Europ.J.Hort.Sci., **72** (3). S. 104–112, 2007, ISSN 1611-4426. © Verlag Eugen Ulmer KG, Stuttgart

Abiotic and Biotic Factors in Slow Filters Integrated to Closed Hydroponic Systems

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Summary

Selected abiotic (electric conductivity, pH, oxygen content, chemical oxygen demand, dissolved organic carbon) and biotic (general bacterial and fungal microflora, fluorescent pseudomonads, filamentous actinomycetes, *Fusarium oxysporum*, *Pythium aphanidermatum*, biochemical oxygen demand, enzyme activities) factors have been monitored in two distinct commercial hydroponic systems with tomato and ornamental plants with integrated slow filters in supernatant, filter skin and effluent in two successive years. In six small scale experimental systems with two slow filters each (nutrient film technique, crop: tomato) the same parameters were followed to establish possible correlations with filter efficacy against *Fusarium oxysporum* f. sp. *cyclaminis*. In half of the systems, the filter skin was enriched with fungal cell wall preparation. Nutrient solutions of the systems showed biggest differences concerning the chemical oxygen demand (COD) (commercial tomato system >> experimental system > commercial ornamental system) and dissolved organic carbon content (DOC). Both factors were positively correlated in supernatant and effluent (e.g. supernatant: r^2=0.813; p<0.001). A significant reduction of COD and DOC due to filtration was visible only in the commercial tomato system. The biological oxygen demand of the nutrient solutions was always very low (<2 mg O_2 l^{-1}). Absolute oxygen content was always reduced by filtration processes. Electric conductivity and pH of nutrient solution remained unaffected by filtration processes. No significant differences between the growing systems concerning biotic factors have been observed in supernatant and filter skin. *Pythium aphanidermatum* and *Fusarium oxysporum* were regularly found in the supernatants and colonized the observed filter skins. All effluents of the systems were free of *P. aphanidermatum*. *Fusarium oxysporum* was detected continuously in the effluents of the experimental system while effluents of commercial systems contained only *F. oxysporum* at one incident. Values for chitinase, cellulase, glucanase and protease enzyme activities of the filter skin were not influenced neither by growing systems nor time. Only xylanase activity showed seasonal influences and activities increased over time (mU = 0.088 (mU $week^{-1}$) * t (week); r^2=0.546; p<0.001). Filter efficacies against *Fusarium oxysporum* f. sp. cyclaminis in the experimental systems varied between 98.3 %±1.0 (treated) and 97.9 %±1.8 (untreated) but no significant differences could be stated.

Key words. Enzyme activities – filter efficacy –microfauna – nutrient solutions – organic matter – oxygen content

Introduction

Closed hydroponic systems are commonly used for the in- and outdoor plant production (Ehret et al. 2001). They inhabit a risk for rapid dispersal of plant pathogens (Stanghellini and Rasmussen 1994) which is considered as a major problem for this production method (Zhang and Tu 2000; Hong et al. 2003; Calvo-Bado et al. 2003). Slow filtration (SF) is one of the oldest, most reliable and simplest water disinfestation technologies (Baker 1948) and has been adopted successfully to horticulture in the early 1990 s (Wohanka 1991, 1995; Runia 1995; Alsanius et al. 2001). Suitability of disinfestation methods is generally expressed in terms of efficacy against a selected pathogen and is of great interest for commercial growers. Therefore a method to assess filter efficacy without pathogen inoculation was demanded (Brand and Alsanius 2004).

As filter efficacy in SF is considered to be a complex interaction between physical, biochemical and biological factors (Yordanov et al. 1996; Brand and Wohanka 2001) and the fundamental principles remain poorly defined (Campos et al. 2002), prediction of filter efficacies can probably expected to be expressed as a multifactor equation. A review by Stevik et al. (2004) showed that straining and adsorption were considered as the main mechanisms of retention of bacterial pathogens in slow filtration and were affected by filter media, organic matter content of the filter, extent of biofilm, temperature, flow rate, ionic strength, pH of the filtered liquid and microbial qualities e.g. shape and size of the pathogen.

Physical factors, such as the flow rate (m h^{-1}) of the nutrient solution and the filter medium affected the filter efficacy against several fungal and bacterial plant diseases. Generally, the filter efficacy was enhanced by low flow

rates and large surface areas in combination with small filter pore diameter (WOHANKA et al. 1995, 1999) but results were not consistent and other major influences were suggested (VAN OS et al. 2001).

