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# Disinfestation of recirculating nutrient solutions in greenhouse horticulture

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**Abstract** – Recirculating nutrient systems offer a good method to control nutrient leaching from greenhouses into the environment. However, the potential for the rapid spread of root diseases is the main hindrance to adoption of recirculating nutrient systems by the greenhouse industry. This review discusses and compares five broadly different methods of disease control in these systems, namely heat, filtration, chemical, radiation and biological control. Each has strengths and weaknesses, but all have been found to be effective in terms of pathogen control. Sterilization (heat, oxidizing chemicals, UV radiation) and membrane filtration methods are generally very effective, but may adversely affect beneficial microorganisms in the recirculated solution. Slow filtration and microbial inoculation methods are less disruptive of the microflora, but effectiveness may vary with the pathogen. Microbial inoculation holds the promise of very targeted disease suppression, but few products are commercially available.

**recirculation / disinfestation / hydroponics / disinfection / root disease**

**Résumé** – Désinfestation des solutions nutritives recyclées en horticulture sous serre. L'utilisation de systèmes de recirculation des nutriments est une bonne façon de contrôler le lessivage des nutriments des serres dans l'environnement. Toutefois, le risque de propagation rapide de maladies des racines est le principal obstacle à l'adoption de tels systèmes par l'industrie serricole. La présente étude examine et compare cinq façons distinctes de contrôler les maladies dans ces systèmes, à savoir le traitement thermique, la filtration, le traitement chimique, le rayonnement et la lutte biologique. Chacune de ces méthodes a ses points forts et ses points faibles, mais toutes se sont révélées efficaces pour combattre les pathogènes. La stérilisation (par la chaleur, l'utilisation d'agents oxydants ou le rayonnement ultraviolet) et la filtration sur membrane sont habituellement très efficaces, mais peuvent nuire aux microorganismes utiles dans la solution recirculée. La filtration lente et l'inoculation microbienne sont moins nuisibles à la microflore, mais leur efficacité peut varier selon le pathogène. L'inoculation microbienne permet une élimination très sélective des maladies, mais peu d'inoculants microbiens sont disponibles dans le commerce.

**recirculation / désinfestation / culture hydroponique / désinfection / maladie des racines**

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## 1. INTRODUCTION

The majority of greenhouse crops are grown using artificial substrates in hydroponic systems. These substrates are preferred to soil-based media for economic reasons, and because of the improved control over water, aeration, nutrition and root distribution. Traditionally, these systems were developed as drain-to-waste or open systems, in which excess nutrient solutions were allowed to drain to the soil and groundwater. Crops grown under these conditions generally are irrigated to excess (up to 40% of the nutrient solution dosed per day may be in excess of crop requirements) to balance the variation in transpiration and nutrient demands of the individual plants and the variation within the system in supplying the plants with nutrient solution [52, 81]. However, hydroponic growers and governments have come to recognize that for environmental reasons, the excess nutrient solutions should not be allowed to drain to waste, but should be collected and re-used within closed or recirculating nutrient systems. As well as enabling good environmental stewardship, these closed systems can reward the grower with savings in water and fertilizer costs.

Root diseases are major problems in the production of greenhouse crops, affecting both yield and quality of the commercial product. Pathogen contamination of the nutrient solution can arise from many sources, including infested rainwater [73], surface water [95], growth media, as well as infected plant material. Research has shown that some root-infecting pathogens are easily spread through the nutrient solutions (Fig. 1), and widespread commercial adoption of recirculating hydroponic systems has been slowed for this reason. Examples of readily-spread diseases are cucumber green mottle mosaic virus, tomato mosaic virus, *Olpidium brassicae* (which vectors tobacco necrosis virus and lettuce big vein agent [60], *O. radiale* [81] which vectors melon necrotic spot virus, *Phytophthora cryptogea* [52, 109], *Pythium* spp. [46, 52, 54], *Fusarium oxysporum* [79, 81] and *Verticillium* spp. [79, 81]. Although some commercial growers who practice recirculating techniques do not have greater disease problems than growers who do not recirculate, treatment of recirculating nutrient solutions is an attractive approach to reduce the possibility of disease dispersal.

Over the years, and most intensely in the last decade, various techniques have been studied for their ability to minimize the spread of root pathogens in recirculating nutrient systems. Some of these techniques are now used successfully in commercial greenhouse facilities but others are still unproven or show problems in a commercial setting. They fall into five broadly based categories, namely heat, chemical, and radiation treatments which



**Figure 1.** Pythium-infected cucumber plants (right) grown in an NFT recirculating system compared to no Pythium (left).

are intended to sterilize the solution, and filtration and microbial inoculation treatments which are non-sterilants and, in theory, less disruptive of the indigenous microflora. Each has its own merits and encumbrances with respect to effectiveness, cost of installation and upkeep, and reliability. The goal of this paper is to review current research on these methods, concentrating less on the economics of each method, but more on efficacy and other technical considerations.

## 2. HEAT TREATMENT (PASTEURIZATION)

The effect of heat on the mortality of pathogen propagules has been studied by many researchers using propagule suspensions in water or a saline solution which are heated to a certain temperature over a relatively long time period. This information is not always relevant in commercial greenhouses, however, because pathogen propagules would be suspended in a solution containing nutrients, plant extracts and other contaminants, and to be commercially viable, heating and the subsequent cooling of recirculating nutrient solutions

would need to be rapid enough to treat large quantities of nutrient solutions ( $\sim 1800 \text{ m}^3 \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$ , [75]).

Runia et al. [79, 81] and McPherson et al. [52] both studied heat disinfestation of nutrient systems using heat exchangers. Runia's system was constructed in cooperation with the Institute of Agricultural and Environmental Engineering at Wageningen, and McPherson et al. used commercially available heat exchangers developed for milk pasteurization and disinfestation of recirculating hydroponic nutrient solutions. Heat exchangers work well in commercial greenhouse circulation systems because the heating and cooling of the nutrient solutions can be done rapidly, large amounts of nutrient solution can be treated and heat exchangers are energy efficient [81]. The heat exchangers used in these experiments were able to heat the nutrient solutions to the desired temperature in approximately two seconds [81].

Runia et al. [81] studied the disinfestation of recirculating nutrient solutions using tomato mosaic virus, *Verticillium dahliae* and *Fusarium oxysporum* f. sp. *melongenae* (FOM) at different temperatures. The data obtained was based on the ability of the pathogens in the nutrient solutions to infect host plants, with and without heat treatment. Tomato mosaic virus was inactivated after treatment at 95 °C for ten seconds and *V. dahliae* propagules were killed after ten seconds at 90 °C. The treatment of FOM-infested nutrient solution at 94 °C for ten seconds did not completely disinfest the solution, but did result in a significant decline in the number of infective propagules. The results of these experiments led Runia et al. [81] to recommend that recirculating nutrient solutions be treated at 95 °C for thirty seconds to inhibit pathogen dispersal.

McPherson et al. [52] studied the control of *Phytophthora cryptogea* and *Pythium aphanidermatum* on tomato and cucumber crops, respectively. When recirculating nutrient solutions were not heat treated, introduced pathogens readily became dispersed throughout the system, infecting a large percentage of the plants. However, if the nutrient solutions were heated to 95 °C for thirty seconds, the inoculated pathogens were confined to the points of inoculation and did not spread through the crop.

The use of heat disinfestation to control pathogen dissemination in recirculating hydroponic systems is now used commercially by greenhouse growers, especially in the Netherlands and the United Kingdom. The commercial systems that have been developed are based on a treatment of the recirculating solution at 95 °C for thirty seconds. Often this is accompanied by an initial filtering of the solutions through a rapid sand filter to remove large bits of plant or other debris. Heating the nutrient solutions to these temperatures causes a build-up of

precipitate of carbonates on the heating coils and pipes, so acid is often added to the nutrient solutions in small amounts prior to heat treatment. In the commercial heat disinfestation systems, this is done automatically. In general, these systems are well accepted by commercial growers. Commercial treatment systems are easy to install, monitoring of the system is easy (using simple thermostats) and growers are comfortable with heat treatment because the concept of heat pasteurization is easy to understand. Heat-treated nutrient solutions have not been found to increase the temperature ( $< 1 \text{ }^\circ\text{C}$ ) of the nutrient solution returning to the crop because of mixing of the treated solution with cooler fresh solution.

### 3. CHEMICAL TREATMENT

While a number of agri-chemical fungicides have been developed which are effective against pathogens found in greenhouse crops, the availability and use of registered products varies among countries. Furthermore, it is also unclear what effects these compounds may have in recirculation systems. As a result, a number of studies have explored the potential use of more general, non-specific chemical treatments for disease control, specifically in closed systems.

#### 3.1. Surfactants

Based on early work which showed that surfactants were fungicidal against apple scab fungus (*Venturia inaequalis*) [19] and apple powdery mildew fungus (*Podosphaera leucotricha*) [39], several studies have since shown that surfactants may also be used to control root pathogens. A number of non-ionic, anionic and cationic surfactants have been shown to be toxic to zoospores of *Olpidium* (vector for lettuce big-vein virus and lettuce ring necrosis disease) [91]. Agral, a non-ionic surfactant, disrupts the plasmalemma of fungal structures such as zoospores which lack a cell wall, and in culture, motile zoospores of *Pythium* and *Phytophthora* will lyse within one minute of exposure to a concentration of  $20 \text{ } \mu\text{g} \cdot \text{ml}^{-1}$  [85]. Further work has shown that this concentration of non-ionic surfactants added to recirculated nutrient solutions effectively controls the spread of *Pythium* in cucumber [87] and *Phytophthora* in pepper [88] with no apparent phytotoxicity. Surfactants work very well in cases such as these where motile zoospores are the sole source of disease spread. Surfactants will degrade over time in recirculating systems, perhaps because of microbial action, so they must be reapplied periodically. Since surfactants will cause foaming, the

lack of foaming may be used as a guide to the timing of additions [88].

