



# EcoClearProx

**BIO H2O2**



*the present and the future*



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# BIO H2O2 : company profile

- BIO H2O2 has been focusing on innovative hygiene solutions 100% Bio solutions. We ave a solution for human , animal, vegetable, plants and flowers against bacterium, virus and mildew. We have a solutions for disinfection of drinking water. We manufacture cleaning agents and disinfectants for the agricultural production (such as dairy, sheep, pig and poultry production), for the transport industries (carwash and truck wash) and for food processing. In lot off laboratories, specialist chemists and engineers work on the development and continuous improvement of formulations and processes. The a stringent quality standards (ISO 9001:2000 and GMP-Pharma) are being followed in production and quality assurance.



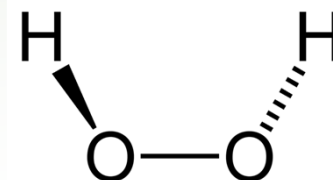
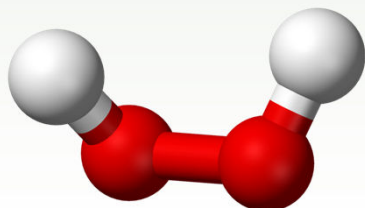
# ECOCLEARPROX





# Hydrogen peroxide: What?

- **Hydrogen peroxide** (H<sub>2</sub>O<sub>2</sub>; CAS No.: 7722-84-1) is a very pale blue liquid which appears colourless in a dilute solution, slightly more viscous than water. It is a weak acid. It has strong oxidizing properties and is therefore a powerful bleaching agent that has found use as a disinfectant and as an oxidizer





# H<sub>2</sub>O<sub>2</sub>: Chemical properties

- H<sub>2</sub>O<sub>2</sub> is one of the most powerful oxidizers known -- stronger than chlorine, chlorine dioxide, and potassium permanganate
- And through catalysis, H<sub>2</sub>O<sub>2</sub> can be converted into hydroxyl radicals ( $\cdot\text{OH}$ ) with reactivity second only to fluorine
- H<sub>2</sub>O<sub>2</sub> decomposes into water and oxygen, without the formation of byproducts

Oxidant	Oxidation potential, V
Fluorine	3,0
Hydroxyl radical	2,8
Ozone	2,1
Hydrogen peroxide	1,8
Potassium permanganate	1,7
Chlorine dioxide	1,5
Chlorine	1,4

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# EcoClearProx

- Stabilized, engineered hydrogen peroxide formulation without addition of silver



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# EcoClearProx

- **EcoClearProx** is composed of 50% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and a stabilizer different from silver. The active ingredient is hydrogen peroxide, where as the stabilizer prevents rapid dissolution of the H<sub>2</sub>O<sub>2</sub> and depending of the circumstances can create a miniscule coating for a short period.
- **EcoClearProx** (H<sub>2</sub>O<sub>2</sub>) dissociates into atomic oxygen and water. Atomic oxygen is responsible for oxidising micro-organisms. Especially on equipment and other materials, the long lasting and preserving effect of **EcoClearProx®** is caused by leaving a coating on the treated product. This coating is formed by the stabiliser. By stabilising, the hydrogen peroxide atomic oxygen is released constantly and so the equipment is prevented from infection and re-infection.
- There are three types of **EcoClearProx®** with different compositions to meet the desired mildness and efficacy.

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# EcoClearProx

- Stabilized, engineered hydrogen peroxide formulation without addition of silver, because:
  - Silver is toxic to all living cells
  - Silver contributes to antibiotic resistance
  - Silver is deposited around nerves and in deeper skin layers and may cause permanent skin damage
  - Silver is intimately associated with environmental contamination of other toxic heavy metals such as mercury and lead
  - Silver sticks to fish gills, potentially choking them to death
  - Silver disturbs bacterial activity when cleaning sewage
  - Silver prevents the use of sludge as fertilizer, needed for nutrient recycling

REF: [http://ec.europa.eu/research/environment/pdf/hylanderhaxton\\_not\\_2906\\_en.pdf](http://ec.europa.eu/research/environment/pdf/hylanderhaxton_not_2906_en.pdf)



# EcoClearProx

## Authorisations

EcoClearProx® has been authorised in agreement with the Biocidal Products Directive 98/8/EC. However, the authorisations can differ depending on the country, a few examples:

### **Belgium** (Registration number: 2408B)

Product-type 1: Human hygiene biocidal products

Product-type 2: Private area and public health area disinfectants and other biocidal products

Product-type 3: Veterinary hygiene biocidal products

Product-type 4: Food and feed area disinfectants

Product-type 5: Drinking water disinfectants

### **The Netherlands** (Notification number: 1321)

Product-type 1: Human hygiene biocidal products

Product-type 2: Private area and public health area disinfectants and other biocidal products

Product-type 3: Veterinary hygiene biocidal products

Product-type 4: Food and feed area disinfectants

Product-type 6: In-can preservatives

Product-type 11: Preservatives for liquid-cooling and processing systems

Product-type 12: Slimicides

REF: [http://ec.europa.eu/environment/biocides/pdf/dir\\_98\\_8\\_biocides.pdf](http://ec.europa.eu/environment/biocides/pdf/dir_98_8_biocides.pdf)