The importance of the biological factors in hydroponic systems with integrated SF were demonstrated by showing the lower filter efficacy of steam sterilized filters (BRAND and WOHANKA 2001) and biologically inactive (fresh) filters (MINE et al. 2003) compared to biologically ripened filters. Further research revealed that amendment of fungal cell wall preparation (FCWP) to filter surfaces resulted in enhanced cell wall-degrading enzymes activities. A correlation between filter efficacy against *Fusarium oxysporum* f. sp. *cyclaminis* and enzyme activities was established but was not considered to be sufficiently strong (r^2=0.495) to predict filter efficacy as a sole factor (BRAND and ALSANIUS 2004).

The main goal of the present investigation was to determine state variables that might predict efficacy of slow filters in hydroponic systems in a future multifactor equation. As a first step, we conducted a survey to limit the number of potential state variables by screening a broad variety of abiotic and biotic factors in the nutrient solution and slow filters integrated into hydroponic greenhouse systems. We hypothesized that physical (e.g., temperature), chemical and biochemical (e.g., pH, Ec, DOC, COD, BOD, oxygen content and saturation, activity of selected hydrolytic enzymes), biological (e.g., general fungal and bacterial flora, selected groups of microorganisms involved biocontrol, selected pathogens) might be candidate state variables for a future multifactor equation.

Materials and Methods

Sampling sites

Samples were taken in two commercial as well as in experimental closed hydroponic growing greenhouse systems (E) with integrated slow filters. In Poppelg rdens Driverier (O) ornamental pot plants (mainly *Euphorbia pulcherrima, Impatiens walleriana, Primula* sp., *Celosia* sp.; production area: 1 ha) were produced in a trough system using a peat-based growing medium. Ingelstorp Trädg rd (T) grew tomatoes (production area: 0.5 ha) in a system with containers filled with pumice stones. In O and T, trough and drip irrigation were used, respectively. The systems differed with respect to the volume of used nutrient solution (ornamental: ca. 18; tomato: 12; experimental: 0.18 m^3), the age of the slow filters (ornamental: 3; tomato: 5; experimental: 0–0.5 a), the flow rate (ornamental: 100–200; tomato: 100; experimental: 300 l m^2 h^{-1}) and the filter body height and surface area (ornamental: 1 m; 5.9 m^2; tomato: 1 m; 5.85 m^2; experimental: 0.5 m; 0.2 m^2). All systems were connected to slow filters with granulated mineral wool (granulaat, 001309, Grodan, The Netherlands).

The two experimental NFT-systems (E) were designed as previously described (BRAND and ALSANIUS 2004). Briefly, six independent nutrient film technique (NFT) systems were set up and each system was connected to two filter units (packing density: ca 107 kg m^{-3}). Tomato (*Lycopersicon esculentum* 'Aromata') was planted at a density of 4 plants m^{-2}. Plants were cultivated for 24 weeks, starting middle of January. Temperature was set to 18 (night) and 19 °C (day) and artificial light was used (200 W m^{-2}) during the first twelve weeks. The slow filters were started six weeks before the first sampling, to allow a biological ripening of the filters. Fungal cell wall preparation (FCWP) was added to six of the filters (treated) to enhance enzyme activity, whereas the six other filters served as control (untreated). Electric conductivity (EC) and pH were measured and adjusted daily. The EC was adjusted to 2.7 (±0.1) mS cm^{-1} with stock solutions (SONNEVELD and STRAVER 1989). The pH was adjusted daily to 5.8 (±0.2) using 2 M sulphuric acid or 2 M potassium hydroxide.

Sampling dates and sampling points within the systems

All investigations were conducted 2004 and 2005. Samplings in the commercial systems were conducted nine times from May till October in 2004 and 2005. In the experimental systems, the sampling period lasted for twelve weeks, starting in the beginning of April. Briefly, samples were taken for the biological and biochemical analysis every two weeks in E. Abiotic factors were monitored permanently (temperature of nutrient solution) or at least weekly (oxygen content, EC, pH). In the commercial systems analysis were conducted ten times during the growing season.

To describe the influence of the filters on the systems, three sampling points were chosen. Nutrient solution from the crop was collected above the filter surface (supernatant) and from the effluent of the filters (effluent). Mineral wool fibre were pulled off the upper part of the filter surface (filter skin).