### 3.2. Elements

A number of essential elements for plants have been found to be toxic to disease-causing microorganisms at high concentrations. However, because of the potential of phytotoxicity at these extreme concentrations, their use in disease control has only sparingly been investigated. High concentrations of Cu ( $4 \mu\text{g}\cdot\text{ml}^{-1}$ ) and Zn ( $10 \mu\text{g}\cdot\text{ml}^{-1}$ ) will kill zoospores of *Olpidium* in culture [91], and  $5 \mu\text{g}\cdot\text{ml}^{-1}$  of Zn reduces incidence of lettuce ring necrosis disease in NFT-grown lettuce [93]. Another element which has received some attention is iodine. An iodine-laden resin has been used in experiments to determine the effects of exposure time and iodine concentration on tobacco mosaic virus (ToMV) and *Fusarium* sp. in recirculated nutrient solutions [77]. Although iodine concentrations of up to  $14 \mu\text{g}\cdot\text{ml}^{-1}$  had no effect on ToMV, *Fusarium* sp. were eliminated in concentrations as low as  $0.7 \mu\text{g}\cdot\text{ml}^{-1}$ . However, iodine appears to readily react with organic matter, which will influence both exposure time and the concentrations needed. A carbon filter was found to remove residual iodine after the disinfection treatment, but also interfered with Fe and Cu in the solution. Any iodine which was reduced to iodide (iodide was not captured by the carbon filter) did not affect the plants.

### 3.3. Oxidants

A problem with the use of oxidants for disinfection is the fact that they are highly reactive with organic solutes, as typically found in hydroponic systems. The oxidation of organic material not only reduces the effectiveness of the disinfection process, but potentially produces halogenated by-products [20]. Oxidation may also counteract the resident microflora, including beneficial (see Sect. 6), neutral and deleterious organisms, and affect the ecological balance in the growing system. One also has to be careful in making sure that residual oxidant does not enter the root zone, at which point phytotoxicity may occur. Having said this, there is abundant evidence that oxidants in recirculating systems can be effective disinfecting agents.

#### 3.3.1. Ozone

Ozone is a very strong oxidizing agent, with a reduction potential which varies from 1.2 to 2.1 V and increases at lower pH. Ozone is relatively unstable, but since it

decomposes completely and without a trace, it has been used successfully for years as a disinfectant for drinking water, waste water and aquaria. In the last ten years or so, ozone has also been developed as a disinfection agent for recirculating nutrient systems. Early work conducted in the laboratory has shown that ozone will effectively kill *Corynebacterium* and *Fusarium* micro and macroconidia, but effectiveness in nutrient solution was reduced because of an interaction of ozone with specific iron chelates [94]. Later in vitro experiments with cultured organisms have also shown that ozone is effective in reducing viable cell numbers of pathogenic bacteria (*Corynebacterium*, *Pseudomonas*, *Erwinia*) and one fungus (*Fusarium*) [105]. Although the ozone concentration which was used was not reported, the time required to reduce the number of viable cells from  $5 \times 10^3$ – $5 \times 10^4$  cells·ml<sup>-1</sup> to 1 cell·ml<sup>-1</sup> was quite high, varying from 60 to 120 minutes. Although other laboratory-based studies [75] have shown somewhat shorter exposure times for *Fusarium* and *Verticillium* (20 minutes in each case), quite lengthy disinfection times are required in nutrient recirculation systems. With the solution pH reduced to 4 to enhance ozone effectiveness, Runia [76] found that cucumber green mottle virus was eliminated after 75 minutes of exposure to ozone (6 g ozone generated·h<sup>-1</sup>, with a redox potential of 673 mV). ToMV was completely eliminated after 1 h of ozone treatment at 20 g ozone·h<sup>-1</sup> although *Verticillium* microsclerotia were still infective after 210 minutes. Since non-pathogenic fungi and bacteria are also killed by ozone, bacterial counts of the treated solution may be used as an indicator of ozone effectiveness [76, 79].

#### 3.3.2. Hydrogen peroxide

Hydrogen peroxide has a reduction potential of 1.8 V and forms water plus O<sub>2</sub> when reduced, making it an attractive sterilant. Working in vitro, Abdou and Galal [1] showed that 1 mM H<sub>2</sub>O<sub>2</sub> would completely prevent conidial germination of three *Fusarium* species and significantly reduce mycelial growth. Preliminary work by Runia [79] shows that conidia of *Fusarium* were completely killed by 100 ppm of activated (catalytically-enhanced) H<sub>2</sub>O<sub>2</sub> after a 5-minute exposure. H<sub>2</sub>O<sub>2</sub> has also been examined as a means to prevent algae growth in hydroponics, but the required dose of 50 ppm is phytotoxic [24]. More work is required on the use of H<sub>2</sub>O<sub>2</sub> in recirculating systems. For example, ozone and H<sub>2</sub>O<sub>2</sub> treatments could be combined to form the hydroxyl radical, which is yet another strong oxidizing agent [20].



### 3.3.3. Chlorine

Chlorine ( $\text{Cl}_2$ ) is often used in disinfestation of drinking water. Although  $\text{Cl}_2$  is not used in disinfestation of hydroponic systems, compounds from which  $\text{Cl}_2$  may be derived have been tried. For example, sodium hypochlorite ( $\text{NaOCl}$ ), the active ingredient in bleach, has been found to be effective against bacterial wilt in sweet pepper caused by *Pseudomonas* [90]. Serious phytotoxicity occurred at concentrations above 50 ppm, and 15 to 25 ppm seemed the best in terms of balancing good bacterial wilt control with minimal phytotoxicity. There is also evidence showing that  $\text{NaOCl}$  is effective against root-knot nematodes in hydroponic systems [89]. Chloramine and chlorine dioxide ( $\text{ClO}_2$ ) are two other chlorine-based disinfectants which have only rarely been used in hydroponic or recirculation systems. In one instance,  $\text{ClO}_2$  was combined with UV radiation to control plant pathogens in recirculating systems [53]. Chloramines (monochloramine and dichloramine) may form from the reaction of ammonia and residual chlorine or  $\text{NaOCl}$  in the nutrient solution, and have detrimental effects on plant growth [26].

## 4. ULTRA-VIOLET RADIATION

Ultra-violet (UV) light is electromagnetic radiation with a wavelength between 100 and 400 nm. It has been demonstrated that wavelengths between 200 and 280 nm (UV-C) have a strong germicidal effect with an optimum at 253.7 nm [35].

The use of UV sterilizer lamps has proven to be highly effective for disinfestation of recirculating systems [3, 21, 27, 31, 86]. UV radiation and submicron filters were evaluated for efficacy and for their effects on lettuce production by Schwatzkopf et al. [83]. Both methods were effective in removing bacteria, but at high intensity, the ultraviolet sterilizer significantly inhibited the production of plants grown in the treated solution. The cause of this inhibition was suspected to be due to ozone and/or free radicals in nutrient solution, which are known to be generated by UV radiation [15]. Stanghellini et al. [86] conducted trials to determine the efficacy of UV radiation of infested water to control root rot of spinach caused by *P. aphanidermatum*. Seedlings were grown in recirculated nutrient solution infested with encysted zoospores and oospores. The solution was either untreated or passed through a UV disinfestation unit at a flow rate of about 124 L·min<sup>-1</sup>. Within seven days of transplanting, all untreated spinach seedlings were dead

whereas no plant death or root infection occurred in the plants grown in UV-treated water.

Interaction of UV light with Fe-chelates can be a problem in the disinfestation of nutrient solutions. However, under certain conditions, and depending on the Fe-chelate, plant growth may be significantly increased by the use of UV light for disinfestation of nutrient solutions [2].

Benoit and Ceustermans [11] tested a UV unit with a 60-W central low-pressure mercury lamp to reduce the *Pythium* root rot infection in lettuce. At an average radiation of 98 mJ·cm<sup>-2</sup>, no lettuce plants were lost, even though the seedlings purchased for planting were found to be infected with *Pythium* sp. Laboratory and greenhouse experiments have also demonstrated that UV light eliminated spread of a single point infection of *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) in ebb and flow, and NFT systems [45].

High-pressure lamps emit UV-C radiation with a wavelength between 200 and 280 nm whereas low-pressure lamps emit UV-C-rays predominantly at the optimum disinfestation wavelength of 253.7 nm. High-pressure lamps are also less energy efficient (about 10% of the power consumption is converted into UV-C compared to about 40% in low pressure lamps). These lamp types have been compared for efficacy against plant pathogens in greenhouse trials [79]. Rapid sand filters were installed to remove organic material before UV treatment since particulate matter can interfere with UV transmission. A UV-dose of 28 mJ·cm<sup>-2</sup> from the high-pressure lamp resulted in a 90% reduction in infectivity of conidia of FORL; a dose of 84 mJ·cm<sup>-2</sup> was required to reduce conidia by 99.9%. Infectivity of ToMV was reduced by 99.8% after a UV dose of about 100 mJ·cm<sup>-2</sup> and by 99.99% after a dose of 277 mJ·cm<sup>-2</sup>. The low-pressure lamp eliminated conidia of FOM at a dose of 70 mJ·cm<sup>-2</sup>. A dose of 100 mJ·cm<sup>-2</sup> reduced the infectivity of ToMV by 99% and a dose of 150–175 mJ·cm<sup>-2</sup> increased the reduction to 99.9% [79]. Hence, both high- and low-pressure lamps can disinfect recirculation water provided that the required UV-dose is achieved.

Standardized lab tests have been developed to establish lethal doses of UV radiation for disinfestation of pathogens in recirculated solutions [110]. In commercial greenhouses, Runia [78] recommends a UV-dose of 100 mJ·cm<sup>-2</sup> for elimination of pathogenic fungi, and 250 mJ·cm<sup>-2</sup> for complete disinfestation, including viruses. These levels are somewhat higher than needed in experiments, largely to build in a margin of safety.

## 5. FILTRATION

Disinfesting irrigation water or nutrient solutions by filtration has to consider the very small size of water transmissible phytopathogens. The size of infectious particles of fungi is mostly in the range of 3 to 50  $\mu\text{m}$ , that of bacteria about 1  $\mu\text{m}$  and of phytopathogenic viruses usually 0.03 to 0.3  $\mu\text{m}$ . Obviously standard filters with a pore size of 80 to 100  $\mu\text{m}$  commonly used to prevent clogging of irrigation lines, drippers etc. are not efficient in eliminating phytopathogens. For disinfesting irrigation water or nutrient solutions, mainly membrane filtration or slow filtration are considered to be suitable filtration techniques.