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# EcoClearProx

- **EcoClearProx®** was introduced to the market as a biocide, i.e. a disinfection and decontamination agent.
- **EcoClearProx®** is dosed in drinking water to disinfect and to prevent any microbial regrowth.
- **EcoClearProx®** is dosed in process water and prevents bacteria from growing during food processing.
- **EcoClearProx®** can be used when processing fish, poultry, vegetables and bread related products. Besides these other applications are: sanitary systems, cooling towers, horticultural and agricultural applications, including treatment of cow claws, consumption and hatching eggs and pig manure processing.
- **EcoClearProx®** is used to disinfect (storage) rooms and warehouses (washing and/or vaporizing)
- **EcoClearProx®** is no antibiotic; the advantage of not being that is no resistance can be built up

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# EcoClearProx

- EcoClearProx® is effective against gram-positive as well as gram-negative bacteria, some viruses, fungi and yeasts, algae and it prevents building up of slime and foam.
- In agreement with this EcoClearProx® has been tested successfully against:
  - Bacteria: *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus hirae*, *Legionella pneumophila*
  - Fungi: *Aspergillus niger*
  - Yeasts: *Candida albicans*
  - Viruses: H5N1

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# EcoClearProx

- The applications of EcoClearProx® can be divided in two categories: *decontamination* and *disinfection*.
  - The main category is *decontamination*: primarily preventing that the present microbial load will increase and secondly reducing the present microbial load. This application is pursued for treatment of food, because it will never be permitted to disinfect food after being contaminated below a certain level. A rather low-concentrated EcoClearProx®-solution is used to prevent growth of the present bacteriological contamination level.
  - The other category is *disinfection*. The purpose is to reduce the microbial load significantly. Disinfection of drinking water, production- and storage rooms, equipment and machinery falls under this category.

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# EcoClearProx vs. Food

- During the last years there is an additional demand for food with low microbial counts, low dosage of preservatives, and high efficiency during processing and long shelf lives due to super market requirements. Nevertheless, many food producing and agri-business companies have problems with the management of their microbiological contaminations. Using **EcoClearProx®** is a possibility to control this problem.
- Legislation will force companies to come up to stated quality requirements. Those requirements will -to a certain extent- depend on the technical possibilities. **EcoClearProx®** helps to meet these requirements (ISO-9000) and in the end both producer and consumer will benefit from this.
- This all requires the use of a decontamination agent. Therefore **EcoClearProx®** is dosed in process water and prevents microbial growth from growing during processing. It is mild and long lasting and protects also against re-infection. **EcoClearProx®** is primarily more preventive oriented then curative.

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# EcoClearProx vs. Food

- **EcoClearProx®** can be applied in those cases in which microbial spoilage of the product is not acceptable and can be used in various fresh, deep-frozen, dried and other sorts of preserved foods (like diced or precooked vegetables, dairy products, mussels, shrimps, various other fish and fish products, meat and meat products as well as ready-made meals). Other possible applications are the treatment of consumption and hatching eggs, stables and areas where food products are prepared, processed, packed and stored.

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# EcoClearProx in practice

- In any (new) application of **EcoClearProx®** the following choices have to be made:
  - I. Which carrier?
  - II. Which concentration?
  - III. Which system?
- In practice the optimal concentration of **EcoClearProx®** has to be determined experimentally. It is not possible to express the time of action of **EcoClearProx®** in absolute terms. The time of action depends on different factors, like exposure to organic pollution and temperature. In case of higher temperatures (above 20 ° C) the processing of hydrogen peroxide will take place faster and therefore one should opt for more stabiliser. If the temperature drops below 0 ° C, there is no bacterial growth and no decomposition of **EcoClearProx®**. **EcoClearProx®** will not dissolve (spontaneously) in a medium that is free from microbial or any organic load. The following slides provide some guide-lines to determine the begin concentration.

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# EcoClearProx in practice

## 1 Which carrier?

- It is not possible to express the time that **EcoClearProx®** is effective in absolute terms. This depends on different factors like the exposure to (organic) pollution and temperature. The higher the temperature and organic pollution is the quicker the dissolvation will be.

## 2 Which concentration?

- The concentration of **EcoClearProx®** determines **how much** active substance (H<sub>2</sub>O<sub>2</sub>) is present. It is important to consider the difference between disinfection and decontamination.
- **Three different categories are distinguished:**
  - **A. living organism:** mussel, eel: 0.01% - 0.05%
  - **B. foodstuff:** vulnerable surface (fish fillet): 0.1% - 0.25%  
less vulnerable surface (oranges): 0.25% - 0.5%
  - **C. non food:** equipment, accommodation: 0.5% - 3%

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# EcoClearProx in practice

## 2 Which concentration? (continued)

		<i>Concentration</i>
<b>1. bacteriological contamination</b>	high low	HIGH LOW
<b>2. organic pollution</b>	much little	HIGH LOW
<b>3. contact-/exposure time</b>	short long	HIGH LOW
<b>4. turbulence</b>	low high	HIGH LOW
<b>5. temperature</b>	low high	LOW HIGH

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# EcoClearProx in practice

## 3 Which system? When dipping / rinsing / spraying / vapourising?

- **Dipping** (instead of spraying):
  - If the foodstuff to be treated is so whimsical that it is impossible to apply **EcoClearProx®** on every part of the surface.
  - If a substantial exposure time is wanted, e.g. in case of a high (microbiological/organic) pollution or when the product is sensitive to higher concentrations of **EcoClearProx®**.
- **Rinsing** (instead of spraying):
  - If the accessibility of a system prevents spraying, e.g. in water supply pipes.
- **Vaporising** (instead of spraying): Spraying is based on water pressure; vapourising works on the basis of air pressure. When vaporising you will obtain microscopically small drops of water (aerosol) in gas form.
  - If the surface may not get (visually) moistened.
  - In most circumstances vaporising is not suitable for surface disinfection because of the low capacity. Vaporising is especially suited for **air-**disinfection to prevent the product being contaminated by micro-organisms present in the air or transferred through the air.