Amendment of fungal cell wall preparation (FCWP)

FCWP was produced according to BRAND and ALSANIUS (2004) and added weekly. Aliquots of 1.75 g (dry weight) were administered to the filters in E. The amendment started at the end of March and continued for twelve weeks. Comparability of starting conditions were checked before the first administrating of FCWP (week 0).

Abiotic factors

Oxygen content (Handy Mk 1, OxyGuard ®, Birker d, Denmark), EC (TD Scan WP 4, Eutech Instruments Pte Ltd, Singapore) and pH (pH meter, Russell pH Ltd, Auchtermuchty, United Kingdom) were manually measured once a week (7 days after inoculation with FCWP) in the supernatant and the effluent of the filters to give an overall picture of the systems. In addition, oxygen content (% and mg l^{-1}), pH and EC in the supernatant were measured every sampling day in the supernatant. Oxygen consumption of filters was calculated by subtracting the oxygen content values of the effluent from the ones of the supernatant.

In the first year, samples for oxygen content were taken right after the effluent left the valve at the bottom of the slow filters. Measured reduction in oxygen content was low probably due to high pressure of the nutrient solution and therefore complicated sampling. Hence, all effluent samples were taken at the inlet of the gutter in the second year. The oxygen content was noted when the display of the oxygen meter was stable for at least 15 s.

Values for BOD_5, COD and dissolved organic carbon (DOC) were determined in accordance to standard meth-

ods using the test kits LCK 555, LCK 314 and LCK 385, respectively (Dr. Lange GmbH, Düsseldorf, Germany). Prior to DOC analysis, the solutions were membrane filtered (Filtropur S 0.2, Sarstedt, Nümbrecht, Germany; pore size: 0.2 µm) before testing.

Temperature of the nutrient solutions in the supernatants were measured continuously by data loggers (HOBO® H08-002-02, Onset Computer Corporation, Pocasset, MA, U.S.A.) and TMC6-HA temperature sensors (Onset Computer Corporation, U.S.A.) in all systems.

Microbiological assays

Aliquots of 1 ml of supernatant and effluent from the filter were serially diluted in NaCl (0.85 %). For dilution of filter skin samples, 5 ml of 0.85 % NaCl solution were added to 50 mg (wet weight) of mineral wool granules and vortexed vigorously for 5 s. Plate counts were conducted to estimate the populations of general bacterial and fungal flora, fluorescent pseudomonads and filamentous actinomycetes as described elsewhere (KHALIL and ALSANIUS 2001) using 100 µl aliquots of the nutrient solution. *Fusarium oxysporum* was quantified using selective medium (KOMADA 1975). Aliquots of 200 µl were spread and incubated for five days (25 °C). For estimation of *Pythium aphanidermatum* 200 µl aliquots were spread on potato dextrose agar media (PDA, Difco, 213400, Sparks, MD, U.S.A.) and incubated at 35 °C for 18 h. All investigations were performed two-fold using two sub samples per dilution step and sample.

Enzyme activity

Enzyme activity measurement of the filter skin (chitinase, cellulase, glucanase, protease and xylanase) was conducted by a method described by BRAND and ALSANIUS (2004). Two kinds of sample preparations were used: (i) during the first five weeks in 2004, aliquots of 50 mg of wet mineral wool were processed for the samples taken in E, (ii) aliquots of 50 mg of lyophilized mineral wool. The latter was considered for all following samples in order to obtain sufficiently high activity levels as the measured enzyme activities for (i) were low. Analysis was done with duplicate parallels and two subsamples.

The samples from commercial systems contained large amounts of muddy substances which solved while incubating in the enzyme substrate and caused clogging of pipette tips and jeopardized the transfer of the enzyme substrate solution. Thereto incubated samples were centrifuged for 5 min using a desk centrifuge (Millipore, U.S.A) before transferring 150 µl of the solution to microtiter plates and stopping the enzyme reaction with 150 µl 2 M HCl.

Efficacy test

Filter efficacy against *Fusarium oxysporum* f. sp. *cyclaminis* was determined eight weeks and twelve weeks (2004) after the first incubation with FCWP. In 2005, one efficacy test was performed twelve weeks after starting the treatment and the first efficacy test was repeated in September 2005. The efficacy test was described by BRAND and ALSANIUS (2004). All tests were performed six days after the last addition of FCWP. Viable counts were enumerated with five replicates per dilution step and sample.