### 5.1. Membrane filtration

Depending on the pore size, membrane filtration is categorized into micro-, ultra- and nanofiltration. Reverse osmosis and various systems of dialysis are also considered to be membrane filtration techniques [14]. The membranes can consist of various materials such as cellulose acetate, various polymers or ceramics, and vary in size and structure. Membrane filtration can be realized as dead end or tangential (“cross-flow”) filtration. The latter, the cross-flow technique (pore sizes 0.01 to 10  $\mu\text{m}$ ) seems to be particularly suitable for eliminating phytopathogens. At a certain pressure the raw water cycles continuously along the membrane, removing particles larger than the pore size and in this way preventing clogging of the membrane. Water and nutrients including the chelates can pass through the membrane. However, over time the concentration of particles remaining in the raw water increases and therefore this concentrate (reject water), has to be wasted from time to time. Runia [75] suggested that the rejected water be returned to the drain water catchment tank thus avoiding the loss of water and nutrients (20–30%).

A membrane filtration system with a pore size of 0.003  $\mu\text{m}$  at a pressure of 3 bar was tested by Runia [75] against FORL (5–12  $\times$  2.2–3.5  $\mu\text{m}$ ), *Verticillium albo-atrum* (3.5–8  $\times$  2–3  $\mu\text{m}$ , cucumber green mottle mosaic virus (CGMMV) and tobacco mosaic virus (TMV). The size of the viruses was 0.018  $\times$  0.3  $\mu\text{m}$ . On a lab-scale basis, the fungi and CGMMV were completely eliminated. However, for TMV some infectivity could be observed in the treated water. In a semi-commercial-scale device, the treated water was completely freed from fungal pathogens and the viruses. McPherson et al. [52] proved a cross-flow micro-filtration unit (0.2  $\mu\text{m}$ ) to be efficient against *P. aphanidermatum* in a soilless cucumber crop. In contrast to other “active” (germicidal agents such as chemicals, heat, or UV radiation) disin-

festation techniques a marked improvement of root quality was observed. Practical use of the membrane technology revealed problems such as clogging and leaking [98]. Based on the technical problems and the comparable high investment, membrane filtration has not been used widely in the horticulture industry.

There have been some attempts to use combinations of various filters sequentially. Darling [25] successfully used a combination of sand filter, cellulose cartridge and ceramic filters to eliminate propagules > 1  $\mu\text{m}$ . Plant parasitic nematodes were retained by passing contaminated water through a series of four filters comprising a gauze cartridge (150  $\mu\text{m}$ ) and three polyester filter bags (80, 1 and 1  $\mu\text{m}$ ) [55].

### 5.2. Slow filtration (“bio-filtration”)

Slow sand filtration was initially developed by John Gibb in Scotland in 1804 to obtain pure water for his bleachery [41]. After improvement of his design, slow sand filtration became a common method of drinking water purification. The most convincing proof of the effectiveness against water-borne diseases of man was provided in 1892 in Hamburg, Germany. People supplied with untreated river water suffered severely from a cholera epidemic while the population of a neighboring city (Altona) escaped by using water treated by slow filtration. In the middle of the 20th century slow sand filtration has been replaced or at least supplemented by other water treatment techniques like chlorination, UV-irradiation, etc. However, slow filtration has recently enjoyed a resurgence for treating drinking water [18]. In the late 1980s Wohanka [108, 110] demonstrated the potential of slow sand filtration for eliminating phytopathogens from reused irrigation water or nutrient solutions. This method has since been widely adopted for closed cultivation systems in the horticulture industry [107].

#### 5.2.1. Principles of slow filtration

The principle of slow filtration is very simple. Raw water percolates very slowly (100 to 300  $\text{L}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ ) through a bed of fine filter sand or other filter material. Soon after the filter process begins, a “Schmutzdecke” (dirt layer or filter skin) forms on the surface of the filter bed. Its consistency varies widely depending on the organic and inorganic material of which it is composed. The “Schmutzdecke” shows a very high biological activity with its population of algae, protozoa, bacteria, fungi, actinomycetes, diatoms, rotifers etc. [30]. Despite the long history of slow sand filtration, the mechanisms of water purification are not well understood. However, it

seems very clear that it is more than a mechanical straining effect. Sedimentation, adsorption, and other physical, chemical and biological factors are suggested to be important processes of slow filtration [41]. Compared to rapid filtration, biological activity is considered to be the most important additional purification mechanism.

During the process of “ripening” or “maturation” of the filter, organic material will be deposited on the surface of the filter grains enabling the formation of microcolonies and biofilms on the surface of the individual filter particles and the filter surface. Brand [17] demonstrated the formation of such slimy, sticky films for slow filters fed by nutrient solutions from a closed hydroponic growing system. Most of the suspended matter in the raw water is trapped at the filter surface, mainly within the “Schmutzdecke”. Because of the low flow rate, inorganic and organic particles including pathogens become attached to the surface of filter grains or filter fibers. The trapped organic impurities are broken down by chemical and microbiological oxidation [41]. Mainly heterotrophic bacteria metabolize the organic material by assimilation and dissimilation. However, other biological processes including feeding by animal inhabitants of the filter bed may be of significance. Dissimilation products will be swept down to act as the substrate for bacteria in deeper layers of the filter bed until complete breakdown and assimilation is achieved [30]. Such biological processes need sufficient time (low flow rate), enough oxygen ( $> 3 \text{ mg}\cdot\text{L}^{-1}$  in the effluent) and adequate temperature. Because of the interactions between biotic and abiotic factors, a slow filter can be considered as an open ecosystem with continuous input of nutrients and output of metabolites [72]. The microbial inhabitants are in biological equilibrium and therefore slow filters are considered to be self regenerating and adaptable to changing environmental conditions.

The microbiological activity decreases with the depth through the filter bed. In the top layers of sand or rock-wool filter beds, bacterial densities of  $10^7$  to  $10^8 \text{ cfu}\cdot\text{cm}^{-3}$  were found, decreasing rapidly within the first centimetres to  $10^6 \text{ cfu}\cdot\text{cm}^{-3}$  and remaining at this level even in deeper layers [17]. The bacterial populations are adapted to the type and amount of food supplied by the passing water, and therefore, fluctuations in raw water quality should be avoided. The contribution of biological activity to the elimination of phytopathogens can vary depending on the particular organism. Comparing a “sterile” slow sand filter with a “biologically ripe” filter, Brand [17] revealed a significant biologically-induced increase in efficiency against bacteria (*Xanthomonas campestris* pv. *pelargonii*) but not against *Fusarium oxysporum* f. sp. *cyclaminis*. Results of Runia et al. [82] and Van Os et al. [101] also indicate that the

biological component of filter activity seems to be of less importance for eliminating fungal pathogens. In contrast to active disinfestation techniques, the effluent from slow filters has relatively high bacterial densities of  $10^3$  to  $10^4 \text{ cfu}\cdot\text{ml}^{-1}$  [106]. The identity of these microorganisms and their role in disease suppression of recirculating nutrient solutions is not well understood. However, they may contribute to the suppression of plant pathogens [52, 70].

### 5.2.2. Construction and maintenance of slow filters

Construction of slow filters for use in the horticultural industry follows the basic design used for small-scale potable water treatment plants [37, 104]. However, implementation of filtration in closed irrigation systems required some adaptations to the needs of horticultural practice [108, 110, 112].

A general scheme of a slow filtration plant is shown in Figure 2.

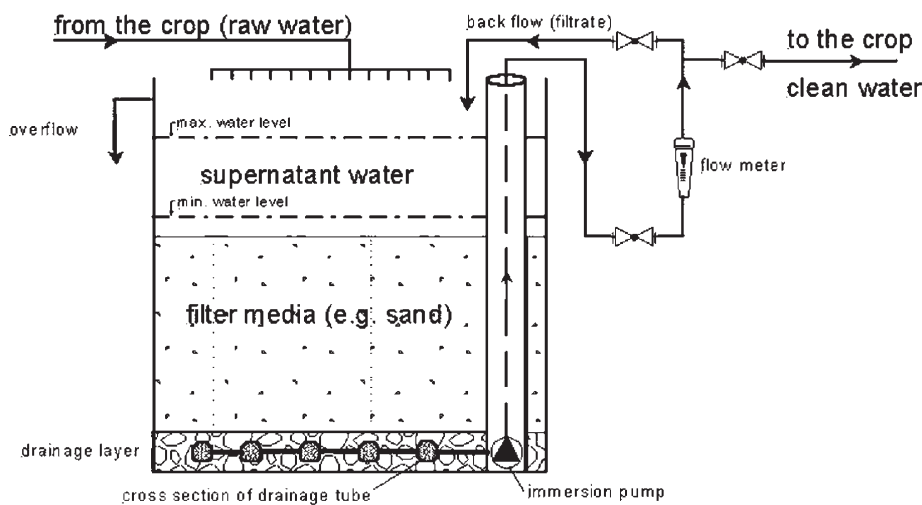
The essential components of a slow filter are:

- a filter box,
- an inlet structure,
- a bed of fine sand or other filter media (with supporting gravel layers if necessary),
- an underdrainage system,
- an outlet structure including a flow meter and control valves to regulate the velocity of water flow through the filter bed.

Slow filters usually are gravity filters consisting essentially of an open-topped filter box. Construction and material of the filter containers vary widely. In European greenhouses, standard water tanks made of corrugated iron with a plastic inlay are mainly used. Simple plastic tanks may often be found on small farms. However, very large pond-like outdoor filters are also used. By varying the filter size, any filter capacity may be obtained according to the requirements of the cropping system.

Gravity filters need a supernatant water layer (raw water) of about 80 to 150 cm to ensure enough pressure for the desired flow rate. The raw water is poured into the filter by an inlet structure. The construction of the inlet structure should prevent damage to the “Schmutzdecke” (filter skin) on the filter surface by the raw water. Hence, the raw water should first flow into a box or a wide pipe and then very gently onto the surface of the filter bed. As an alternative, the raw water may be sprinkled onto the supernatant surface (see Fig. 2). The latter system has the advantage of oxygen enrichment.





