Variables Limiting Efficacy of Slow Filters Integrated into Closed Hydroponic Growing Systems

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Abstract

For sustainable production of greenhouse vegetables and ornamentals, losses of water and nutrients must be avoided.

Closed hydroponic systems are highly efficient in their use of water and nutrients, produce high yields and minimise the needs for soil sterilization. However, they carry a risk of dispersal of root-borne pathogens, mainly zoosporic oomycetes, which can cause major crop losses. To prevent this, nutrient solution is of disinfested before reuse by pasteurisation, UV-light treatment, oxidation with ozone or peroxides, or filtration.

Slow filtration is a low-cost technique with high efficacy against oomycetes and bacterial pathogens. However, tools for easy monitoring of slow filter efficacy are currently unavailable. Abiotic and biotic factors are critical for slow filter performance. Enzyme activity in the filter skin is a key factor and is a possible predictor of filter efficacy, explaining 50% of the filter efficacy. A number of state variables, e.g. polysaccharides, total organic carbon and oxygen content, were examined in this study in attempts to explain the remaining 50% of efficacy. Abiotic and biotic state variables affecting filter efficacy were monitored for filters subjected to sub-optimal factors such as high electric conductivity or at massive pathogen bombardment. Supporting studies were performed in commercial greenhouses.

The main conclusions were that electric conductivity of the nutrient solution affects the efficacy of slow filters, whereas bombardment of the filter skin with pathogen mycelia had minor importance on filter function. The state variables total polysaccharides, biofilm polysaccharides, total organic carbon and oxygen content did not support prediction of filter efficacy. Interactions were found between enzyme activity and the microbial colonisation of the filter skin.

Keywords: Electric conductivity, enzyme activity, *Fusarium oxysporum* f.sp. *cyclaminis,* hydroponics, polysaccharides, *Pythium aphanidermatum*, slow filter

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Utan tvivel är man inte klok
Tage Danielsson

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List of Publications

This thesis is based on the work contained in the following papers, which are referred to in the text by their Roman numeral:

I Furtner, B., Bergstrand, K.-J., Brand, T., Jung, V. & Alsanius, B.W. (2007) Abiotic and Biotic Factors in Slow Filters Integrated to Closed Hydroponic Systems. *European Journal of Horticultural Sciences* 72 (3) 104-112.

II Alsanius, B.W., Bergstrand K.-J., Furtner B., Jung V. & Brand T. Electrical conductivity as a limiting factor for efficacy of horticultural slow filters. *Submitted manuscript.*

III Bergstrand, K.-J., Khalil, S., Hultberg, M. & Alsanius, B.W. Cross response to dual pathogen bombardment in closed hydroponic growing systems. *Manuscript.*

Abbreviations

AOT	Advanced oxidation technology
BOD	Biological oxygen demand
bPs	Biofilm polysaccharides
cfu	Colony forming units
COD	Chemical oxygen demand
DFT	Deep flow technique
DIT	Drip irrigation technique
DOC	Dissolved organic carbon
EC	Electric conductivity
f.sp.	*forma specialis*
KB	King´s agar B
MA	Malt extract agar
mU	MilliUnit
NFT	Nutrient film technique
PDA	Potato dextrose agar
SAT	Static aerated culture
SF	Slow filtration
TOC	Total organic content
tPs	Total polysaccharides

1 Introduction

Greenhouse systems provide the potential to produce vegetables and ornamental plants of high quality and high yields in areas where outdoor production would have been impossible or would have resulted in poor-quality products and low yield (Bjelland, 1972). Greenhouse production mainly employs some type of hydroponic system, where plants are kept in a growing medium and fed with nutrient solutions containing all the minerals essential for growth. The hydroponic system can be either solid or liquid, i.e. with or without a growing medium (Göhler & Molitor, 2002) and can be open or closed. In open hydroponic systems, excessive nutrient solution is drained into the surrounding environment, leading to economic losses and to eutrophication of water courses and groundwater. It has been estimated that the average Swedish greenhouse tomato production unit with an open hydroponic system loses 850 kg nitrogen, 80 kg phosphate and 850 kg potassium annually by releasing spent nutrient solution (Hansson, 2003). In closed hydroponic systems, these nutrients are recycled, and approx. 20-25% of water is saved (Hansson, 2003). However, recycling of nutrient solution can mediate dispersal of root-borne pathogens (Stanghellini & Rasmussen, 1994), while closed hydroponic systems also demand high raw water quality and accuracy in nutrient supply (Göhler & Molitor, 2002). Closed hydroponic systems are recommended by authorities and extension officers in Europe, mainly for environmental reasons but also for strictly economical reasons for the grower.

According to the EU Water Framework Directive (2000/60/EC) all release of pollutants that can harm the marine environment is prohibited. The Swedish Environmental Protection Law (*miljöbalken,* SFS 1998-808) gives the government the right to restrict or prohibit disposal of wastewater into drains, water courses or groundwater (Sect. 3, Ch. 9, 4 §). It also states (Sect. 1, Ch. 2, 5 §) that all businesses are obliged to recycle when possible

and to be as efficient as possible in the use of any resource, and to use the best technology available for any application. These statements could be interpreted as an obligation to use closed systems in greenhouse crop production (Naturvårdsverket, 2005).

1.1 Hydroponic growing systems

A number of different techniques are available for hydroponic systems. Drip irrigation technique (DIT), nutrient film technique (NFT), static aerated culture (SAT), deep flow technique (DFT) and capillary uptake (CU) are commonly used methods (Göhler & Molitor, 2002).