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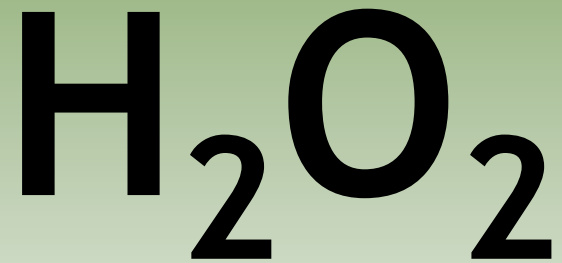


# EcoClearProx in practice

## Measuring H<sub>2</sub>O<sub>2</sub> concentrations:

- Peroxide test-strips
  - [H<sub>2</sub>O<sub>2</sub>]-range: 0 - 25 ppm (0,0025%)
- Amperometric Hydrogen Peroxide micro-sensor
- others

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Worldwide applications:  
Literature overview

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# Industrial applications

## End Use Industries

- Landfills
- Oil refining
- Mining / metallurgy
- Machining
- Textiles
- Power production
- Composting
- Potable water
- Chemicals and resins
- Food processing
- Electronics
- Pulp and paper
- Timber products
- Hazardous wastes
- Site remediation
- Municipal wastewater

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# Industrial applications

## Stand-Alone Applications (1 of 2)

- *Odor control* - Oxidizes hydrogen sulfide, mercaptans, amines and aldehydes. H<sub>2</sub>O<sub>2</sub> may be applied directly to aqueous wastes containing these odorants, or to wet scrubbers used to remove them from airstreams. If the odors are the result of biological activity, H<sub>2</sub>O<sub>2</sub> may instead be added as a preventative to eliminate the anoxic conditions which favor the generation of odors.
- *Corrosion control* - destroys residual chlorine and reduced sulfur compounds (thiosulfates, sulfites, and sulfides) which form corrosive acids when condensed onto processing equipment and oxidized by air.
- *BOD/COD removal* - Oxidizes both organic and inorganic pollutants which contribute to BOD and COD -- catalytic, H<sub>2</sub>O<sub>2</sub> may be needed to oxidize the more resistant substances. H<sub>2</sub>O<sub>2</sub> may also affect BOD/COD removal by enhancing the performance of other processes (see below).
- *Inorganic oxidation* - Oxidizes cyanides, NO<sub>x</sub>/SO<sub>x</sub>, nitrites, hydrazine, carbonyl sulfide, and other reduced sulfur compounds mentioned above (odor/corrosion control).

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# Industrial applications

## Stand-Alone Applications (2 of 2)

- *Organic oxidation* - Hydrolyzes formaldehyde, carbon disulfide, carbohydrates, organophosphorus and nitrogen compounds, and various water-soluble polymers; and (with catalysis) destroys phenols, BTEX pesticides, solvents, plasticizers, chelants, and virtually any other organic requiring treatment.
- *Metals oxidation* - Oxidizes ferrous iron, manganese, arsenic, and selenium to improve their adsorption, filtration, or precipitation from process waters and wastewaters.
- *Toxicity reduction/Biodegradability improvement* - With catalysis, chemically digests complex organics into smaller, less toxic and more biodegradable fragments.
- *Disinfection* - Control excess microbial growth in water supplies and cooling circuits, and disinfects process waters and biological effluents, e.g. slurry disinfection (Tofant et al., 2006)

REF: Tofant et al., 2006, The hydrogen peroxide, as a potentially useful slurry disinfectant. Livestock Science 102, 243-247

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# Industrial applications

## Enhancement (Combination) Applications

- *Flocculation/precipitation* - Oxidizes metal complexes and improves the performance of inorganic flocculants. *Air Flotation* - Releases evenly dispersed microbubbles which entrain emulsified fats, oils and greases to enhance their removal in air flotation units and grease traps.
- *Biotreatment* - As a pretreatment - degrades toxic, refractory or bio-inhibitory organics, rendering them more amenable to biodegradation. In conjunction with - provides a supplemental source of dissolved oxygen *in-situ* (penetrating both soil columns and bioflocs, eliminating the sludge bulking phenomenon). As a polishing step - destroys trace levels of organics that pass through biotreatment, providing the ancillary benefit of disinfection.
- *Filtration* - Controls biofouling of UF and RO membranes while eliminating foul odors from media filters.
- *Carbon adsorption* - Enhances the adsorption of many pollutants while providing dissolved oxygen to support biologically-active carbon beds (improving removal efficiencies still further).
- *Air scrubbers* - Replaces chlorine for deodorizing offgases and controlling VOC's. Depending on the target pollutant(s), catalytic or Advanced Oxidation Processes may be required.
- *Incineration* - Provides supplemental oxygen to improve combustion efficiencies and lower operating temperatures.

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# Consumer applications

## Consumer Products

- Diluted H<sub>2</sub>O<sub>2</sub> (around 30%) is used to bleach human hair
- Hydrogen peroxide is used in laundry bleaches that are gentle to fabrics and dyes, topical solutions which fight germs without staining
- Contact lens cleaning solutions
- Chlorine-free household cleaners (detergents) and wood bleach formulations.