**Figure 2.** Functional scheme of a slow (sand) filter.

The flow rate should be in the range of 10 to 30 cm per hour, which means a filter capacity of 100 to 300 L·m<sup>-2</sup>·h<sup>-1</sup>. A low flow rate is essential for optimum efficiency of the biological component in particular. Usually the flow rate is controlled by special outlet structures. In its simplest form, only a valve at the outlet is necessary to reduce the flow rate. The outlet opening should be at a higher level than the filter surface to prevent total drainage and thus drying up of the filter bed. In horticultural practice another outlet structure is often used: This may consist of a wide pipe (20 to 40 cm in diameter) extending through the filter bed into the drainage layer (see Fig. 2). A plunger pump is often installed in the pipe. The capacity of this pump should not be higher than the maximum filtration rate of the slow filter. Otherwise, a regulation valve is necessary. To monitor the flow rate, a flow meter is absolutely necessary. To ensure a more or less continuous water flow through the filter bed, a back flow device is recommended. It should be activated when there is no raw water supply, or when no clean water is needed.

The standard filter medium is sand which must meet certain requirements (Tab. I). Often "local sand" with a grain size of 0–2 mm is used [114]. The minimum thickness of the filter bed should be 50 to 60 cm, but 80–120 cm are recommended. Depending on the raw water quality, cleaning of the filter bed will be necessary after a few weeks or months to prevent clogging. This is done by scraping off only the top layer. The filter sand is supported by three layers of graded gravel (2–8, 8–16 and 16–32 mm) and some kind of underdrainage (see Fig. 2). Rockwool granules have proven to be a highly effective alternative filter material [113, 114] although

**Table I.** Quality of filter sand.

Effective grain size	0.15–0.30 mm
Uniformity coefficient (UC)	< 3, max. 5
Silt content	< 1%
Acid solubility	< 5% after 30 min

Effective grain size ( $d_{10}$ ): sieve opening through which 10% (by weight) of the grains will pass.

Uniformity coefficient (UC): ratio between the sieve opening through which 60% (by weight) of the grains will pass and the effective grain size;  $UC = d_{60}/d_{10}$ .

some problems with preferential flow in large filters may occur.

There is virtually no effect of slow filtration on the physical and chemical parameters of nutrient solutions such as content of individual nutrients, pH and the EC-values [99, 111]. However, depending on the biological activity, the oxygen content may be drastically reduced.

### 5.2.3. Effectiveness of slow filtration against phytopathogens

It has been shown that slow filtration is a highly effective means of removing bacteria, viruses and other human pathogens from water. Elimination rates are often in the range of 99 to 99.99% [30, 71]. However, fluctuation in temperature or other factors can reduce the efficiency of slow filtration considerably. For evaluation of the effectiveness against plant pathogens, long-term experience from the drinking water industry cannot be simply transferred to horticulture. The main

phytopathogens are different from human pathogens and drainage water from a crop is very different from drinking water. Furthermore, the running conditions for a slow filter in horticulture are quite different from those of a community water supplier. For these reasons, specific experimental work has been necessary to evaluate the effectiveness of slow filtration against phytopathogens under specific horticultural conditions.

Basically there are two ways to evaluate the effectiveness of slow filtration against phytopathogens. Integrating a slow filter into a closed cropping system with a definite host-pathogen system (infestation by inoculated host plants) allows the direct transfer of results to practical horticulture. Effectiveness of slow filtration is measured by monitoring disease development in the crop irrigated with treated water. The second method is to calculate efficiency rates by comparing relatively high concentrations of a pathogen in the inlet supernatant with the concentration in the effluent. In this case usually a cropping system with non-host plants is used to ensure a series of experiments without destroying the test system (host plants). Such experiments are especially suitable to optimize the filtration technique by comparing various filter media, flow rates, etc.

Initial experiments on the efficacy of slow filters against phytopathogens were focused on *Phytophthora* species. Friedel et al. [33] demonstrated a complete elimination of *Phytophthora cinnamomi* from the recycled irrigation water of a *Erica gracilis* crop. The result was confirmed by Behrens et al. [10] and Van Kuik [96] testing slow sand filters against *P. cinnamomi* in a closed system of container-grown *Chamaecyparis lawsoniana*. Furthermore, it has been shown that slow sand filtration can safely prevent the dissemination of *Phytophthora cryptogea* by the recirculated nutrient solution on *Gerbera jamesonii* grown in rockwool slabs [110]. Runia et al. [82] tested the slow sand filtration technique against *P. cinnamomi* by artificial inoculation of the supernatant with zoospores at flow rates of 100 and 300 L·m<sup>-2</sup>·h<sup>-1</sup> and three grades of grain size (0.15–0.30, 0.2–0.8 and 0.5–1.6 mm). Only by using the low flow rate of 100 L·m<sup>-2</sup>·h<sup>-1</sup> and the “fine” and “middle” filter sand could a complete elimination of *P. cinnamomi* be achieved. These results were confirmed by Van Os et al. [101].

Ehret et al. [29] have shown that slow sand filtration<sup>1</sup> is very effective over long time periods against *P. aphanidermatum*. There is no information available about the efficiency of slow filtration against other

zoosporic fungi (e.g. *Olpidium* spp.), but if one considers the similarities of these pathogens, results obtained from *Phytophthora* and *Pythium* trials could be used to speculate on the effects of slow filtration on these fungi. Further work is needed to verify this.

In a filtration process in which air is pumped through the filter material and the water is constantly moving, the “Shieer-Biofilter” (lava granules) uses long retention times and biological working mechanisms to eliminate pathogens from water. Early results have shown that *P. cinnamomi* can be completely eliminated [80].

Until recently, the efficacy of slow filtration against *F. oxysporum* f. spp. has only been demonstrated by incorporating slow filters into non-host cropping systems or lab-scale filter systems. The very first experiments with a non-optimised slow sand filter produced efficiency rates of only 70–80% [108]. Later experiments with an improved filter design revealed efficiency rates of approximately 99.9% against *F. oxysporum* f. sp. cyclaminis [111]. Comparing various filter media at a flow rate of 200 L·m<sup>-2</sup>·h<sup>-1</sup>, Wohanka and Helle [113] achieved efficiency rates higher than 99%. The best results (99.9%) could be obtained by sand and granulated rock wool as filter media. Effects of pumice and an open porous clay material (Seramis®) were slightly but significantly lower. Further experiments [102] with various filter media (sand, rock wool, glass wool, polyurethane foam) at two flow rates (100 and 300 L·m<sup>-2</sup>·h<sup>-1</sup>) confirmed the high efficiency of slow filtration against *F. oxysporum*. Efficiency rates obtained by a series of experiments were in the range of 97.3 to 100%. In lab-scale filters, *F. oxysporum* was eliminated to a greater extent at pH 7 than at pH 5 [69]. Runia et al. [82] demonstrated efficiency rates of 99.9% against FORL at a flow rate of 100 L·m<sup>-2</sup>·h<sup>-1</sup> within the first three days after inoculation. With a higher flow rate (300 L·m<sup>-2</sup>·h<sup>-1</sup>) the test fungus was eliminated by 94 to 99%. However, in the effluent of some filters, high pathogen concentrations (10 to 40% of the inoculated propagules) were detected 57 or 116 days after inoculation, indicating reduced efficacy over long time periods. Similarly, Ehret et al. [29] found that long-term sand filtration<sup>2</sup> of FORL varied between 93 and 97%, decreasing somewhat over time. Trials with a “Shieer-Biofilter” revealed similar reduction rates as standard slow filtration [80].

In a soilless closed cropping system of geranium mother stocks, dissemination of *Xanthomonas campestris* pv. *pelargonii* (Xcp) was completely prevented by using slow sand filtration at a flow rate of

<sup>1</sup> link: [http://res2.agr.ca/parc-crapac/english/1agassiz/crop\\_science/sand.htm](http://res2.agr.ca/parc-crapac/english/1agassiz/crop_science/sand.htm).

<sup>2</sup> link: [http://res2.agr.ca/parc-crapac/english/1agassiz/crop\\_science/sand.htm](http://res2.agr.ca/parc-crapac/english/1agassiz/crop_science/sand.htm).

200 L·m<sup>-2</sup>·h<sup>-1</sup> [110]. However, inoculation of the inlet supernatant of slow filters with a high concentration of *X. campestris* revealed that a low amount of the bacteria can pass the filter bed. Wohanka [110], using a slow sand filter at a flow rate of 200 L·m<sup>-2</sup>·h<sup>-1</sup>, found a reduction of *X. campestris* from  $1.1 \times 10^5$  to  $1.3 \times 10^3$  cfu·ml<sup>-1</sup> (efficiency rate 98.8%). Comparing various filter media at the same flow rate, sand, pumice and anthracite produced efficiency rates of only 81 to 83% [114]. However, with granulated rockwool a mean efficiency rate of 98.6% could be obtained. Trials with somewhat lower inoculation densities at a flow rate of 100 L·m<sup>-2</sup>·h<sup>-1</sup> showed efficiency rates of 99.9 to 100% for sand, 91.9 to 100% for granulated rock wool and 99.7 to 99.9% for poly urethane foam, respectively [102]. There was no significant reduction in efficiency at a higher flow rate of 300 L·m<sup>-2</sup>·h<sup>-1</sup>. This is in contrast to results of Wohanka et al. [114] who demonstrated a significant negative correlation between flow rate (100 to 300 L·m<sup>-2</sup>·h<sup>-1</sup>) and efficiency rate. Efficiency of slow filtration may be influenced by pH. The elimination of *Erwinia carotovora* was significantly higher at pH 5 than at pH 7 [69].