In DIT systems plant roots are supported by a growing medium. Inorganic media such as mineral wool, polystyrene foam or volcanic material can be used, or organic media including peat, coconut fibre or wood chips. The growing medium is kept in a container and nutrient solution is intermittently supplied individually to each plant by a small capillary hose. Excessive nutrient solution drains from the container. This system is regularly used in commercial production of all common greenhouse crops such as tomatoes, cucumbers and sweet peppers, and cut flowers such as roses and gerbera (Ehret *et al.*, 2001).

In NFT systems (Burrage, 1992; Göhler & Molitor, 2002), plants are kept in gutters with a constant flow of nutrient solution. The roots are forming a compact mat that is permanently soaked in this solution. The nutrient solution is kept aerated through the constant movement. With increasing plant age, the decomposition of roots at the bottom of the root mats competes for oxygen, leading to partial oxygen depletion of the root mat. As NFT systems require large amounts of water, commercial feasibility demands that this technology is combined recirculation of the nutrient solution. NFT systems are very well adapted for experimental uses, but are less common in commercial production in Scandinavia, partly because they are regarded as high risk systems since the spread of root-borne pathogens is enhanced (Wohanka, 1993). Disinfestation of the nutrient solution before reuse is therefore regarded as mandatory in NFT-systems (Runia, 1994b)

In the sub irrigation with capillary mat technique, plants are kept in containers with growing medium in gutters, where nutrient solution is

supplied intermittently. Water and nutrients are absorbed to the growing medium by capillary forces.
In this study, systems employing DIT, NFT and sub irrigation were studied.

1.2 Disinfestation of nutrient solutions

A number of water-borne plant pathogens are of significant interest in horticultural crops. They belong to different groups, i.e. oomycetes (*Pythium,* (Tu *et al.*, 1999), *Phytophthora,* (McPherson et al., 1995), *Olpidium, (Paludan, 1985))*; true fungi (*Fusarium oxysporum* (Runia & Amsing, 2001; Jarvis, 1988) and *Verticillium (Runia & Amsing, 2001)*); bacteria (*Clavibacter michiganensis* (Huang & Tu, 2001), *Pseudomonas corrugata* (Scarlett et al., 1978) and *Ralstonia* (Alsanius & Bergstrand, 2007); and viruses (tomato mosaic virus (Broadbent, 1976), cucumber green mosaic virus (van Dorst, 1988), or pepino mosaic virus (Fakhro *et al.*, 2005). Nematodes, for example *Prathylenchus* and *Meloidogyne* can also cause problems (Alsanius & Brand, 2000).
These pathogens can cause major economic losses (Ehret *et al.*, 2001) and are the reason why which disinfestation of the nutrient solution is crucial in closed hydroponic systems.

A number of different types of disinfestation techniques have been developed and are commercially available. UV-radiation, slow filtration, ozone treatment, pasteurisation, photocatalytic reactions, membrane filtration, chemical treatments such as peroxides, tensides or metals are methods used to some extent for horticultural purpose (Ohtani *et al.*, 2000; Ehret *et al.*, 2001; Ikeda *et al.*, 2002; Alsanius & Bergstrand, 2007). This study focuses on slow filtration. Some form of slow filtration has been used for potable water production since ancient times, and was adopted for horticultural use during the 1980's by Wohanka and is described by Wohanka (1995). A filter bed of sand or mineral wool with a depth of 40-100 cm and a drainage layer in the bottom is constructed. The filter tank can be made out of steel, concrete or plastic. The nutrient solution passes through the filter bed driven by gravity at a speed of 100-300 L m^{-2} h^{-1}. A filter skin ("*Schmutzdecke*") consisting of organic materials and microorganisms forms on the filter surface (Campos *et al.*, 2002). Physical and biological processes occurring in the filter, mainly on the filter skin, eliminate fungal spores and bacteria. Filter efficiency of >99% have been reported for *Fusarium oxysporum* and *Pythium aphanidermatum* (Déniel *et al.*,

2004; Wohanka *et al.*, 1999). However, efficiency rates are reported to be lower for viruses and nematodes (van Os *et al.*, 1999; Berkelmann *et al.*, 1995). Slow filtration does not change the constitution of the nutrient solution in any significant way, except for consuming the dissolved oxygen (Wohanka, 1995) and immobilising of manganese during anoxic conditions in the filter column (Li *et al.*, 2005). Slow filtration provides an efficient and low-cost solution for disinfestation and is especially well-suited for smaller systems.

Treatment with ultraviolet (UV) light has been used commercially for a number of years. Like slow filtration, the technology was first used for municipal drinking water production, but has already been used in hydroponic systems for decades. Ultraviolet light (100-400 nm) is transmitted through the nutrient solution, scattering the DNA of the pathogens and thus inactivating them. Assuming that radiation dose is sufficient, the method is effective against fungal spores, bacterial cells and viruses (Buyanovsky & Gale, 1981; Zhang & Tu, 2000). Runia (1994b) found that *Fusarium* can be completely eradicated from nutrient solution subjected to UV-light at a dose of 70 mJ cm^{-2}. However, clarity of the nutrient solution is crucial and therefore suspended solids >80 μm need to be removed by pre-filtering. UV treatment has the advantages of being compact and having relatively low energy consumption.