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# Drinking water disinfection

- Controlling biofilm growth in drinking and wastewater pipelines has attracted considerable scientific and technological attention over recent years. In this work, the biofilm control effectivity of a combined disinfectant comprised of hydrogen peroxide and silver ions was examined. The performance of the combined disinfectant was compared to the effectivity of each of the ingredients alone. Hydrogen peroxide alone (30 ppm) was as effective as a combination of hydrogen peroxide (30 ppm) and silver ions (30 ppb) in preventing biofilm growth. Both compositions showed significant biofilm prevention effectivity as compared to silver ions alone.

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# Impact on biofilms

- Controlling biofilm growth in drinking and wastewater pipelines has attracted considerable scientific and technological attention over recent years. In this work, the biofilm control effectivity of a combined disinfectant comprised of hydrogen peroxide and silver ions was examined. The performance of the combined disinfectant was compared to the effectivity of each of the ingredients alone. Hydrogen peroxide alone (30 ppm) was as effective as a combination of hydrogen peroxide (30 ppm) and silver ions (30 ppb) in preventing biofilm growth. Both compositions showed significant biofilm prevention effectivity as compared to silver ions alone.

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# Swimming pool disinfection

- The application of peroxides for swimming pool disinfection was limited. However, recently, more stable forms have been developed, which can be used for application in swimming pools. Through stabilizer addition, the decomposition of hydrogen peroxide is delayed and the disinfection ability can be maintained.
- Hydrogen peroxide disinfection of swimming pools requires a higher dose.

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# Aquaculture

- The American Food and Drug Administration (FDA) has classified hydrogen peroxide as a Low Regulatory Priority (LRP) drug for use in controlling fungus on fish and fish eggs (Meyer, 2003).
- H2O2 have shown to have fungicidal and disinfection capacities (Lilley and Inglis, 1997).
- H2O2 can be used as a general disinfection method for treating water culture and surface of tanks before the introduction of fish (Avendano-Herrera et al., 2006).
- REF: Meyer, 2003. Regulation changes, policies and guidelines for Alaska fish and shellfish health and disease control. Alaska Department of Fish and Game. Regional Information Report No. 5J03-07
- REF: Lilley and Inglis, 1997. Comparative effects of various antibiotics, fungicides and disinfectants on *Aphanomyces invaderis* and other saprolegniaceous fungi. *Aquaculture-Research* 28(6), 461-469.
- REF: Avendano-Herrera et al., 2006. Use of hydrogen peroxide against the fish pathogen *Tenacibaculum maritimum* and its effect on infected turbot (*Scophthalmus maximus*). *Aquaculture* 257, 104-110.

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# Food processing

- **Food Processing**  
Hydrogen peroxide is Generally Recognised As Safe (GRAS) as an antimicrobial agent, an oxidizing agent and more by the US Food and Drug Administration.  
(<http://vm.cfsan.fda.gov/~dms/eafus.html>)
- Food Grade hydrogen peroxide is approved for use in such FDA approved applications as bleaching instant tea, tripe and dietary fiber and as a sanitizer of polymeric food-contact services used in packaging (see 21 CFR sections 133 through 184 for a complete list of approved uses). When used in food applications, hydrogen peroxide dosing is controlled to minimize the amount of residual hydrogen peroxide. However, any remaining residual normally decomposes to oxygen and water in any subsequent processing steps.



# Food processing

- Pavoni et al., 2004. Efficacy of Certain Disinfectants Towards Enteroviruses: Kinetics of in vitro and in vivo Inactivation. *Veterinary-Research-Communications* 28(1, Suppl. 1), 265-267.
  - The purpose of this study was to evaluate the inactivation kinetics of enteroviruses with sodium hypochlorite, peracetic acid, chlorine dioxide, and hydrogen peroxide tested on viral dilutions and then inoculated in cell cultures. The results showed that the three types of viruses had similar kinetics when treated with sodium hypochlorite while, in the presence of chlorine dioxide, poliovirus 1 was more resistant than coxsackievirus B5 and echovirus 30. This difference was also confirmed on treatment with peracetic acid, while hydrogen peroxide, considering its low concentrations, was the most effective inactivator.



# Food processing

- Wirtanen et al., 2001, Efficacy testing of commercial disinfectants against foodborne pathogenic and spoilage microbes in biofilm-constructs. *European-Food-Research-and-Technology* 213(4-5), 409-414.
  - The most effective formulations in these tests were the oxidising disinfectants based on hydrogen peroxide.
- Cliffe-Byrnes and O'Breine, 2008. Effects of washing treatment on microbial and sensory quality of modified atmosphere (MA) packaged fresh sliced mushroom (*Agaricus bisporus*). *Postharvest Biology and Technology* 48, 283-294.
  - The effects of different washing treatments combined with modified atmosphere packaging (MAP) on the quality and storage life of sliced mushrooms were determined. Trials with anti-microbial treatments involved a disinfectant step followed by a rinse, and investigated the effectiveness of different concentrations. Overall, the most effective treatment was 3% H<sub>2</sub>O<sub>2</sub> for up to 60 s prior to slicing followed by a spray application of 4% sodium d-isoascorbate monohydrate or 1% H<sub>2</sub>O<sub>2</sub>. Subsequent storage under modified atmospheres at 4 °C maintained quality and enhanced shelf-life.