The efficiency of slow sand filtration against tomato mosaic virus (ToMV) was tested by Runia et al. [82] in a series of trials with various grain sizes and flow rates. At a flow rate of 100 L·m<sup>-2</sup>·h<sup>-1</sup> ToMV was reduced by 91 to 99% within three days after inoculation. In one trial the reduction rate decreased from 98 to 97% (grain size: 0.15–0.30 mm) and from 91 to 87% (grain size: 0.5–1.6 mm) six days after inoculation. In the filters with the high flow rate of 300 L·m<sup>-2</sup>·h<sup>-1</sup> the efficiency rates decreased from 80 and 90% one day after inoculation to 23 and 70% six days after inoculation for large and small grain size, respectively. Van Os et al. [100] found reduction rates of 78.3 to 91.2% with “fine” sand (0.15–0.35 mm) and of 62.8 to 86.7% with “middle” sand (0.2–0.8 mm) within twelve days after inoculation with ToMV. In earlier trials with repeated circulation of the treated water through a slow sand filter at approximately 200 L·m<sup>-2</sup>·h<sup>-1</sup>, reduction rates of 83, 90 and 97% could be observed after one, two and five passages, respectively [79]. A “Shieer-Biofilter” provided reduction of ToMV by 91.5 to 98.5% [80]. The efficiency rates of slow filtration or similar bio-filtration seem not to be sufficient for preventing dissemination of root infecting viruses in closed irrigation systems. However, as shown by Berkelmann et al. [13], slow sand filtration markedly inhibited the development of a virus disease (pelargonium flower break virus) in soilless-grown geranium mother stocks.

Nematodes are not very common in soilless culture. However, *Pratylenchus vulnus* on roses [7] and

*Radopholus similis* on anthuria [8] have become a serious threat. Using slow sand filtration, *R. similis* was eliminated by 91.1 to 96.4% without significant influence of grain size or biological load [101]. Passage through the filter bed did not influence the ability of *R. similis* to infect plant roots and to reproduce.

#### 5.2.4. Conclusion

Membrane filtration theoretically can remove all phytopathogens from recirculated water. However, because of clogging and leaking the first generation of such filters have failed in practical horticulture. The current generation is more reliable, but problems with removal of the concentrate (brine) and the high investment are still preventing their common use [98].

Slow filtration with fine sand or granulated rockwool has proven to be highly efficient against the most relevant phytopathogens in soilless, closed cropping systems. It has the advantage of a low energy input and low cost and ease of construction and operation. It does not need waste water for back-flushing the filter bed. As a “passive” disinfestation technique, positive effects on the resident microflora of nutrient solutions can be expected. Limitations are the insufficient efficiency against viruses and nematodes, the large areas needed for high capacity filters, and in the absence of a pre-filter, the potential for frequent clogging in cases of high loads of silt, peat, etc. in the raw water. In winter frost regions, filters must be inside the greenhouses or require precautions against freezing.

## 6. MICROBIAL INOCULATION

Biological control agents (bca) with fungi and/or bacteria as the active organisms can have experience conditions when used in protected cultivation with closed hydroponic systems [61, 63]. Many different organisms and strains have been studied with respect to potential disease control in closed hydroponic systems but only a few products for disease management are commercially available.

### 6.1. Pythium

Various microorganisms have been tested for control of plant pathogenic *Pythium* and *Phytophthora* species occurring in closed systems. Because of the poor competitive abilities, colonization patterns [72] and etiology of fungi, primary root colonizers have predominantly been studied. Root microorganisms, which have high rhizosphere competence and rapidly utilize critical

carbon sources otherwise used for propagation of pathogenic fungi and/or their zoospore germination, are interesting biocontrol agents. Most of them involved strains belonging to species of *Pseudomonas* and *Bacillus*. Apart from abundance in the root zone, pseudomonads also make up a substantial part of the culturable heterotrophic microflora found in the nutrient solution of closed systems [12]. In experiments regarding the control of *P. aphanidermatum*, substantial growth promotion, yield increase and disease reduction were obtained using *Bacillus subtilis* BACT-0 [92], increase in number of fruits and reduction in percentage of unmarketable fruits with *Pseudomonas fluorescens* PF15 and *Pseudomonas corrugata* Pc13 [72], reduction in both fungal density and disease symptoms using *P. fluorescens* strain CH31 and CH1 [56], as well as disease suppression using *P. fluorescens* WCS365 and *Streptomyces griseoviridis* (Mycostop®) [68]. *Pythium ultimum* causes damping-off in seedling plants of many plant species and root rot on older plants. Under Scandinavian conditions, *P. ultimum* is a significant pathogen in hydroponically-grown tomato during low light conditions in spring and autumn [50]. *Pseudomonas fluorescens* strain 5.014 as well as its mutant (5-2/4) with reinforced production of 2,4 diacetyl phloroglucinol controlled *P. ultimum* damping-off and root rot in hydroponic culture of tomato [42, 43]. A multiple strain treatment including *P. fluorescens* 5.014, *Xanthomonas maltophilia* 18.013 and an unidentified Gram-positive strain 19.018 increased the yield of tomato by 2 kg·m<sup>-2</sup> compared to the non-inoculated control [5].

## 6.2. Fusarium

In contrast to pathogens causing root rots, microbial inoculants controlling *Fusarium* sp. should be able to colonize vascular tissue and to induce resistance [63]. Microorganisms studied for control of *Fusarium* spp. include non-pathogenic *Fusarium* spp., and species of *Pseudomonas* and *Bacillus* [4, 16, 32, 47, 64–67]. Control of different forma speciales of *F. oxysporum* using non-pathogenic *Fusarium* sp. was readily obtained and there was a high degree of disease suppression [47, 67]. However, the number of inoculations and site of inoculation appears to be of importance [67].

Lemanceau and Alabouvette [47] compared a multiple strain treatment using non-pathogenic *F. oxysporum* 47 and *P. fluorescens* C7 with single strain treatments on tomato in the presence of *F. oxysporum*. The multiple strain treatment was superior with respect to the percentage of wilted plants during the observation period of 13 weeks. Further, the amount of crown and root rot was reduced with a multiple strain treatment as well as the

single treatment with *F. oxysporum* 47, whereas the single treatment with *P. fluorescens* C7 was less competitive.

## 6.3. Mechanisms

The effect of microbial inoculation on disease control may depend on various mechanisms, such as competition for space and nutrient sources, production of metabolites, i.e. antibiotics [6, 42, 43], siderophores [59, 62], HCN, biosurfactants [84], extracellular enzymes [44, 57] as well as induced resistance [22, 28, 57, 59]. The diversity of mechanisms allows tailoring of bca for specific diseases. However, other physical, chemical and biological factors prevailing in closed hydroponic systems, and especially in the root zone, are important for the success of protection. This implies that changes in environmental and cultural conditions within the growing system may modify the effect of microbial inoculation.

### 6.3.1. Siderophores

Siderophores are a group of microbial metabolites which chelate iron. These compounds may be exuded by most microorganisms under low iron availability conditions. The siderophores sequester ferric iron in the environment [58] and the resulting iron-siderophore complex is recognized as a receptor, mediating uptake of iron into the microbial cells. For many pseudomonads with biocontrol ability, siderophore production is recognized as an important factor for biocontrol of different plant pathogens. *Pseudomonas putida* WCS358 inoculated on carnation together with non-pathogenic *F. oxysporum* 47 displayed an enhanced control of pathogenic *F. oxysporum* compared to a *Pseudomonas* mutant lacking the ability to produce siderophores [48]. This was caused by the siderophore's (pseudobactin 358) effect on the fungi's iron metabolism [49]. Interestingly, although mutants lacking the ability to produce siderophores failed to control *P. ultimum* in vitro, they could protect cucumber roots against the disease in in vivo-experiments [62]. In hydroponic greenhouse systems, iron is supplied as synthetic chelates to the nutrient solution. Therefore, these systems should not be characterized by low iron conditions. However, Bakker [9] emphasizes that the choice of applied synthetic chelate might be important for effective disease suppression mediated by siderophores. He points to the fact that iron availability for microorganisms will depend on the affinity for iron of the applied chelate [103]. Chelates with low affinity would be available for both the biocontrol agent and pathogen, resulting in a lack of competition for iron.



### 6.3.2. Antibiotics

Antibiotics produced by the inoculant or by members of the root-inhabiting microflora may contribute to disease suppression in hydroponic systems. Bochow et al. [16] showed that peptide antibiotics formed by *Bacillus subtilis* affected the growth of FORL. Alsanius et al. [6] and Hultberg et al. [42, 43] inoculated tomato seeds with *P. fluorescens* 5.014 and its diacetyl phloroglucinol (phl)-producing mutant (5-2/4) and found that the mutant was superior in antagonizing *P. ultimum* in both in vitro and in vivo studies. The phl<sup>+</sup>-mutant was superior in controlling higher densities of *P. ultimum* compared to the wild type. Furthermore, preliminary results on characterization of microbial metabolites in the nutrient solution of a closed hydroponic greenhouse system indicate the occurrence of both pyoluteorin and phenazine-1-carboxylic acid (Alsanius, unpublished).

### 6.3.3. Biosurfactants

Biosurfactants may be produced by a variety of different microorganisms, such as *Pseudomonas* spp., *Bacillus* spp., *Arthrobacter*, *Rhodococcus*, *Acinetobacter* and *Corynebacterium* [40, 74, 84] and induce lysis of zoospores. All of these species were found in the disease-free nutrient solution of closed tomato systems [12]. Further, rhamnolipid-producing strains of *Pseudomonas aeruginosa* and *P. corrugata* were found in the root zone of hydroponically-grown cucumber, tomato and cantaloupe [84]. Spontaneous formation of rhamnolipids was reported in the nutrient solution from closed hydroponic systems with cucumber and pepper after infestation with *P. aphanidermatum* and *Phytophthora capsici*, respectively. Purified mono- and di-rhamnolipids showed lytic activity towards different zoosporic pathogens. Concentrations of 5 to 30 µg·mL<sup>-1</sup> of the tested compounds were critical for causing decrease in motility and lysis of zoospores. However, necessary concentrations of the compounds were dependent on the nature of, as well as zoospore sensitivity to, the active compound. Rhamnolipid production is also influenced by some carbon sources, such as glucose [38, 84] and olive oil [85] that may serve as precursors.