Pasteurisation is considered to be the most efficient disinfestation method for most pathogens. Viruses, bacteria and fungal spores are eliminated to a very high extent (Runia & Amsing, 2001; Göhler & Molitor, 2002).
The nutrient solution is heated by an oil or gas burner to 95 °C for at least 30 seconds and then passes through a heat-exchanger where heat is transmitted to the cool incoming nutrient solution. To prevent the heat-exchanger from becoming clogged with precipitate, the pH of the nutrient solution is lowered to 4.5 before passage through the unit.
This technique is energy-consuming, requiring around 8 kWh m^{-3} of treated nutrient solution (A. Nilsson, pers. comm. 2009). The current commercial equipments for flame heating rely on fossil fuels, although the carbon dioxide produced can be used for carbon dioxide enrichment of the greenhouse air (Runia & Amsing, 2001). The method is also expensive and maintenance-intensive.

Different types of oxidation techniques are available: ozone-treatment, photocatalytic treatment using advanced oxidation technology (AOT) and chemical treatment using stabilised hydrogen peroxide or sodium hypochlorite. In ozone treatment, ozone (O_3) is used to oxidize organic

matter in the nutrient solution, thereby eliminating pathogens of various kinds. The oxidation methods have advantages such as low space requirements and high efficiency (Alsanius & Brand, 2000). Ozone treatment has been proven to be effective against *Verticillium*, tomato mosaic virus and cucumber green mottle mosaic virus (Runia, 1994a). Equipments for photocatalytic treatment are available on the market, but little experience has been gained to date about their efficiency against pathogens of interest for horticultural crops (Alsanius & Bergstrand, 2007).

1.3 Objectives

Slow filters represent a cheap and efficient way of reducing the dispersal of root-borne pathogens by the circulating nutrient solution. However, growers consider the lack of tools for assessment of filter efficacy during operation to be a drawback. Brand (2000) ascribed 20 % of slow filter efficacy to biological properties, while other studies have described the impact of enzyme activity and Biofilm polysaccharides on filter efficacy (Brand & Alsanius, 2004a; Furtner *et al.*, 2007). In the framework of the present thesis, we aimed to define additional state variables explaining variations in filter efficacy. The starting hypotheses were that:

I: Candidate state variables for prediction of filter efficacy may be found among physical, chemical, biochemical and biological properties of the nutrient solution and/or filterskin.

II: Exposure of slow filters to suboptimal conditions decreases filter efficacy.

III: Electrical conductivity limits the efficacy of slow filters connected to closed greenhouse systems with respect to their disinfecting properties and their reduction of dissolved organic compounds.

IV: Massive bombardment of slow filters with plant pathogens decreases their efficacy

2 Materials and methods

2.1 Experiments

The experiments were conducted in an experimental greenhouse at SLU Alnarp, Sweden and at two commercial sites in southern Sweden. For Paper I, two treatments were employed, addition or no addition of fungal cell wall preparations to the filter skin. For Paper II, three treatments were used, electrical conductivity of 2, 3 and 5 mS cm^{-1}, with the aim of this gaining more knowledge about factors limiting the efficacy of slow filtration. For Paper III, two treatments were employed, addition or no addition of mycelia from *Pythium aphanidermatum* to the filter skin of the slow filter. The overall objective of these studies was to identify factors might affect the efficacy of a slow filter, and to test the limits for of slow filter performance during suboptimal conditions.

The following section gives a brief overview of the methods used for the different experiments. For more specific information, see the Papers I to III.

2.2 Experimental setup

2.2.1 Commercial systems

(Paper I)
Slow filters in two commercial greenhouse market gardens were examined. The first system produced ornamental pot plants in a capillary-irrigation system on a production area of 1 ha. The recirculating nutrient solution was disinfested using a slow filter with mineral wool (filter depth 1 m, filter surface 5.9 m^2). The second commercial greenhouse produced tomatoes on an area of 0.4 ha. Plants were grown using DIT, with plastic containers

filled with pumice. Excess nutrient solution was collected in open gutters and was filtered through a slow filter (depth of filter bed 1 m, surface area 10 m^2, flow rate 100 m^{-2} h^{-1}) with mineral wool as the filter medium. The filtered solution was mixed with fresh water, and EC and pH were adjusted automatically by addition of stock solutions to crop-specific values before the solution was supplied to the crop.

2.2.2 Greenhouse experiments

(Papers I-III)

Greenhouse experiments were conducted in a 90 m^2 experimental greenhouse chamber at SLU Alnarp. Plants (tomato, *Lycopersicon esculentum* cv. Aromata) were grown in NFT systems (Figure 1). Each system was equipped with two slow filters consisting of a 200 l plastic container filled with rockwool (Granulaat, Grodan BV., the Netherlands) at a density of 136 kg m^{-2} (Papers I, II) or 190 kg m^{-2} (Paper III). Filter surface was 0.19 m^2 and the filter depth was 0.4 m + 0.1 m drainage layer. Nutrient solution was filtered through the filter bed at a flow rate of 300 L h^{-1} m^{-2}. Nutrient solution was prepared according to Sonneveld & Straver (1989) and contained KNO_3 8.01 mM, NH_4NO_3 0.6 mM, $MgSO_4$ 1.88 mM, $Ca(NO_3)_2$ 4.2 mM, Fe-EDTA 18.73 µM, $MnSO_4$ x $7H_2O$ 6.25 µM, $ZnSO_4$ x 7 H_2O 12.5 µM, H_3BO_3 31.25 µM, $CuCl_2$ x $2H_2O$ 0.94 µM, Na_2MoO_4 x $2H_2O$ 0.63 µM. EC was adjusted daily to 2.5±0.2 mS cm^{-1} and pH 5.8±0.1, respectively. In Paper II, slow filter efficacy was challenged by different EC-regimes: EC = 2, 3 or 5 mS cm^{-1}. The day/night temperature of the greenhouse was set to 19ºC/18ºC (Papers I, II) or 22ºC/21ºC (Paper III) day time/night time, respectively. Vents opened when temperature exceeded setpoints by 2ºC. Additional light (SON-T, 200 W^{-2}, 11 h per day) was given for the first eight weeks of the experiment (Papers I, III) or during the whole growing period (Paper II).