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# Food processing

- The National Aeronautics and Space Administration (NASA) is working towards future long duration manned space flights beyond low earth orbit. During these missions, vegetable crops may be grown on the transit vehicle as well as the lunar or planetary surface. The vegetable crops will provide the crew with added nutrition and variety. The vegetable crops, unlike the prepackaged foods, will add bright colors, fresh textures (e.g., crispiness), and fresh aromas to the crew's menu. Ten vegetable crops have been identified for possible use in long duration missions. They are lettuce, spinach, carrot, tomato, green onion, radish, bell pepper, strawberries, fresh herbs, and cabbage. Since these crops do not require any cooking or food processing prior to consumption, careful sanitation procedures need to be performed. Since hydrogen peroxide degrades into water and oxygen and is generally recognized as safe (GRAS), hydrogen peroxide has been recommended as the sanitizer (Perchonok and French, 2005).
- REF: Perchonok and French, 2005. Hydrogen peroxide treatment of vegetable crops. SAE transactions 114(1), 362-366.



# Disinfection of eggs

The concentration of *Escherichia coli* in the inoculum was determined to be  $1.25 \times 10^8$  CFU/mL. The mean concentration of *E. coli* in the wash solution of eggs after treatment and the corresponding percent destruction is shown in Table 1.

**TABLE 1. Relative efficiency of egg sanitizers**

TREATMENT	BACTERIA (PER ML IMMERSION SOLUTION)	% DESTRUCTION
Post ethanol wash (negative control)	0	Not applicable
Post <i>E. coli</i> contamination (positive control)	$6.067 \times 10^5$	Not applicable
1.5% hydrogen peroxide solution	0	100.0
0.2% oxyhalogen solution	$2.83 \times 10^3$	99.53
0.4% Tek-Trol® solution	$1.07 \times 10^3$	99.82
250 ppm chlorine from 5.25% hypochlorite solution	$1.91 \times 10^4$	98.65
Distilled water wash	$1.02 \times 10^5$	83.19
Phosphate buffer saline wash	$3.17 \times 10^5$	47.75

**Conclusion:** Only hydrogen peroxide achieved complete disinfection of the shell surface.



# Disinfection of eggs

- Sander, J.E., and J.L. Wilson. 1999. Effect of hydrogen peroxide disinfection during incubation of chicken eggs on microbial levels and productivity. *Avian Dis.* 43:227-233.
  - Hydrogen peroxide was also evaluated in the presence of a severe challenge with *Staphylococcus aureus*-contaminated eggs. A significant reduction was found in aerosol bacterial counts within the hatcher when incubators were fogged with 3% hydrogen peroxide when compared with water-fogged machines even in the face of high bacterial challenge.
  - The use of hydrogen peroxide as a hatchery sanitizer did not affect hatchability, broiler livability, body weight, or feed conversion but did reduce the incidence of retained yolk sacs in 42-day-old chickens



# Disinfection of eggs

- Sheldon, B.W., and J. Brake. 1991. Hydrogen peroxide as an alternative hatching egg disinfectant. *Poult. Sci.* 70:1092-1098.
  - The present study examined the effectiveness of H<sub>2</sub>O<sub>2</sub> at different concentrations to disinfect broiler hatching eggshell surfaces and to maintain hatching potential. Under pure culture conditions, 0.50% H<sub>2</sub>O<sub>2</sub> yielded over a 6 log kill in 30 s of three potential eggshell bacterial contaminants. Under higher H<sub>2</sub>O<sub>2</sub> demands, such as occurs on eggshell surfaces, H<sub>2</sub>O<sub>2</sub> concentrations of 5% (vol/vol) were required to disinfect the shell surfaces (approximately 5 log reduction). Hatchability of fertile eggs from a 44-wk-old flock was significantly increased by 2% following spraying 5% H<sub>2</sub>O<sub>2</sub> in comparison to untreated controls.
  - Level of contaminated eggs and "early-dead" embryos were significantly reduced in the H<sub>2</sub>O<sub>2</sub>-treated eggs.
  - In comparison with formaldehyde fumigation, no significant difference in hatchability due to H<sub>2</sub>O<sub>2</sub> treatment was detected in eggs from a 30- or 56-wk-old flock. Eggshell permeability, as measured by egg moisture loss in an incubator, was not significantly affected by H<sub>2</sub>O<sub>2</sub> (5%) or formaldehyde fumigation when compared with untreated or water-sprayed control eggs.
  - These results demonstrated that H<sub>2</sub>O<sub>2</sub> compared favorably to formaldehyde as a hatching egg disinfectant without adversely affecting hatching potential. Under some conditions, H<sub>2</sub>O<sub>2</sub> actually improved the hatching potential of fertile broiler eggs compared with hatchability of untreated eggs.

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# Applications in horticulture

- Some gardeners and hydroponics implementers have professed the value of hydrogen peroxide in their watering solutions. They claim its spontaneous decomposition releases oxygen that can enhance a plant's root development and also help treat root rot, which is cellular root death due to lack of oxygen