### 6.3.4. Induced systemic resistance

Induced systemic resistance (ISR) and systemic acquired resistance (SAR) are found to play an important role in biological control of fusarioses. They have also been stated as factors in the control of pythiaceous disease by some microbial inoculants [22, 23]. Bacterially-induced systemic resistance was found to be involved in control of *F. oxysporum* on carnation using

*P. fluorescens* strains WCS374 and WCS417, as reviewed by van Loon [97], and FORL using the endophytic *P. aureofaciens* strain 63-28 [57] or WCS417r [28]. Induced resistance by non-pathogenic *Fusarium* has been found to be involved in control of several formae speciales of *F. oxysporum* [34]. However, the induction and degree of protection due to induction appears to be dependent on the inoculated non-pathogenic *Fusarium* strain [28, 63].

### 6.4. Commercial use

Many studies demonstrate that results generated in vitro cannot always be repeated in vivo in hydroponic systems. This may be explained by the fact that multiple mechanisms are involved in biological control of root pathogens using artificial microbial inoculation. Apart from the inoculants' effect on the pathogen, there is some evidence that bca also change the composition of the rhizobacterial community structure, with respect to both functional diversity and taxonomic characterization [6, 42].

For commercial application of bca, several factors, such as formulation, dose-response relationship, single vs. multiple strain treatment, as well as multiple target bca, must be considered. Microbial inoculation may also be varied with respect to the mechanisms involved. For example, inoculation of biosurfactant-producing organisms would preferably take place in the nutrient solution, whereas inoculation of a competitor for space or nutrients would probably be most appropriate on the root itself. However, the question should be raised about artificial microbial inoculation being the only approach to biologically counteract plant pathogens in closed systems. In 1994, Gertsson et al. [36] stated that closed irrigation systems could be managed without disinfestation treatment as long as good hygienic and environmental conditions were maintained in the system. There are indications that a natural establishment of disease suppressive agents towards important pathogenic fungi may develop in closed systems [51, 52, 70]. Regardless of how the suppressive agents arise, however, the mechanisms of disease suppression must be understood in order to use biological control as an operative strategy in closed irrigation systems.

## 7. DISCUSSION

Research over the years has developed and validated a large number of different principles for disinfestation of hydroponic recirculating nutrient systems. Many of these principles have been developed into methods which have

**Table II.** List of currently used or researched disinfestation methods for recirculation systems, with advantages and disadvantages of each.

Method of disinfestation	Advantages	Disadvantages
Filtration		
1. Membrane	Highly effective	Frequent plugging and leaks High capital costs
2. Slow filtration	Low cost	High space requirement Effectiveness varies with pathogen
Pasteurization	Highly effective	High capital costs High maintenance costs
Chemical		
1. Ozone	Highly effective	High capital costs High maintenance costs Efficiency drops with high organic matter Interaction with some micronutrients
2. Other than ozone	Low cost	Phytotoxicity at high concentrations
UV light	Low space requirement	Efficiency drops with high organic matter and bulb age Interaction with micronutrients
Microbial inoculation	Environmentally-friendly May be tailored to both target and infection sites	Commercial availability Consistency

made their way into commercial applications. However, each has advantages and disadvantages (Tab. II). In our view, there is no obvious “best” solution for pathogen control in recirculating hydroponic systems. The sterilization methods of heat, radiation and oxidizing agents are generally the most effective; however, these are often the most costly and may negatively influence populations of indigenous beneficial microorganisms in the root zone. The more “microorganism-friendly” methods of slow filtration and microbial inoculation are currently not as effective, but are potentially less disruptive of the resident microflora. Microbial inoculation is perhaps the most pathogen-specific of all the methods. All except microbial inoculation are “spot-disinfestation” methods, reducing the spread of pathogens in the nutrient solution but having no effect on pathogens already present in the growing media. In this respect, microbial inoculation holds the most potential for future development. Further, some technical approaches might not be a solution for smaller greenhouse companies as they are too expensive.

In evaluating these various methods, one must ask how important is it to achieve complete sterilization or removal of the pathogens in a closed system? Does less than 100% effectiveness by any of these methods result in epidemic spread of the disease, or in a significant economic loss? In this respect, measurement of absolute lev-

els of the disease organism (such as propagule concentrations) in recirculation water, in conjunction with knowledge of how those levels relate to disease incidence [54], is probably a more important indicator of disease threat than are reduction rate or efficacy figures which are sometimes calculated for a given control method. Furthermore, populations of disease organisms in the recirculated solution should be related to an economic threshold, something which is rarely done. Hence, less than 100% eradication of the pathogen may be adequate if the pathogen levels in the treated solution do not reach a biological or economic threshold. What also is the role of pathogen latent period, crop condition, and environmental factors on the epidemiology of pathogens when efficacy of the control method is less than 100%? More wide-spread adoption of these systems will likely generate this information. This should result in improved use of closed systems and enhanced environmental, agricultural and economic sustainability of the greenhouse industry.

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## REFERENCES

- [1] Abdou E.S., Galal A.A., Sensitivity of *Fusarium moniliforme*, *F. oxysporum* and *F. solani* to superoxide anion and hydrogen peroxide in vitro, Egypt. J. Microbiol. 32 (1997) 523–535.
- [2] Acher A., Heuer B., Rubinskaya E., Fischer E., Use of ultraviolet-disinfected nutrient solutions in greenhouses, J. Hort. Sci. 72 (1997) 117–123.
- [3] Adams R.P., Robinson I., Treatment of irrigation water by ultraviolet radiation, in: Lovelock D.W. (Ed.), Plant Pathogens, Academic Press, New York, 1979, pp. 91–97.
- [4] Alabouvette C., Lemanceau P., Steinberg C., Recent advances in the biological control of fusarium wilts, Pestic. Sci. 37 (1993) 365–373.
- [5] Alsanius B.W., Gertsson U.E., Einfluss der Bakterisierung von Tomatenpflanzen auf Zuwachs und Ertrag in geschlossenen Kultursystemen, BDGL-Schriftenreihe 16 (1998) 148.
- [6] Alsanius B.W., Khalil S., Hultberg M., Englund J.E., Sundin P., The influence of introduced *Pseudomonas* isolates on the rhizobacterial community of hydroponically cultivated tomato: a comparison of different methods of assessment, in: Hultberg M. (Ed.), Seed bacterization with *Pseudomonas fluorescens* and interactions with *Pythium ultimum* on tomato in soilless systems, Acta universitatis agriculturae sueciae, Agria 159 (1999).
- [7] Amsing J.J., Bestrijding *Pratylenchus vulnus*: In steenwol wortelaaltjes makkelijk de baas, Vakblad voor de Bloemisterij 45/45 (1990) 38–39.
- [8] Amsing J.J., Runia W.Th., Disinfestation of nematode-infested recirculation water by ultra-violet radiation, Meded. Fac. Landbouwwet. Rijksuniv. Gent 60/3b (1995) 1087–1092.
- [9] Bakker P.A.H.M., The potential role of siderophores in disease suppression in closed hydroponic systems, International Congress of Plant Pathology, Edinburgh, Scotland, August 9–16, 1998, Abstract 3.8.4S.
- [10] Behrens V., Kemp J., Wohanka W., Wasser wiederverwenden trotz Pilz-Gefahr. Langsam-Sandfilter können das Ausbreiten von *Phytophthora cinnamomi* wirksam verhindern, Dtsch. Baumsch. 92 (1992) 310–312.
- [11] Benoit F., Ceustermans N., Low pressure UV disinfection also effective for NFT-lettuce. European Vegetable, R. D. centre, Sint-Katelinje-Waver, Belgium, 1993, 9 p.
- [12] Berkelmann B., Charakterisierung der Bakterienflora und des antagonistischen Potentials in der zirkulierenden Nährlösung einer Tomatenkultur (*Lycopersicon esculentum* MILL.) in Steinwolle, Geisenheimer Ber. 10 (1992) 119.
- [13] Berkelmann B., Wohanka W., Krczal G., Transmission of *Pelargonium* Flower Break Virus (PFBV) by recirculating nutrient solutions with and without slow sand filtration, Acta Hort. 382 (1995) 256–262.
- [14] Bernard J., Water treatment handbook, Lavoisier Publishing, Paris, 1991, pp. 208–217.
- [15] Blazka P., Prochazkova L., Mineralization of organic matter in water by U.V. radiation, Water Res. 17 (1983) 355–364.
- [16] Bochow H., Colej S., Fischer I., Alemayehu M., Modes of action in biocontrolling fungal seedling and root rot diseases by, IOBC wprs 19 (1996) 99–108.
- [17] Brand T., Beurteilung der Bakterienflora von Langsamfiltern als biologische Filtrationskomponente in geschlossenen Bewässerungssystemen des Gartenbaus. Geisenheimer Ber. 41 (2000) 112.
- [18] Brink D.R., Parks S., Update on slow sand/advanced biological filtration research, in: Graham N., Collins R. (Eds.), Advances in slow sand and alternative biological filtration, John Wiley & Sons, Chichester, New York, Brisbane, Toronto, Singapore, 1996, pp. 11–18.
- [19] Brown I.F., Sisler H.D., Mechanisms of fungitoxic action of n-dodecylguanidine acetate, Phytopathology 50 (1960) 830–839.
- [20] Bull R.J., Gerba C., Rhodes Trussel R., Evaluation of the health risks associated with disinfection, Crit. Rev. Environ. Contr. 20 (1990) 77–113.
- [21] Buyanovsky G. J., Gale J., Degani N., Ultra-violet radiation for the inactivation of microorganisms in hydroponics, Plant and Soil 60 (1981) 131–136.
- [22] Chen C., Bélanger R.R., Benhamou N., Paulitz T.C., Induced systemic resistance (ISR) by *Pseudomonas* spp. Impairs pre- and post-infection development of *Pythium aphanidermatum* on cucumber roots, Eur. J. Plant Pathol. 104 (1998) 877–886.
- [23] Chen C., Bélanger R.R., Benhamou N., Paulitz T.C., Role of salicylic acid in systemic resistance induced by *Pseudomonas* spp. against *Pythium aphanidermatum* in cucumber roots, Eur. J. Plant Pathol. 105 (1999) 477–486.
- [24] Coosemans J., Control of algae in hydroponic systems, Acta Hort. 382 (1995) 263–268.
- [25] Darling D.D., A mobile filtration plant to eliminate fungal spores in irrigation water for plant propagation, Aust. For. Res. 7 (1977) 273–274.
- [26] Date S., Hataya T., Namiki T., Effects of nutrient and environmental pre-treatments on the occurrence of root injury of lettuce caused by chloramine, Acta Hort. 481 (1999) 553–559.
- [27] Daughtrey M.L., Schippers P.A., Root death and associated problems, Acta Hort. 98 (1980) 283–289.
- [28] Duiff B.J., Pouhair D., Olivain C., Alabouvette C., Lemanceau P., Implication of systemic induced resistance in the suppression of *Fusarium* wilt of tomato by *Pseudomonas fluorescens* WCS417r and by nonpathogenic *Fusarium oxysporum* Fo47, Eur. J. Plant Pathol. 104 (1998) 903–910.
- [29] Ehret D.L., Bogdanoff C., Utkhede R., Lévesque A., Menzies J.G., Ng K., Portree J., Disease control with slow filtration for greenhouse crops grown in recirculation, Pacific Agri-Food Research Centre Technical Report 155, 1999, 37 p.
- [30] Ellis K.V., Slow sand filtration, Chemical Rubber Company, Crit. Rev. Environ. Contr. 15 (1985) 315–354.