Statistical analyses

The results from viable counts, expressed as mean ± SD after log transformation (ANGLE et al. 1996), were analysed by using one-way ANOVA combined with Tukey B test ($p<0.05$). For detection of significant differences between treated and untreated systems in the experimental systems, ANOVA or the nonparametric Wald-Wolfowitz sequence test were used. For testing of correlations between factors the Pearson correlations coefficient (two-folded) was calculated and testing on differences between supernatant and effluent two-pair sample t-test was used. To describe differences between the systems concerning oxygen consumption of slow filters these data were expressed as ppm h^{-1} as suggested by MERMILLOD-BLONDIN et al. (2005). These values enable a better comparability because of different flow rates and filter heights of the systems. The level of significance in all tests was $\alpha=0.05$. All calculations were done using SPSS 11.5 (SPSS Software GmbH, Germany).

Results

Abiotic factors

For the experimental systems, the temperature (°C) of the nutrient solutions (2004: 21.6 °C±1.6; min. 18.7; max. 27.5; 2005: 22.2 °C±1.9; min. 18.7, max. 28.7) did not differ between treated and untreated filters. Comparable values were found in the commercial system for tomato (2004: 21.6 °C±1.6; min.: 17.1; max.: 25.6; 2005: 20.0 °C±1.4; min.: 16.8; max.: 25.6) while lower temperatures were monitored in the ornamental system in 2004 (19.7 °C±2.3; min.: 13.7; max.: 29.9). In this system data were lost due to data logger failure in 2005.

The pH of the nutrient solution was not affected by the passage through any of the filters. No significant differences in EC between experimental systems with or without treatment and similar values (mS cm^{-1}) were found in both years. In the commercial ornamental system, considerable variations were found in the supernatant (1.1±0.4) and effluent (1.2±0.8) in 2004; during 2005 the EC displayed a more homogenous picture (supernatant: 1.0±0.3; effluent: 1.0±0.3). In the commercial tomato system EC-levels were higher (supernatant: 3.38±0.75; effluent: 3.17±0.65) in 2004 than in 2005 (supernatant: 3.3±0.3; effluent: 3.3±0.4).

Oxygen contents (mg $^{-1}$) of the supernatant were 5.7±0.7 and 6.7±0.9) in the experimental systems in 2004 and 2005, respectively. Irrespective of the filter treatment, oxygen consumption (mg l^{-1}) of filters was detectable and statistically significant (2004: 0.3±0.1; 2005: 1.0±0.3) in both years. In contrast to the experimental systems, the oxygen content in the supernatant of both commercial systems was significantly higher (ornamentals: 2004: 8.6±0.7; 2005: 9.1±0.9; tomato: 8.0±1.2; 2005: 8.2±0.7) and oxygen consumption was significantly enhanced (Ornamental: 2004: 3.4±1.4; 2005: 2.8±1.2; tomato: 2004: 2.3±1.4; 2005: 2.7±0.9) compared to the experimental systems. Absolute oxygen consumption was ornamental > tomato > experimental systems in both years. However, comparison of oxygen consumption per h of the filters demonstrated that filters in the experimental systems had highest oxygen consumptions per h (Fig. 1) in 2005.

In all observed systems and both of the years, the biochemical oxygen demand (BOD_5) was very low and mostly below (<2 mg O_2 l^{-1}) of the lower test range of the used test kit. The chemical oxygen demand (mg O_2 l^{-1}) in treated and untreated experimental systems was at the same level in supernatant and effluent and not influenced over time (average values 2004: supernatant: 31.8±6.5 (treated), 33.1±4.6 (untreated); effluent: 33.0±4.4 (treated) and 36.2±5.4 (untreated); 2005: supernatant: 36.1±8.7 (treated), 33.5±9.8 (untreated); effluent: 29.4±10.6 (treated) 27.6±9.4 (untreated)). In the ornamental system, there were no differences in COD between supernatant (2004: 14.0±8.6; 2005: 19.3±9.0) and effluent (2004: 12.3±5.6; 2005: 14.1±2.0). In the commercial system for tomato, measured COD was highest. In both years, the COD in the supernatant (2004: 76.7±26.5; 2005: 65.3±11.4) was reduced significantly (2004: 17.9; p=0,014; 2005: 9.4; p=0.02) to 58.8±18.1 and 55.9±12.6 in 2004 and 2005. Moreover, in 2004 the COD was decreasing over time but this was not the case 2005.