# Applications in agriculture

- Bhattarai et al., 2004. Aerated subsurface irrigation water gives growth and yield benefits to zucchini, vegetable soybean and cotton in heavy clay soils. *Ann. appl. Biol.* 144, 285-298.
  - Inadequate oxygen concentration in the root zone is a constraint to plant performance particularly in heavy, compacted and/or saline soils. Sub-surface drip irrigation (SDI) offers a means of increasing oxygen to plant roots in such soils, provided irrigation water can be hyper-aerated or oxygenated.
  - Hydrogen peroxide (HP) at the rate of 5 litre ha<sup>-1</sup> at the end of each irrigation cycle was injected through SDI tape to a field-grown zucchini (courgette) crop (*Cucurbita pepo*) on a saturated heavy clay soil in Queensland, Australia. Fruit yield, number and shoot weight increased by 25%, 29% and 24% respectively due to HP treatment compared to the control.
  - Two pot experiments with vegetable soybean (*Glycine max*) and cotton (*Gossypium hirsutum*) compared the effectiveness of HP and air injection using a Mazzei™ air injector (a venturi), throughout the irrigation cycle in raising crop yield in a heavy clay soil kept at saturation or just under field capacity. Fresh pod yield of vegetable soybean increased by 82-96% in aeration treatments compared with the control. Similarly cotton lint yield increased by 14-28% in aeration treatments compared with the control. The higher lint yield was associated with more squares and bolls per plant which accompanied greater above ground biomass and an increase in root mass, root length and soil respiration.

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# Applications in horticulture

## Hydrogen peroxide (USA)

- The basic premise behind H<sub>2</sub>O<sub>2</sub> when used, as anti-microbials is that bacteria and fungi are comprised of proteins and proteins are highly subject to oxidation. Disruption of the protein by oxidation results in loss of protein function and ultimately cell death. When using this category of oxidizers for disease control, there are a couple of important facts to consider:
  - First, H<sub>2</sub>O<sub>2</sub> have little or no residual activity due to the fact that it is rapidly degraded in the environment and are broken down to their constituent component- water (H<sub>2</sub>O) and oxygen (O<sub>2</sub>). Since H<sub>2</sub>O<sub>2</sub> have no residual activity, coverage is essential because if the chemical doesn't contact the organism at the time of spraying and in the right concentration there is no effect.
  - Secondly, in order to be effective, non-residual chemistry should be able to kill not only actively growing disease organisms but also spores. Lastly the oxidizing chemical must be stable enough to exist for some time in the environment and at the same time not harm the plant material.

BIO H<sub>2</sub>O<sub>2</sub>



# Applications in horticulture

## Hydrogen peroxide (USA)

- Stabilized, engineered formulations of hydrogen peroxide has sporicidal properties, while the stabilizer allow H<sub>2</sub>O<sub>2</sub> to be applied as a spray or drench without the rapid loss of the active ingredient or phytotoxicity. H<sub>2</sub>O<sub>2</sub> is labeled for use on the following crops: beans, broccoli, cauliflower, cabbage, cucurbits, onions, peppers, potatoes, and tomatoes. H<sub>2</sub>O<sub>2</sub> is also labeled for use in *apples, grapes, blueberries, cranberries, strawberries, brambles, pears, cherries, peaches, nectarines, and plums*. The label lists control of a broad range of fungal and bacterial diseases.

BIO H<sub>2</sub>O<sub>2</sub>



# Applications in horticulture

## Hydrogen peroxide (USA): Controlling Powdery Mildew

- Powdery mildew is different from most other plant diseases caused by fungi, because the fungus that causes it lives almost entirely on the surface of infected plant parts. The fungus may penetrate only one cell layer deep into the plant. Thus, it is exposed to eradication following topical treatment with a range of products that do not affect many other pathogenic fungi that colonize deeper into infected plant tissues. Research in New York and other locations has demonstrated that many new and “alternative materials can provide effective control of powdery mildew if applied often enough (7 day schedule) through the growing season. These materials burn out the fungus growing on the surface, but do not provide protection against new infections; thus, repeated applications are important. These materials include: Nutrol (manopotassium phosphate); Kaligreen and Armicarb (potassium bicarbonate-baking soda); oils such as Stylet Oil and Trilogy; and dilute solutions of hydrogen peroxide.

BIO H<sub>2</sub>O<sub>2</sub>



# Applications in horticulture

## Hydrogen peroxide (the Netherlands):

- Biocide used for removal of green precipitate;
- Disinfection of greenhouses, stores and warehouses;
- Disinfection of concrete floors, cultivation tables, irrigation mats, drip irrigation, plant containers, nursery containers, anti root screens and hand tools used for the cultivation of agricultural and horticultural crops;
- Disinfection of machinery used for the harvest, transport and processing of potatoes;
- Disinfection of surfaces and materials used for the cultivation of mushrooms, although in the Netherlands the use of H<sub>2</sub>O<sub>2</sub> is prohibited during cultivation of the mushrooms itself or in warehouses used for storing mushrooms;
- Use as fungicidal drench for treatment of flower bulbs and turnips.

Ref:

<http://www.groeiservice.nl/bestanden/5106/Overzicht%20veiligheidsaanbevelingen%20herbetreding%20glastuinbouw.xls>

BIO H2O2



# Applications in organic horticulture



- Subpart G: The National List of Allowed and Prohibited Substances § 205.600-205.619
- Subpart G of the NOP Act limits the inputs for agriculture production and food processing to the substances, which are mentioned in the following tables. In the Regulation 2092/91 EU inputs are limited by Annex II A to E and Annex VI A to C.
- Please consider the meaning of the different writing:
- NOP-allowed substances written **in green** are not granted by the Regulation 2092/91 EU.
- Or, NOP-prohibited substances highlighted with black letters are allowed in the Regulation 2092/91 EU.
- Notes, which are written with ⇒ Bradley Hand letters are additional comments, referring to the Regulation 2092/91 EU.