- [31] Ewart J.M., Chrimes J.R., Effects of chlorine and ultraviolet light in disease control in NFT, *Acta Hort.* 98 (1980) 317–323.
- [32] Fravel L.D., Larkin R.P., Biocontrol of *Fusarium* wilt of hydroponically-grown basil (*Fusarium oxysporum* f. sp. *basilici*) using *F. oxysporum* CS-20, *Phytopathology* 89 (1999) S26.
- [33] Friedel S., Wohanka W., Molitor H.-D., Erica: *Phytophthora* in Fließbrinnen bekämpfen - Langsandsfilter eine wirksame Methode, *Gärtnerbörse + Gartenwelt* 91 (1991) 69–72.
- [34] Fuchs J.G., Moëne-Loccoz & Défago G., Non-pathogenic *Fusarium oxysporum* Fo47 induces resistance to *Fusarium* wilt in tomato, *Plant Dis.* 81 (1997) 492–496.
- [35] Gelzhaeuser P., Holm K., Jung K.D., Martiny H., Steuer W., Desinfektion von Trinkwasser durch UV-Bestrahlung, second edition, Expert-Verlag, Ehningen, 1989, 128 p.
- [36] Gertsson U.E., Hansson I., Waechter-Kristensen B., Lundquist S., Svedelius G., Weich R., Tomato grown in circulating nutrient solution using rockwool and as hydroponics, *Acta Hort.* 361 (1994) 237–244.
- [37] Graham N., Collins R. (Eds.), *Advances in slow sand and alternative biological filtration*, John Wiley and Sons, New York, 1996.
- [38] Guerra-Santos L., Kämpeli O., Fiechter A., *Pseudomonas aeruginosa* biosurfactant production in continuous culture with glucose as carbon source, *Appl. Environ. Microbiol.* 48 (1984) 301–305.
- [39] Hislop E.C., Clifford D.R., Eradication of apple powdery mildew (*Podosphaera leucotricha*) with dormant season sprays of surface-active agents, *Ann. Appl. Biol.* 8a (1976) 557–568.
- [40] Hommel R.K., Ratledge C., Biosynthetic mechanisms of low molecular weight surfactants and their precursor molecules, *Surfactant Ser.* 48 (1993) 3–63.
- [41] Huisman L., Wood W.E., *Slow sand filtration*, World Health Organization, Geneva, 1974.
- [42] Hultberg M., Khalil S., Alsanian B., Sundin P., The influence of introduced *Pseudomonas* isolates on the rhizobacterial community in biocontrol of *Pythium ultimum* on hydroponically grown tomato, in: Hultberg M. (Ed.), *Seed bacterization with Pseudomonas fluorescens and interactions with Pythium ultimum on tomato in soilless systems*, *Acta Univ. Agric. Sueciae. Agaria* 159 (1999).
- [43] Hultberg M., Alsanian B., Sundin P., Suppression of damping-off in tomato seedlings; in vivo and in vitro interactions between *Pseudomonas fluorescens* 5.014 and 5-2/4 and *Pythium ultimum*, *Biol. Control* (2000) in press.
- [44] Inbar J., Chet I., Evidence that chitinase produced by *Aeromonas caviae* is involved in the biological control of soil-borne plant pathogens by this bacterium, *Soil Biol. Biochem.* 23 (1991) 973–978.
- [45] Jamart G., Kamoen O., Bakonyi J., UV disinfection of recirculating nutrient solution in closed horticulture systems, *Meded. Fac. Landbouwkunden Toegep. Biol. Wet. Univ. Gent (Belgium)* 59 (1994) 1071–1078.
- [46] Jenkins S.F., Averre C.W., Root diseases of vegetables in hydroponic culture systems, *Plant Dis.* 67 (1983) 968–970.
- [47] Lemanceau P., Alabouvette C., Biological control of *fusarium* diseases by fluorescent *Pseudomonas* and non-pathogenic *Fusarium*, *Crop Prot.* 10 (1991) 279–286.
- [48] Lemanceau P., Bakker P.A.H.M., de Kogel W.J., Alabouvette C., Schippers B., Effect of pseudobactin 358 production by *Pseudomonas putida* WCS358 on suppression of *Fusarium* wilt of carnations by nonpathogenic *Fusarium oxysporum* Fo47, *Appl. Environ. Microbiol.* 58 (1992) 2978–2982.
- [49] Lemanceau P., Bakker P.A.H.M., de Kogel W.J., Alabouvette C., Schippers B., Antagonistic effect of nonpathogenic Fo47, and pseudobactin 358 upon pathogenic *Fusarium oxysporum* f. sp. *dianthi*, *Appl. Environ. Microbiol.* 59 (1993) 74–82.
- [50] Lundquist S., Svedelius G., Studie av svampar i tomatododling med cirkulerande näringslösning, *Växtskyddsnotiser* 55 (1991) 14–23.
- [51] McPherson G.M., Root diseases in hydroponics – their control by disinfection and evidence for suppression in closed systems, *Int. Congr. Plant Pathology*, Edinburgh, Scotland, August 9–16, 1998, Abstract 3.8.1S.
- [52] McPherson G.M., Harriman M.R., Patisson D., The potential for spread of root diseases in recirculating hydroponic systems and their control with disinfection, *Meded. Fac. Landbouww. Univ. Gent* 60/2b (1995) 371–379.
- [53] Mebalds M., Hepworth G., Van der Linden A., Beardsell D., Disinfestation of plant pathogens in recycled water using UV radiation and chlorine dioxide, in: *Recycling water in the Australian nursery and flower industries: Managing water quality and pathogen disinfestation*, Final report for HRDC project No. NY320, 1995, pp. 101–127.
- [54] Menzies J.G., Ehret D.L., Stan S., Effect of inoculum density of *Pythium aphanidermatum* on the growth and yield of cucumber plants grown in recirculating nutrient film culture, *Can. J. Plant Pathol.* 18 (1996) 50–54.
- [55] Moens M., Hendrickx G., Drainwater filtration for the control of nematodes in hydroponic systems, *Crop Prot.* 11 (1992) 69–73.
- [56] Moulin F., Lemanceau P., Alabouvette C., Suppression of *Pythium* root rot of cucumber by a fluorescent pseudomonad is related to reduced root colonization by *Pythium aphanidermatum*, *J. Phytopathol.* 144 (1996) 125–129.
- [57] M'Piga P., Bélanger R.R., Paulitz T.C., Benhamou N., Increased resistance to *Fusarium oxysporum* f. sp. *radicislycopersici* in tomato plants treated with the endophytic bacterium *Pseudomonas fluorescens* strain 63-28, *Physiol. Mol. Plant Pathol.* 50 (1997) 301–320.
- [58] Neilands J.B., Microbial iron compounds, *Annu. Rev. Biochem.* 50 (1981) 715–731.
- [59] Ongena M., Daayf F., Jacques P., Thonart P., Benhamou N., Paulitz T.C., Cornélis P., Koedam N., Bélanger R.R., Protection of cucumber against *Pythium* root rot by



fluorescent pseudomonads: predominant role of induced resistance over siderophore and antibiosis, *Plant Pathol.* 48 (1999) 66–76.

[60] Paludan N., Spread of viruses by recirculated nutrient solutions in soilless cultures, *Tidsskr. Planteavl.* 89 (1985) 467–474.

[61] Paulitz T.C., Biological control of root pathogens in soilless and hydroponic systems, *HortScience* 32 (1997) 193–196.

[62] Paulitz T.C., Loper J.E., Lack of a role for fluorescent siderophore production in the biological control of *Pythium* damping-off of cucumber by a strain of *Pseudomonas putida*, *Phytopathology* 81 (1991) 930–935.

[63] Postma J., Mechanisms in disease suppression in soilless cultures, *IOBC wprs* 19 (1996) 85–94.

[64] Postma J., Luttikholt A.J.G., Nonpathogenic *Fusarium* isolates from carnation suppressing wilt caused by *F. oxysporum* f. sp. *dianthi*, *Meded. Fac. Landbouwwet. Univ. Gent* 61/2b (1996) 499–505.

[65] Postma J., Luttikholt A.J.G., Colonization of carnation stems by a nonpathogenic isolate of *Fusarium oxysporum* and its effect on *Fusarium oxysporum* f.sp. *dianthi*, *Can. J. Bot.* 74 (1996) 1841–1851.

[66] Postma J., Rattink H., Biological control of *Fusarium* wilt of carnation with nonpathogenic *Fusarium* isolates, *Meded. Fac. Landbouwwet. Rijksuniv. Gent* 56/2a (1991) 179–183.

[67] Postma J., Rattink H., Biological control of *Fusarium* wilt of carnation with a nonpathogenic isolate of *Fusarium oxysporum*, *Can. J. Bot.* 70 (1992) 1199–1205.