Average DOC contents evolved comparably to the chemical oxygen demand in all studied systems and a positive correlation between DOC and COD in the supernatant (Fig. 2) or effluent could be established.

Microbial assessment

The general bacterial flora counts were not influenced by the season or the year or by the different systems. The general fungal flora showed a similar pattern, but at a lower level. To maintain a concise picture mean values (± std) for all systems and all assessed microbial parameters are shown for 2005 only (Fig. 3). The general bacterial flora exceeded the number of fluorescent pseudomonads in all systems and both years (Fig. 3). The number of fluorescent pseudomonads in the effluents of the commercial systems was very low and often below the detection limit. However, filter skin and supernatant were always colonized by fluorescent pseudomonads. Similar observations were made for filamentous actinomycetes. The

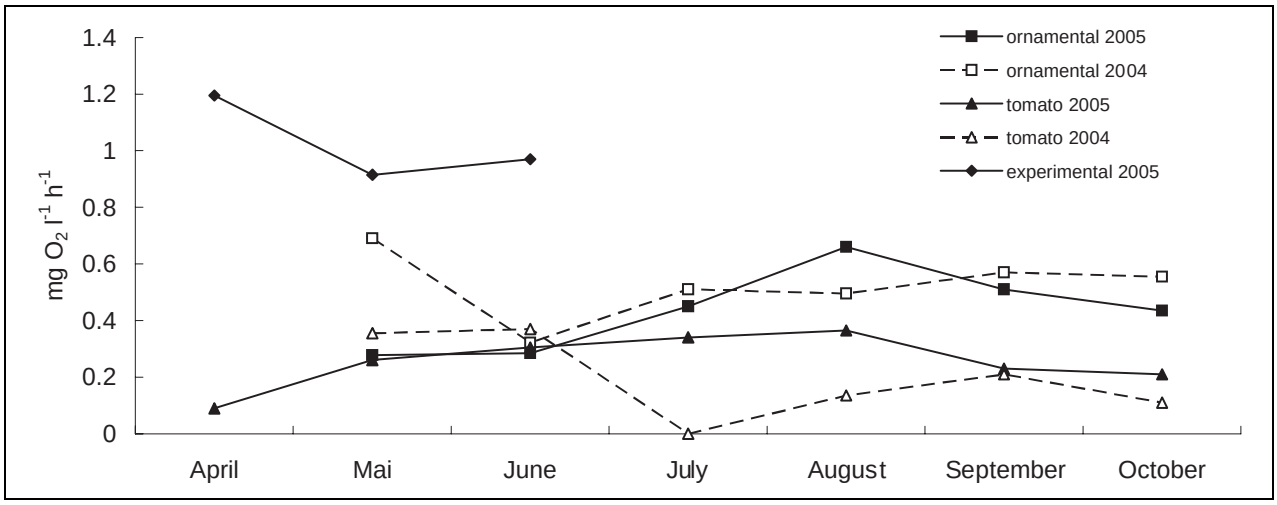


Fig. 1. Oxygen consumption (mg O_2 l^{-1} h^{-1}) of slow filters integrated to two commercial systems with tomato and ornamentals for 2004 and 2005. Displayed values for experimental systems are obtained 2005.