The upper layer of the filter was enriched weekly with preparations of fungal cell walls as described by Sivan & Chet (1989) (In paper I, only one of two treatments), modified according to Brand & Alsanius (2004a). Fungal cell wall preparations was added at a density of 9.2 m^{-2} (Papers I, II) or 10.5 g m^{-2} (Paper III). In brief, aliquots (20 mL) of yeast medium (per L. aqua dest. 10 g glucose (Fluka 49150), 5 g yeast extract (Merck 1.03753.0500), and 5 g Proteose peptone No 3 (Difco 0122-17-4)) were inoculated with conidial suspensions of *Fusarium oxysporum* f.sp. *cyclaminis*. After five days of incubation at 25ºC in the dark, mycelium was harvested and washed four

times in deionised water with intermediate resuspension and centrifugation. Mycelium was autoclaved, lyophilised and stored at 4°C until use.
Before addition to the filters, the pellet was resuspended in 0.5 L tap water using a high-frequency blender (Polytron, Kinematica GmbH, Switzerland). The flow through the filter was stopped and the suspension was added to the supernatant and allowed to settle for 20 min before flow through the filter was restarted.

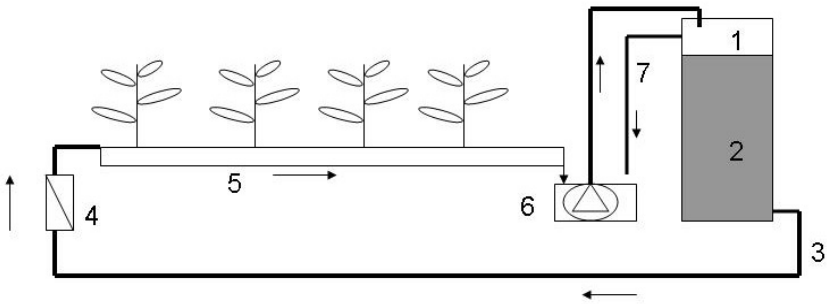

Figure 1. Schematic view of the system used for greenhouse experiments. 1) Water supernatant 2) Filter column 3) Outflow from filter 4) Flow meter 5) NFT-gutters with plants 6) Reservoir with plunger pump 7) Overflow.

2.3 Sample collection

(Papers I-III)
On each sampling occasion, samples were taken from the nutrient solution before (supernatant) and after (effluent) filtration and from the filter skin of the slow filters. In the experimental systems and in the commercial system with slow filtration, nutrient solution sample before filtration was taken from the supernatant of the filter. Samples from the filtered solution were taken directly at the outlet from the filter. Samples for TOC analysis were frozen at -20°C and stored until analysis.

Filter skin specimens were taken from the upper layer of the filter (0-0.5 cm, Figure 2) with a pair of tweezers and transferred to plastic petri dishes. After sampling, specimens were cooled immediately, transported to the laboratory, frozen at -80°C for 30 minutes and lyophilised for 48 hours. Lyophilised samples were stored at -20°C until analysis.

Figure 2. Cross-section of the upper layer of a slow filter consisting of compressed mineral wool granules. Filter skin samples were taken from the upper 5 mm of the filter column (indicated by arrow). Picture: Karl-Johan Bergstrand.

2.4 Analyses

2.4.1 Microbial assessment

(Papers I-III)

The microflora of the nutrient solution was investigated with respect to the general fungal and bacterial flora and fluorescent pseudomonads a using spread-plate technique. The following media were used for all systems:

For the general bacterial flora, R2A (Difco 218263) was prepared according to the manufacturer's instructions. Plates were inoculated with 100 µL of sample suspension, serially diluted from 10^{-1} to 10^{-4}, and were incubated at 25°C for three days.

For the general fungal flora, diluted malt extract agar (MA) was prepared according to manufacturers instructions (per L. aqua dest 10 g Malt extract, Difco 218630, 20 g Bacto agar, Difco 0140-01). Plates were inoculated with 100 µL of serially diluted suspension (10^{0} to 10^{-3}), and incubated 25°C for four days.

For fluorescent pseudomonades, King´s agar B was prepared according to King (King *et al.*, 1954). Plates were inoculated with 100 µL of diluted suspension (10^{0} to 10^{-3}) and were incubated at 25°C for 48 h. Number of

colonies was counted under ultraviolet light (366 nm, Blak Ray UVL-56, Ultra Violet products, Inc., Upland, U.S.A.).
The occurrence of *Pythium aphanidermatum* was analysed on potato dextrose agar (PDA, Difco 213400, prepared according to the manufacturer's instructions) supplemented with rifampicin (100 μg mL^{-1}, Duchefa, Haarlem, the Netherlands, Paper I) or rifampicin (100 μg mL-1) and kanamycin (30 μg mL^{-1}) Paper III). Plates were incubated at 35°C for 18 h.
Occurrence of *Fusarium oxysporum* was monitored using Komada medium (Komada, 1975) (Paper I). A 200 μL portion of sample suspension was spread on each plate and plates were incubated at 25°C for five days.