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# Applications in organic horticulture



**Table 1: Irrigation and cleaning systems**

Substances allowed to use within irrigation systems:	
As algicide, disinfectants and sanitizer	<ul style="list-style-type: none"><li>➤ Ethanol</li><li>➤ Isopropanol</li><li>➤ Calciumhypochlorite</li><li>➤ Chlorine dioxide</li><li>➤ Sodium hypochloride</li><li>➤ <b>Hydrogen peroxide</b></li><li>➤ Soap based algicide</li></ul>

⇒ The Regulation 2092/91 EU mentions in Annex II subpart E the products granted for cleaning and disinfections of livestock buildings and installations, including equipments for production.

⇒ The Regulation 2092/91 EU does not point out the irrigation system explicitly.

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BIO H2O2



# Applications in organic horticulture



The NOP grants the following synthetic substances. The substances with green background are prohibited in the Regulations 2092/91 EU:

Use	Substances	Restrictions / Remarks
As plant disease control	<ul style="list-style-type: none"><li>➤Coppers, fixed - copper hydroxide, copper oxide, copper oxychloride, includes products exempted from EPA tolerance.</li><li>➤Copper sulfate</li><li>➤Hydrated lime</li><li>➤Hydrogen peroxide</li><li>➤Lime sulfur</li><li>➤Oils</li><li>➤Potassium bicarbonate</li><li>➤Elemental sulfur</li><li>➤Streptomycin</li><li>➤Tetracycline (oxiytetracycline calcium complex)</li></ul>	<p>Provided, that, copper-based materials must be used in a manner that minimizes accumulation in the soil and shall not be used as herbicides.</p> <p>Substance must be used in a manner that minimizes accumulation of copper in the soil.</p> <p>horticultural, narrow range oils as dormant, suffocating, and summer oils</p> <p>for fire blight control in apples and pears only</p> <p>for fire blight control only</p>

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BIO H<sub>2</sub>O<sub>2</sub>



# Applications in potato production

## Organic potato production (USA)

- H<sub>2</sub>O<sub>2</sub> is listed by the Organic Materials Review Institute (OMRI) for disease control for pathogen suppression

## Management of potato viruses

- For disinfection of equipment use a suitable disinfectant frequently and between seed lines, and ensure adequate chemical contact with equipment for 10 minutes. Corrosive chemicals must be rinsed off. Suitable disinfectants currently include: bleach (2.4% a.i. sodium hypochloride), 1% benzoic acid or 10% hydrogen peroxide sanitisers
- REF: Crop & Food Research INFOSHEET No 3-43 2007 (New Zealand)

BIO H<sub>2</sub>O<sub>2</sub>



## H<sub>2</sub>O<sub>2</sub> is naturally generated in plants as a result of oxidative stress

- H<sub>2</sub>O<sub>2</sub> is a form of reactive oxygen species (ROS) generated as a result of oxidative stress.
- Several roles for ROS during pathogen infections have been proposed: as direct antimicrobial agents, as activators of defense genes, as agents for crosslinking proteins to limit pathogen infections, and as producers of the hypersensitive response (HR), cell death, salicylic acid production, and systemic acquired resistance (SAR).
- Plants release H<sub>2</sub>O<sub>2</sub> in response to the presence of a fungal invasion, which attacks by piercing the cell wall of a plant and breaking it down. H<sub>2</sub>O<sub>2</sub> generated in response to wounding can be detected at wound sites and in distal leaf veins within 1 hr after wounding. The response is systemic and maximizes at about 4-6 hr in both wounded and unwounded leaves, and then declines.

BIO H<sub>2</sub>O<sub>2</sub>



# Medical applications

- Hydrogen peroxide is used as an antiseptic by hospitals, doctors and dentists in sterilizing, cleaning and treating everything from floors to root canal procedures.
- 3% H<sub>2</sub>O<sub>2</sub> is used medically for cleaning wounds, removing dead tissue, or as an oral debriding agent. Most over-the-counter peroxide solutions are not, however, suitable for ingestion.

REF: [http://en.wikipedia.org/wiki/Hydrogen\\_peroxide](http://en.wikipedia.org/wiki/Hydrogen_peroxide)

REF: Nhanes, 2004. Dental examiners procedure manual, 323p.

BIO H<sub>2</sub>O<sub>2</sub>



# Medical applications

- Perez et al., 2005. Activity of selected oxidizing microbicides against the spores of *Clostridium difficile*: Relevance to environmental control. *American Journal of Infection Control* 33(6), 320-325.
- Feuerstein et al., 2006. Synergic antibacterial effect between visible light and hydrogen peroxide on *Streptococcus mutans*. *Journal of Antimicrobial Chemotherapy* 57, 872-876.
- Ahmad and Iranzo, 2003. Treatment of post-burns bacterial infections by Fenton reagent, particularly the ubiquitous multiple drug resistant *Pseudomonas* spp. *Medical Hypotheses* 61(4), 431-434.

BIO H<sub>2</sub>O<sub>2</sub>



# Medical applications

- Sattar et al., 2002. Combined application of simulated reuse and quantitative carrier tests to assess high-level disinfection: Experiments with an accelerated hydrogen peroxide-based formulation. *American Journal of Infection Control* 30(8), 449-457.
- Tuttlebee et al., 2002. Effective control of dental chair unit waterline biofilm and marked reduction of bacterial contamination of output water using two peroxide-based disinfectants. *Journal of Hospital Infection* 52(3), 192-205.
- Linger et al., 2001. Evaluation of a hydrogen peroxide disinfectant for dental unit waterlines. *Journal of the American Dental Association* 132(9), 1287-1291.



