but at 0.15% concentration this risk is probably minor. However do not store or mix in aluminum vessels.

Safety

The active component of Vinoxide-HTC is a stronger oxidizing agent than chlorine, so needs to be treated with the same respect or more. Use caution when handling and avoid skin contact (especially to the eyes) or inhaling. Do not use as a mist. Vinoxide-HTC will have a strong vinegar smell, but avoid sniffing it.

There are no MSDSs for this concentration of PAA or this mix. However, in line with the various MSDSs for peracetic acid, take precautions against skin contact (nitrile or latex gloves), eye contact (safety glasses), inhalation (mask), or ingestion (don't leave the colourless stuff lying around for children to drink or taste). If drunk, give water and seek medical advice. Particularly avoid splashing it on your eyes or inhaling it.

Do not buy 35% PAA as a shortcut or even 5%. Do not buy glacial (>90%) acetic acid. Unless you operate a WorkSafe laboratory or are trained to fully comply with lab protocols, these are simply too dangerous to health. Use the vinegar and hydrogen peroxide mixture.

In the USA, the FDA considers PAA to be safe for accidental contact at concentrations less than 1%. It is not clear that it is used for sanitisation of drinking water (potable water) in the USA (asserted by Gregorio), and it does not seem to be in the European Union[4] or Australia. At low concentrations, PAA is used to sanitise waste-water and cooling towers. Check with your local

---

[2] Clean means *clean*, not sterilized. It is traces of transition metals, including the iron in blood or fingerprints, that you are trying to remove, as well as organic residue. These will break down the peroxide and eventually reduce the PAA.

[3] Even at this concentration PAA is classified in the Poisons Act of Tasmania and Vinoxide-HTC must be labelled POISON. The situation in the USA is different, and it is considered safe for sale by the FDA below 1% concentration. The situation in the EU is not known.

[4] See http://www.lenntech.com/processes/disinfection/regulation-eu/eu-water-disinfection-regulation.htm

authority if in doubt. Remember that PAA is a stronger oxidant than sodium hypochlorite (household bleach) or calcium hypochlorite (swimming pool disinfectant) at equivalent concentrations.

Disposal

Please do not dispose of Vinoxide-HTC where it can get into a fresh water way such as a farm dam or a stream. PAA is fatal to fish in moderate amounts and also aquatic plants and invertebrates. We do not want it in the food chain either, especially as fish, fresh-water crustaceans (for example Australian yabbies) and molluscs (and platypus in Australia) may accumulate it. Its effectiveness varies with pH, and fresh water environments vary a lot. The safe way is to dilute it a lot and dispose of it in a toilet/sewer where it will be broken up oxidizing the sewage. Peracetic acid is used for waste-water sanitisation in the USA and the EU. Disposal direct to the sea (salt water) may be safe as the components of Vinoxide-HTC should be broken down in minutes.

The USA's EPA allows disposal of PAA under 1% into waterways, but in Tasmania it is not allowed because of the risk to endemic and other rare native species.

All components of Vinoxide-HTC are biodegradable, producing acetic acid (the weak acid of vinegar) and water.

Effectiveness

The mechanism of sterilization is generalized oxidation, which attacks sulfur bonds, thereby destroying cell walls and thus the cell contents. Peroxides should theoretically be better than chlorine bleach in this role, since hydrogen peroxide (2.4% in Vinoxide-HTC, and 3% in the bought solution) is a source of stronger oxidizing radicals than hypochlorite, and peracetic acid (0.15% in Vinoxide-HTC) is stronger still.

Oxidizer		**Oxidation potential (V)**
Peracetic acid	$CH_3.COOOH$	1.81
Hydrogen peroxide	H_2O_2	1.80
Sodium hypochlorite (bleach)	NaOCl	1.36

Bear in mind that sterilization is a balancing act. You want the sterilant to kill all living bacteria and fungus cells, as well as their spores, but you do not want to damage the explant cells or seeds. Stronger oxidizing radicals go with shorter treatment times and lower concentrations. It is not simply a question of which sterilant to use, but the concentration and the time too. Vinoxide-HTC has the capacity to damage some cells of explants.

There is not yet any validated scientific evidence to indicate that adding acetic acid to hydrogen peroxide improves disinfectant properties in tissue culture. Although the low concentration of PAA may improve sterilization, it is also possible that undiluted 3% hydrogen peroxide might be just as effective. ***Researchers are requested to test this, preferably with a blind test.***

Recommended uses and protocols

1. To disinfect the surface of media, spray the surface with Vinoxide-HTC and discard any excess liquid. Make your media with somewhat higher values of pH than your protocol suggests. The application of Vinoxide-HTC over the media will lower the pH and result in the degradation of the ingredients of Vinoxide-HTC if the pH is too low.

2. Before use on any valuable plants a timing protocol should be performed to determine the optimum time that the plant can survive the sterilization process and yet kill all the contamination. We referred earlier to the balancing act of sterilization.

3. To disinfect explant soft tissue, use between 1 to 4 min exposure. For woody plants, 5 to 10 min will be required to penetrate the surface tissue. Some explants require rinsing with sterile water; otherwise you can leave the solution on the explants.

4. Rescue of orchids seedlings with contamination. Wash the seedling in distilled water, then in Vinoxide-HTC for 30s to 1 min. Then rinse three times with sterile distilled water.

This document is available on the Open Web at http://ahjs.biz/pdfs/Vinoxide-HTC.pdf*, and in the password-protected files section of the Home Tissue Culture group in Yahoo Groups (hometissueculture).*