In the commercial systems (Paper I), filamentous actinomycetes were analysed using COA agar (Postma *et al.*, 1999). Plates were covered with a membrane filter (Optitran BA-S 83, Whatman GmbH, Dassel, Germany) and inoculated with 100 μL of suspension. After five days of incubation at 25°C, filters were removed. Incubation was resumed for five additional days before colonies were counted. For all analyses, duplicate samples with two parallels were analysed and plates containing 30-300 colonies were enumerated.

2.4.2 Enzyme activity

(Paper I-III)
The activity (mU) of five enzymes (protease, chitinase, cellulase, β-1,3-glucanase and xylanase) in the filter skin was determined using the colorimetric method described by Brand & Alsanius (2003). Lyophilised samples (0.02 g) from the filter skin were incubated with 0.5 mL of enzyme substrate (Blue Substrates, Göttingen, Germany CM-chitin-RBB, Gelatine-RBB, CM-curdlan-RBB, CM-cellulose-RBB, and CM-xylan-RBB for chitinase, protease, β-1,3-glucanase, cellulase and xylanase, respectively) in Eppendorf tubes at 37°C for 2 hours. After incubation, 150 μL of the enzyme substrate were transferred to a microtitre plate. For samples from the commercial systems, the Eppendorf tubes were centrifuged for 5 min using a desk centrifuge (Millipore, Billerica, U.S.A.) before the enzyme substrate was transferred, in order to prevent clogging of pipette tips with organic materials. The pH was lowered by adding 150 μL of sulphuric acid (2 M), causing degraded molecules of enzyme substrate to precipitate, whereas unaffected molecules remained in solution. After centrifugation (3250 x g, 20 min), supernatant solution was transferred to an additional microtitre plate and extinction of the solution was measured spectrophotometrically (Asys Hitec, Linz, Austria, software Digiwin) at 590 nm (chitinase) or 550 nm (cellulase, β-1,3-glucanase, protease, xylanase).

Non-incubated samples prepared as described above were used as controls. Enzyme activity was calculated as

$$\text{MilliUnit [mU]} = E_s - E_c \times 1000 \times t^{-1} \quad (1)$$

where E_s= extinction of the sample, E_c= extinction of the control and t= incubation time (min).

2.4.3 Polysaccharide content

(Paper III)

Total polysaccharide and biofilm polysaccharide content (mg g^{-1}) of the filter skin was analysed using the modified Dubois method (Dubois *et al.*, 1956; Furtner *et al.*, 2007). For total polysaccharide (tPs), lyophilised filter skin samples were incubated with 5% phenol solution and concentrated sulphuric acid at 25°C for 25 minutes. After centrifugation, supernatant solution was transferred to plastic cuvettes, and light absorbance was measured at 488 nm (Cary 50, Varian Inc. Palo Alto, U.S.A.). Content of tPs was calculated on the basis of a glucose standard curve.

Biofilm polysaccharide (bPs) content was analysed as described by (Furtner *et al.*, 2007). Lyophilised samples (0.05 g) were incubated with 12.5 mL of a solution of 0.23% formaldehyde and 0.85% NaCl at 4°C. After 1 hour, 5 mL of NaOH solution (1 M) were addedand incubation resumed for further three hours at 4°C. After centrifugation (20.000 x g, 20 min.), the solution was membrane filtered (Minisart, 0.2 μm, Sartorius, Göttingen, Germany). The bPs content in the filtered solution was determined as described for tPs.

2.4.4 DNA analysis

(Paper III)

DNA content (mg g^{-1}) of the filter skin was measured using photometrical method of Hoechst as described by Furtner *et al.* (2007). In brief, filter skin samples were subjected to the same extraction process as described for bPs analysis, except that wet samples (3.4 g) were used. Aliquots of 2 μL of the solution were transferred to a cuvette with 2 mL of DNA assay solution (90 mL aqua dest., 10 mL TNE buffer (1000 mL aqua dest, 12.11 g (trishydroxymethyl)aminomethane, 3.72 g EDTA $Na_2 \times 2H_2O$), and 10 μL of dye stock solution (10 mL aqua dest, 10 mg Hoechst dye H 33258).

DNA content was then measured photometrically (Hoefer DyNA Quant 200, Hoefer Scientific Instruments, San Francisco, USA) and expressed as mg g^{-1}.

2.5 Chemical/physical assessment

(Paper I-III)
Total Organic Carbon (TOC, mg L^{-1}) was measured using a test kit system (LCK 385, Hach-Lange AG, Düsseldorf, Germany). In brief, organic compounds in the sample suspension were oxidised. The carbon dioxide developed caused a colour reaction in a reagent, which was measured spectrophotometrically using a photometer (Xion 500, Hach-Lange AG, Düsseldorf, Germany).