# Medical applications

- Segal et al., 2001, Disinfection of Goldmann Tonometers After Contamination With Hepatitis C Virus. American-Journal-of-Ophthalmology 131(2), 184-187.
  - **METHODS:** Hepatitis C virus was placed on Goldmann tonometer tips, air dried, and then disinfected by dry gauze wipes, isopropyl alcohol wipes, cold water washes, povidone iodine 10% wipes, and hydrogen peroxide or isopropyl alcohol soaks followed by a cold water wash and dry. Hydrogen peroxide and isopropyl alcohol disinfection techniques followed the Centers for Disease Control and Prevention guidelines for prevention of possible transmission of human immunodeficiency virus (HIV). After disinfection, samples from tonometer tips were amplified by polymerase chain reaction to quantitate the amount of hepatitis C virus RNA remaining.
  - **RESULTS:** Percentage of hepatitis C virus RNA remaining after disinfection: dry gauze wipes 95.65%, isopropyl alcohol 5-second wipes 88.91%, cold water wash 4.78%, povidone iodine 10% 5-second wipes 0.72%, hydrogen peroxide soak with cold water wash 0.07%, and isopropyl alcohol soak and cold water wash 0.02%.
  - **CONCLUSIONS:** After inoculation of Goldmann tonometer tips with hepatitis C virus, a 5-minute soak in 3% hydrogen peroxide or 70% isopropyl alcohol followed by washing in cold water resulted in the greatest reduction in hepatitis C virus RNA



# Veterinary applications

- Kuramochi et al., 1997. *Pneumocystis carinii* cysts are susceptible to inactivation by chemical disinfectants. *Experimental Animals* 46(3), 241-245.
- Leslie et al., 2006. Efficacy of Two Hydrogen Peroxide Teat Disinfectants Against *Staphylococcus aureus* and *Streptococcus agalactiae*. *Journal of Dairy Science* 89(9), 3696-3701.
- Treatment of cow claws against digital dermatitis (Mortellaro disease) and foot rot ([http://www.ivis.org/proceedings/wbc/wbc2004/abstr\\_1\\_100.pdf](http://www.ivis.org/proceedings/wbc/wbc2004/abstr_1_100.pdf))

BIO H2O2



# Veterinary applications

- Nicol and Billerey, 2008. Drainage of a peritoneal abscess in practice. *Point Veterinaire* 39(282), 73-+

Three weeks after omentopexy surgery by the right flank, which was performed twice due to fixation rupture, a peritoneal abscess at the wound site was diagnosed in a cow. The animal's general health had declined. The abscess was opened and explored with the animal standing. Two drains were placed, such that the two lobes of the cavity could remain open, and rinsed with hydrogen peroxide daily for 20 days. The system was well tolerated and removed after six weeks. There was no condemnation of any parts when the animal was culled. This type of drainage is an option to be considered for treatment of a peritoneal abscess but should only be used for diffuse peritonitis after failure of medical treatment.

BIO H2O2



# Decontamination of spaces with H2O2 vapors

- Rogers et al., 2005. Decontamination assessment of *Bacillus anthracis*, *Bacillus subtilis*, and *Geobacillus stearothermophilus* spores on indoor surfaces using a hydrogen peroxide gas generator. *Journal of Applied Microbiology* 99(4), 739-748.
  - This study demonstrates the decontamination efficacy of hydrogen peroxide gas on spores from *B. anthracis* Ames and the two simulants *B. subtilis* and *G. stearothermophilus*. The capacity for decontamination appears to be influenced in part by the porosity of the contaminated surfaces and differences in decontamination efficacy are prevalent between the biological warfare agent *B. anthracis* and surrogates. The hydrogen peroxide gas exposure demonstrated significant efficacy in spore decontamination on the seven types of surface materials evaluated.



# Decontamination of spaces with H<sub>2</sub>O<sub>2</sub> vapors

- Jeanes et al., 2005. Eradication of persistent environmental MRSA. *Journal of Hospital Infection* 61(1), 85-86.

**Table 1** Environmental sampling for methicillin-resistant *Staphylococcus aureus* on a surgical ward over a four-week period

Sampling method	Before cleaning				After deep cleaning		After decontamination <sup>a</sup>
	Contact plates				Contact plates	Surface swabs	Surface swabs
Time	Week 1	Week 2	Week 3	(Overall)	Week 4		Week 4
No. sites	9	6	13	(28)	15	50	50
No. positive	3	6	1	(10)	2	8	0
% positive	33.3	100.0	7.6	(35.7)	13.3	16.0	0

Surface sampling was conducted by either contact plates or surface swabbing, as indicated.

<sup>a</sup> Sampling after hydrogen peroxide vapour decontamination and after deep cleaning was conducted using swabs to sample adjacent sites, then processed using a selective broth enrichment protocol.

- Conclusion: No MRSA was cultured from 50 adjacent sites after hydrogen peroxide vapour decontamination, and the ward was available for re-occupation.

BIO H<sub>2</sub>O<sub>2</sub>



# Decontamination of spaces with H<sub>2</sub>O<sub>2</sub> vapors

- Johnston et al., 2005. Evaluation of hydrogen peroxide vapour as a method for the decontamination of surfaces contaminated with *Clostridium botulinum* spores. *Journal of Microbiological Methods* 60(3), 403-411.
  - HPV was found to be effective at deactivating spores of toxigenic *Cl. botulinum*, non-toxigenic *Clostridium* spp. and *Geobacillus stearothermophilus* dried onto stainless steel surfaces.



# BIO H2O2

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