[68] Postma J., Willemsen-de Klein M.J.E.I.M., Hoogland E.E., Biocontrol of *Pythium aphanidermatum* in closed culture systems, *IOBC wprs* 19 (1996) 42–46.

[69] Postma J., van Os E.A., Kritzman G., Prevention of root diseases in closed soilless growing systems by microbial optimization, *Meded. Fac. Landbouwkund. Toegep Biol. Wet. Univ. Gent* 64/3b (1999) 431–440.

[70] Postma J., Willemsen-de Klein M.J.E.I.M., van Elsas D.J., Effect of the indigenous microflora in the development of root and crown rot caused by *Pythium aphanidermatum* in cucumber grown on rockwool, *Phytopathology* 90 (2000) 125–133.

[71] Preuss G., Schwarz D., Elimination von Mikroorganismen und Parasiten bei der Langsamfiltration. *Berichte aus dem Rheinisch-Westfaelischen Institut für Wasserchemie und Wassertechnologie, Band 14* (1995) 65–89.

[72] Rankin L., Paulitz T.C., Evaluation of rhizosphere bacteria for biological control of *Pythium* root rot of greenhouse cucumbers in hydroponic culture, *Plant Dis.* 78 (1994) 447–451.

[73] Rattink H., Epidemiology of *Fusarium* crown and root rot in artificial substrate systems, *Meded. Fac. Landbouwwet. Rijksuniv. Gent* 56/2b (1991) 423–430.

[74] Rosenberg E., Microbial surfactants, *CRC Crit. Rev. Biotechnol.* 3 (1986) 109–132.

[75] Runia W.T., Elimination of plant pathogens in drainwater from soilless cultures, *ISOSC Proc. 7th Int. Congress on Soilless Culture* (1988) 429–445.

[76] Runia W.T., Disinfection of recirculation water from closed cultivation systems with ozone, *Acta Hort.* 361 (1994) 388–396.

[77] Runia W.T., Disinfection of recirculation water from closed cultivation systems with iodine, *Meded. Fac. Landbouwwet. Univ. Gent* 59/3a (1994) 1065–1070.

[78] Runia W.T., Elimination of root-infecting pathogens in recirculation water from closed cultivation systems by ultra-violet radiation, *Acta Hort.* 361 (1994) 361–369.

[79] Runia W.T., A review of possibilities for disinfection of recirculation water from soilless cultures, *Acta Hort.* 382 (1995) 221–229.

[80] Runia W.Th., Lavafiltratie geschikt tegen phytophthora en pythium, *Vakblad voor de Bloemisterij* 51 (1996) 52–53.

[81] Runia W.T., van Os E.A., Bollen G.J., Disinfection of drainwater from soilless cultures by heat treatment, *Neth. J. Agric. Sci.* 36 (1988) 231–238.

[82] Runia W.Th., Michielsen J.M.G.P., van Kuik A.J., van Os E.A., Elimination of root-infecting pathogens in recirculation water by slow sand filtration, *Proc. 9th Int. Congr. on Soilless Culture, St. Helier, Jersey, 1996*, pp. 395–407.

[83] Schwartzkopf S.H., Dudzinski D., Minners R.S., The effect of nutrient solution sterilization on the growth and yield of hydroponically grown lettuce, *HortScience* 22 (1987) 873–874.

[84] Stanghellini M.E., Miller R.M., Biosurfactants – their identity and potential efficacy in the biological control of zoospore pathogens, *Plant Dis.* 81 (1997) 4–12.

[85] Stanghellini M.E., Tomlinson A., Inhibitory and lytic effects of nonionic surfactant on various asexual stages in the life cycle of *Pythium* and *Phytophthora* species, *Phytopathology* 77 (1987) 112–114.

[86] Stanghellini M.E., Stowell L.J., Bates M.L., Control of root rot of spinach caused by *Pythium aphanidermatum* in a recirculating hydroponic system by ultraviolet irradiation, *Plant Dis.* 68 (1984) 1075–1076.

[87] Stanghellini M.E., Rasmussen S.L., Kim D.H., Rorabaugh P.A., Efficacy of nonionic surfactants in the control of zoospore spread of *pythium aphanidermatum* in a recirculating hydroponic system, *Plant Dis.* 80 (1996) 422–428.

[88] Stanghellini M.E., Kim D.H., Rasmussen S.L., Rorabaugh P.A., Control of root rot of peppers caused by *Phytophthora capsici* with a nonionic surfactant, *Plant Dis.* 80 (1996) 1113–1116.

[89] Stanton J.M., O'Donnell W.E., Hatching, motility and infectivity of root-knot nematode (*Meloidogyne javanica*) following exposure to sodium hypochlorite, *Aust. J. Exp. Agr.* 34 (1994) 105–108.

[90] Teoh T.S., Chuo S.K., The possible use of sodium hypochloride for bacterial wilt control in the hydroponic cultivation of sweet pepper, *Singapore J. Prim. Ind.* 6 (1978) 102–112.

- [91] Tomlinson J.A., Faithfull E.M., Effects of fungicides and surfactants on the zoospores of *Olpidium brassicae*, Ann. Appl. Biol. 93 (1979) 13–19.
- [92] Utkhede R.S., Koch C.A., Menzies J.M., Rhizobacterial growth and yield promotion of cucumber plants inoculated with *Pythium aphanidermatum*, Can. J. Plant Pathol. 21 (1999) 265–271.
- [93] Vanachter A., Development of *Olpidium* and *Pythium* in the nutrient solutions of NFT grown lettuce, and possible control methods, Acta Hort. 382 (1995) 187–196.
- [94] Vanachter A., Thys L., Van Wambeke E., Van Assche C., Possible use of ozone for disinfestation of plant nutrient solutions, Acta Hort. 221 (1998) 295–300.
- [95] Van Dorst H.J.M., Surface water as source in the spread of cucumber green mottle mosaic virus, Neth. J. Agric. Sci. 36 (1988) 291–300.
- [96] Van Kuik A.J., Eliminating *Phytophthora cinnamomi* in a recirculated irrigation system by slow sand filtration, Meded. Fac. Landbouwwet. Rijksuniv. Gent 59/3a (1994) 1059–1063.
- [97] Van Loon L.C., Disease-suppressive actions of *Pseudomonas* bacteria: induced resistance, IOBC wprs 19 (1996) 53–61.
- [98] Van Os E.A., Nutrient cleaning? the Dutch approach, Practical Hydroponics and Greenhouses 7/9 (1999) 67–70.
- [99] Van Os E.A., Bruins M.A., van Buuren J., van der Veer D.J., Willers H., Physical and chemical measurements in slow sand filters to disinfect recirculating nutrient solutions, Proc. 9th Int. Congr. on Soilless Culture, St. Helier, Jersey, 1996, pp. 313–327.
- [100] Van Os E.A., Amsing J.J., Runia W., Aaltjes ontsnappen aan langzaam zandfilter, Vakblad voor de Bloemisterij 52 (1997) 44–45.
- [101] Van Os E.A., Amsing J.J., van Kuik A.J., Willers H., Slow sand filtration: A potential method for the elimination of pathogens and nematodes in recirculating nutrient solutions from glasshouse-grown crops, Acta Hort. 481 (1999) 519–526.
- [102] Van Os E.A., Wohanka W., Bruins M., Seidel R., Slow filtration: A technique to minimise the risks of spreading root-infecting pathogens in closed hydroponic systems, Proceedings of the Intern. Symp. on Protected Cultivation in Mild Winter Climates: Current trends for sustainable techniques, Cartagena, Spain, 2000, in press.
- [103] Van Peer R., van Kuik A.J., Rattink H., Schippers B., Control of *Fusarium* wilt in carnation grown on rockwool by *Pseudomonas* sp. strain WCS417r and by Fe-EDDHA, Neth. J. Plant Pathol. 96 (1990) 119–132.
- [104] Visscher J.T., Parmasivan R., Raman A., Heijnen H.A., Slow sand filtration for community water supply: Planning, design, construction, operation and maintenance, International Reference Centre for Community Water Supply and Sanitation, The Hague, 1987.
- [105] Yamamoto H., Terada T., Naganawa T., Tatsuyama K., Disinfection effect of ozonation on water infested with several root-infecting pathogens, Ann. Phytopathol. Soc. Jap. 56 (1990) 250–251.
- [106] Waechter-Kristensen B., Berkelmann-Loehnertz B., Sundin P., Wohanka W., Gertsson U.E., Hultberg M., Khalil S., Jensen P., Management of microbial factors in the rhizosphere and nutrient solution of hydroponically grown tomato, Acta Hort. 450 (1997) 335–340.
- [107] Wijchman G., Langzame zandfiltratie wint snel terrein, Vakblad voor de Bloemisterij 49 (1994) 26–29.
- [108] Wohanka W., Geschlossene Kulturverfahren im Zierpflanzenbau aus der Sicht des Pflanzenschutzes, in: Düngen im Zierpflanzenbau, TASPO-praxis, No. 16, 1989, pp. 80–85.
- [109] Wohanka W., Geschlossene Kulturverfahren im Zierpflanzenbau aus Sicht des Pflanzenschutzes, Taspo Praxis 18, Geschlossene Kulturverfahren Zierpflanzenbau, 1990, pp. 57–61.
- [110] Wohanka W., Slow Sand Filtration and UV radiation: Low cost techniques for disinfection of recirculating nutrient solutions or surface water, ISOSC Proc. 8th Int. Congr. on Soilless Culture, Rustenburg, 1992, pp. 497–511.
- [111] Wohanka W., Disinfection of recirculating nutrient solution by slow sand filtration, Acta Hort. 382 (1995) 246–255.
- [112] Wohanka W., Preventing disease in closed irrigation systems, Flower TECH 3 (2000) 8–11.
- [113] Wohanka W., Helle M., Suitability of various filter media for slow filtration, Proc. 9th Int. Congr. on Soilless Culture, St. Helier, Jersey, 1996, pp. 551–557.
- [114] Wohanka W., Luedtke H., Ahlers H., Luebke M., Optimization of slow filtration as a means for disinfecting nutrient solutions, Acta Hort. 481 (1999) 539–544.