Organic matter (OM) content of the filter skin (%) was determined at the end of the experiment. Pieces of the filter skin were carefully lifted using a spatula. Samples were incinerated (LMI AB, Helsingborg, Sweden) and organic matter content was calculated as:

$$OM\ (\%) = 1 - (Wt\ sample_{\text{after incineration}}) \times (Wt\ sample_{\text{before incineration}} \times 100)^{-1} \quad (2)$$

Chemical oxygen demand (mg L^{-1}) was determined (Papers I, II) using a test from Hach-Lange AG, Düsseldorf, Germany (LCK 314). Samples were added to test tubes with prepared oxidiser solutions. After incubation at 148°C for two hours, colorimetric analyses were performed using a photometer (Xion 500, Hach-Lange AG, Düsseldorf, Germany). In Paper I, dissolved organic carbon (DOC) was determined as described for TOC, except that the sample solution was membrane filtered (Filtropur S 0.2, Sarstedt, Nümbrecht, Germany; pore size 0.2 µm) before analysis. For Paper I, biological oxygen demand (BOD_5, mg L^{-1}) was determined using the test LCK 555 (Hach-Lange AG, Düsseldorf, Germany) and measured after five days of incubation.

Levels of dissolved oxygen (mg L^{-1}) in the nutrient solution before and after filtration were measured once a week (experimental systems) or in conjunction with sampling (commercial systems). Measurements were conducted using a portable O_2-meter (OxyGuard Handy Mk1, OxyGuard A/S, Birkeröd, Denmark). Oxygen consumption of the filters was calculated

(Papers I, II) by subtracting the oxygen content in the effluent from the content in the supernatant.
The temperature of the nutrient solution before filtration was logged every 30 minutes with a logger (HOBO H8, external sensor TMC6-H, Onset computer corp., Bourne, USA).

2.6 Efficacy tests

(Papers I-III)
Experimental systems were exposed to efficacy tests three times during each experiment. *Fusarium oxysporum* f.sp. *cyclaminis* was grown on PDA agar plates (PDA, Difco 213400, prepared according to the manufacturer's instructions) for two weeks. Plates were supplied with 10 mL of sterile NaCl solution (0.85% v/w). Fungal mycelia were rubbed off with a sterile spatula. Conidial suspension was filtered through cheese-cloth to a concentration of approximately 10^7 cfu mL^{-1}. The conidial suspension was introduced into the system to a final concentration of approximately 10^4 cfu mL^{-1}. Samples were taken before and after filtration every 30 minutes for the next 2 h (before filtration) or 4.5 h (after filtration). Samples were analysed using Komada medium as described above. The numbers of colonies were counted after five days of incubation at 25°C and efficacy was calculated as

$$\text{Efficacy (\%)} = 1 - (\text{cfu}_{\text{after filtration}}) \times (\text{cfu}_{\text{before filtration}})^{-1} \times 100 \quad (3)$$

2.7 Crop assessment

(Paper III)
In the experimental systems, crop development was monitored weekly. The following state variables were monitored: plant height (cm), leaf number, leaf length, stem diameter (mm), number of trusses, number of flowers/truss, harvest. Plant height was recorded through measurements of the apical growth. The position of the apex was marked on the plant support string, after seven days growth was measured. The numbers of fully developed leaves (under flowering truss) were counted. Stem diameter was measured at two opposing positions of the stem (where apex position was seven days before) using a calliper. The numbers of flowering or fruit-bearing trusses and the number of buds/flowers on each newly developed truss were counted.

2.8 Statistical analyses

(Papers I-III)
Duplicate experiments were run in two consecutive years, with three independent replicates per run. For Paper III, data from the second year are used for the calculations. Microbial counts were log-transformed before calculations (Angle *et al.*, 1996).
Data were analysed with one-way ANOVA, with Fisher's test (paper III) or Tukey's test (paper I, II) with $p<0.05$ considered as significant, or with the non-parametric Wald-Wolfowitz sequence test. For correlations, step-wise regression analysis was employed. To determine on differences in crop assessments and oxygen contents, two-sided t-tests were performed.
Calculations were made using SPSS 11.5 (SPSS Software GmbH, München, Germany) (Paper I) or Minitab software (ver. 15.1.20.0, Minitab inc., State College, USA) (Papers II, III).

3 Results and discussion

3.1 Efficacy of slow filter

(Papers I-III)
Raising the EC-levels of the nutrient solution to 5 mS cm^{-1} caused significant reduction in filter efficacy against *Fusarium oxysporum* f.sp. *cyclaminis* (Fig. 3, Paper II). This findings might be of interest in many modern cropping practices, for example ornamental pot plant production where osmotic stress is used as a tool for growth regulation (van Leeuwen, 1993) or in tomato production where modern cultivars often require higher EC levels than have traditionally been employed (Sonneveld & Straver, 1989).

However, addition of *Pythium aphanidermatum* to the filter skin did not affect filter efficiency against *F. oxysporum*, nor did the addition of FCWP to the filter skin. In the experiment with *Pythium* addition (Paper III), filter efficacy was high (99.9%) on all test incidents and due to the high and consistent efficacy, no correlations with other parameters were observed. Filter efficacy was higher in Paper III, than in paper I and II. Different packing density of the filter medium in paper III, compared to paper I and II are the most probable reason, indicating that physical properties of the filter column may be an important factor for filter efficacy in addition to the proposed determining factors enzyme activity (Brand & Alsanius, 2004b) and polysaccharide content (Furtner *et al.*, 2007).

3.2 Microbial assessment

(Papers I-III)
The results from the microbial assessment showed that the general bacterial flora and general fungal flora of the nutrient solution or the filter skin were

not affected by the addition of FCWP to the filter skin. No differences with respect to season or repetition were observed (Paper I). The effluent nutrient solution from the commercial systems contained low counts of fluorescent pseudomonads, in contrast to the supernatant and to the filter skin, which were always densely colonised with fluorescent pseudomonads. In the experimental systems, supernatant and effluent nutrient solution generally contained *Fusarium oxysporum* and the supernatant also contained *Pythium aphanidermatum*, whereas the effluent from commercial systems was consistently free from both *F. oxysporum* and *P. aphanidermatum* except on one single sampling occasion. The presence of *F. oxysporum* on the filter skin was verified in both commercial and experimental systems (Paper I).

No significant differences in the bacterial flora, fungal flora or fluorescent pseudomonads in the nutrient solution were found with respect to addition of *P. aphanidermatum* to the filter skin. Microbial colonisation was generally not affected by different EC levels, either in the nutrient solution or on the filter skin. However, bacterial counts were significantly higher in the nutrient solution after filtration at EC 3. We expected bacterial colonisation to be densest in the treatment with the lowest EC, as increasing salinity is normally expected to inhibit microbial growth (Rietz & Haynes, 2003) (Paper II).

For the filter skin, a significant trend of decreasing density of general bacterial colonization was observed during the experiment in Paper III (Fig. 2, Paper III). Simultaneously, the fungal flora on the filter skin remained quantitatively unaffected, as did the flora of fluorescent pseudomonads. *P. aphanidermatum* was not detected in the nutrient solution in Paper III.

3.3 Biochemical assessment

(Papers I-III)

The activity of chitinase, cellulase, β-1,3-glucanase and protease in the filter skin was generally in the range reported by Brand & Alsanius (2004b), and was significantly enhanced by the addition of FCWP, confirming the findings by Brand & Alsanius (2004a). Xylanase activity was detected only sporadically in Paper I, in contrast with the results obtained in Paper III where xylanase activity peaked at around 1.5 mU. Enzyme activity was unaffected by addition of *P. aphanidermatum* to the filter skin (Fig. 1, Paper III), but was significantly affected by different EC regimes (Paper II), with higher EC levels having a negative effect on chitinase, cellulase and xylanase activity in the filter skin (Fig. 1, Paper II), as was also proposed by

García & Hernández (1996). For the higher EC levels studied in paper II (3 and 5 mS cm^{-1}), the activity of cellulase and xylanase decreased towards zero, after initial values at 1.5 to 1 mU, respectively (Fig. 2, Paper II). A similar trend of high initial enzyme activities, followed by a stagnation phase, was observed for protease in Paper I. Chitinase activity together with EC level was found to be a predictor of filter efficacy.

$$Eff = 102 - 1.2\ chi - 1.14\ EC\ (R^2=0.913,\ p<0.001) \quad (4)$$

where Eff=filter efficacy, chi=chitinase activity and EC =electrical conductivity of the nutrient solution. These findings further support the theory that microbial metabolism rather than microflora composition is changed by elevated salinity. In the commercial systems with ornamental plant production, activity of chitinase on the filter skin and occurrence of *F. oxysporum* and *P. aphanidermatum* in the nutrient solution were found to be linked to EC as described by the equation (5):

$$EC = 2.42 - 0.804\ chi + 0.438\ FO\ sup + 0.081\ PA\ sup\ (R^2=0.61,\ p=0.008) \quad (5)$$

where chi = chitinase activity, FO sup=viable count of *F. oxysporum* and PA sup = viable count of *P. aphanidermatum* in the supernatant nutrient solution.

Brand & Alsanius (2004a) found that 5 % of filter efficacy is explained by chitinase, cellulase and protease activity. The findings reported in the present work move a step closer towards fully understanding and predicting slow filter mechanisms and function. The conductivity of the nutrient solution might be the missing link that has not been accounted for in previous studies on slow filters. In Paper III, no correlations between filter efficacy and enzyme activity could be calculated due to high and consistent efficacy of the filters. However, certain relationships were found between biological and biochemical factors were found. For example, chitinase, protease and xylanase activity was found to be linked to microbial and biochemical factors as described by equations (6) to (9):

$$chi = -0.144 + 0.0239\ R2A - 0.0287\ MA + 0.0589\ KB + 0.00561\ tPs - 0.00509\ bPs - 0.128\ DNA\ (R^2=0.509,\ p=0.037) \quad (6)$$

$$prot = 2.47 + 0.362\ R2A - 0.237\ MA - 0.670\ KB - 0.078\ tPs + 0.0343\ bPs + 3.34\ DNA\ (R^2=0.564,\ p=0.016) \quad (7)$$

$$xyl = 0.68 + 0.025\ R2A - 0.344\ MA + 0.507\ KB + 0.104\ tPs - 0.00509\ bPs - 0.128\ DNA\ (R^2=0.473,\ p=0.035) \quad (8)$$

$$bPs=23.1 - 38.6\ chi - 1.27\ prot + 2.67\ cell + 0.65\ gluc - 4.42\ Xyl\ (R^2=0.514,\ p=0.016) \quad (9)$$

where chi, prot, cell, gluc, and xyl = chitinase, protease, cellulase, β-1,3-glucanase and xylanase activitiy in the filter skin, respectively. tPs = total polysaccharide content, bPs = biofilm polysaccharide content and R2A, MA and KB represents plate counts from general bacterial and fungal flora and fluorescent pseudomonads in the filter skin, respectively. DNA = DNA amount in the filter skin.

Bacterial and fungal cells are considered to be a minor part of the TOC of nutrient solutions from hydroponic systems (Waechter-Kristensen *et al.*, 1999). In the present study, no correlations between viable count and TOC levels were found. This supports the findings that dissolved organic compounds and not microorganisms accounted for the majority of organic compounds in the nutrient solution. Both TOC and COD levels were higher in the treatments with EC 5 mS cm^{-1}, compared with the treatments with 2 and 3 mS cm^{-1} (Paper II). This indicates that degradation of organic compounds is retarded by saline conditions, as was also suggested by Rietz & Haynes (2003). In this respect, alteration of the metabolism of the microflora is more probable than a shift in composition. However, only viable count analysis was performed and it is possible that analysis with molecular methods would have given a different picture, since only a minor part of the microflora is considered culturable (Bakken, 1997). The filtration process had a very limited influence on TOC/DOC levels and on viable count. The BOD_5 values (Paper I) were low, often below the detection level for the analytical method used. BOD_5-analysis is suggested to be of minor interest for nutrient solutions.

Results from the commercial system established a correlation between COD and DOC (Fig. 2, Paper I). Similar observations (R^2 = 0.918; $p<0.001$) were made in Paper III.

Content of total polysaccharides on the filter skin did not change during the experiment and no differences were found between treatments (Paper III). This conflicts with findings by Furtner (Furtner *et al.*, 2007), who found that tPs was highly correlated with time and with the addition of

FCWP. In our experiments, the sampling period was shorter, possibly explaining why no correlations were obtained in this study. For the biofilm polysaccharide, content was higher on the last sampling incident for both treatments. The increase of bPs was negatively correlated with enzyme activity. Total polysaccharides and biofilm polysaccharides were not correlated to each other.

3.4 Physical factors

(Papers I-III)
Addition of FCWP had no effect on oxygen saturation or oxygen consumption of the filters. Oxygen consumption of the filters was higher in the commercial systems compared with the experimental systems, as was oxygen saturation in the supernatant (Paper I). The filtration process decreased oxygen saturation of the nutrient solution significantly, as has also has been demonstrated in previous studies (Wohanka, 1995). Different EC-levels did not affect oxygen saturation of the nutrient solution or the oxygen consumption of the filters (Paper II). The treatment where *P. aphanidermatum* was added to the filter skin had significantly lower levels of dissolved oxygen in the nutrient solution both before and after filtration ($p=0.002$, $p=0.004$, respectively). The difference in oxygen saturation could not be explained by any of the state variables, TOC or microbial community (Paper III). The temperature of the nutrient solution did not differ between the treatments in any case. For the commercial systems examined in the present study, the system with ornamental crops had lower temperatures than the tomato system and the experimental systems which were probably due to lower cultivation temperatures and shorter retention time in the greenhouse. Neither pH nor EC was affected by the filtration process, except for the commercial system with pot plants, where differences in EC due to passage through the filter were found for one of the years studied. Variations in EC in the inflowing nutrient solution, combined with insufficient time interval between sampling for supernatant and effluent samples, probably explains this phenomenon.

3.5 Crop development

(Paper III)
In the experimental systems plant height described a linear regression at R^2 = 0.99 (Fig. 4, Paper III). The number of trusses and leaves increased in the first part of the experiment and then stabilised after harvesting started. Plant development was not affected by addition of *P. aphanidermatum*.

3.6 Conclusions and outlook

From this study we can conclude that the main state variables suggested as limiting for the efficacy of slow filtration in closed hydroponic systems do not affect efficacy to such an extent that they are worth considering for normal hydroponic cropping. However, high EC-levels affect filter efficacy negatively and should be taken into account in systems with high EC-levels. The finding that elevated EC-level is negatively correlated with filter efficacy is novel and is of great value in prediction of filter efficacy.

The hypothesis that a massive influx of pathogens to the filter would compromise the filtration process could be rejected. Even in the presence of high loads of *P. aphanidermatum* and *F. oxysporum* efficacy remained unaffected. Abundance of pathogen cell walls have can to trigger the enzyme activity on the filter skin and since high enzyme activity is positively correlated with efficacy, our finding seems very logical.

Our target of finding additional state variables of value in predicting for filter efficacy was not completely fulfilled. It is possible that factors not investigated in this study have a greater impact than generally supposed. Production of antimicrobial compounds, including biosurfactants, by the microflora inhabiting the filter skin are such parameters suggested for future research.

In horticultural applications, slow filters have shown a high efficacy towards relevant root pathogens. This efficacy is determined by flow rate, column particle size and the presence of an intact filterskin. In the light of these facts, and the results from the present series of studies, its is worth questioning whether tools for monitoring filter efficacy are necessary at all in connection with traditional slow filters. We proved that slow filtration is effective even when subjected to massive loads of pathogens in the nutrient solution. The experimental systems in this study were sub-optimal in that we used NFT systems, which are highly conducive to root diseases; the filters were managed at high flow rates and the filter column was not as deep

as generally recommended. Nevertheless, the plants remained healthy even when *P. aphanidermatum* was inoculated onto the filters. Our studies of the commercial systems shows that large changes in the biological, chemical or physical parameters of the nutrient solution in such systems are rare and that only slow changes associated with season occurs. However, different crops and cultural practices might challenge the filters with different conditions. The results from this and previous studies should be sufficient to remove doubts about closed growing systems in general, and slow filtration in particular, as a good practice in managing greenhouse crops. However, for the past decade viral and bacterial diseases have developed into one of the major concerns for vegetable crops, whereas most of the studies on closed systems have mainly focused on fungal and oomycete root pathogens. In future investigations, preventing the spread of viral and bacterial pathogens in recirculating nutrient solutions should be given priority.

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