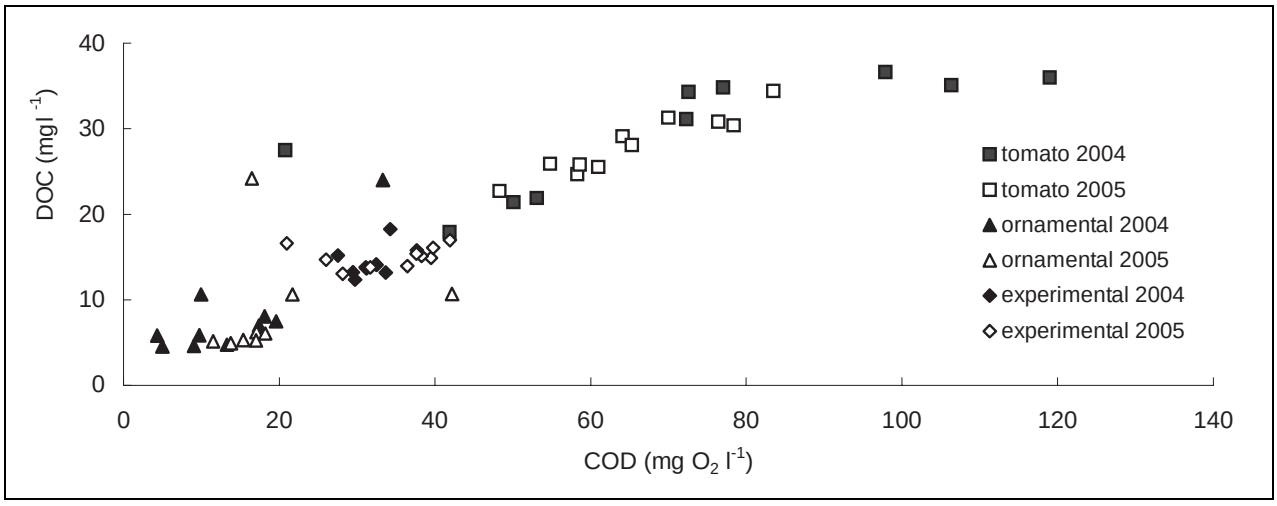


Fig. 2. Mean values of DOC (mg l^{-1}) and COD (mg O_2 l^{-1}) in the supernatant of all systems in 2004 and 2005.

difference in the general fungal microflora and in filamentous actinomycetes between supernatant and effluent was more expressed for the commercial than the experimental growing systems.

In the experimental systems, the filter skin was colonized by *Fusarium oxysporum* in a range (cfu g^{-1})of 5.6±0.9 (2.1±0.4) to 4.7±0.9 (1.7±0.7) for treated and untreated filters in 2005 (2004). The results obtained in the commercial tomato system (2005: 5.4±0.3; 2004: 5.2±0.7) and of the ornamental system (2005: 5.4±0.5; 2004: 4.9±0.1) were similar to the ones of the experimental ones for 2005. The supernatants of the commercial and experimental tomato systems contained always *F. oxysporum* in 2005. Yet, *F. o.* was only detected in irregular intervals the ornamental system (2004 and 2005) and in the tomato and experimental system in 2004. Effluents of experimental systems always contained *F. oxysporum* irrespective of the treatment, except one time in treated systems in 2004. The effluent of the ornamental system was free of *F. oxysporum* in 2004 and 2005. *F. oxysporum* was found once (2005) in the commercial tomato system.

In the experimental filters, the *Pythium aphanidermatum* (*P. a.*) densities of the supernatant and filter skin increased over time in 2004 and 2005 and were positively correlated (r^2=0.65; p=0.012; n=24) (Fig. 4). In the supernatant of commercial systems, *P. aphanidermatum* occured sporadically. The effluents of all systems were free of *P. aphanidermatum* in both years.

Enzyme activities of experimental and commercial systems

In the experimental systems, hydrolytic enzyme activity in the filter skin was influenced by the weekly amendment of FCWP, reflecting higher levels in treated than in non-treated filters (Fig. 5) in both years.

In 2004, the chitinase activities (mU) of treated and untreated filters rose from 0.5±0.7 and 0.1±0.5 in week 0 to 1.6±0.6 and 0.6±0.3, respectively, within twelve weeks. After twelve weeks of treatments a significant difference between untreated and treated filters was visible. Compared to 2004, the chitinase activities obtained in 2005 were more balanced and ranged between 1.8 (week 1; treated filters) and 0.6 (week eleven, untreated filters). Compared to week 1, the chitinase activities of untreated filters decreased significantly from 1.8±0.2 to 1.2±0.2.

Cellulase activities (mU) of treated filters increased from 0.0 to 0.7 during 2004. Untreated filters showed low activities between 0.0 and 0.2. In 2005 the cellulase activities ranged from 0.3±0.1 to 0.7±0.3 in the treated filters and 0.1±0.4 to 0.5±0.1 in the control.

Glucanase activity (mU) in untreated filters was low in 2004 and increased from 0.0 (week 1: ±0.1) to 0.2 (week 13: ±0.0). Treated filters showed higher and significant activity increase over time with 0.0±0.1 (week 1) and 0.6±0.2 (week 13). In 2005, the similar patterns were ob-

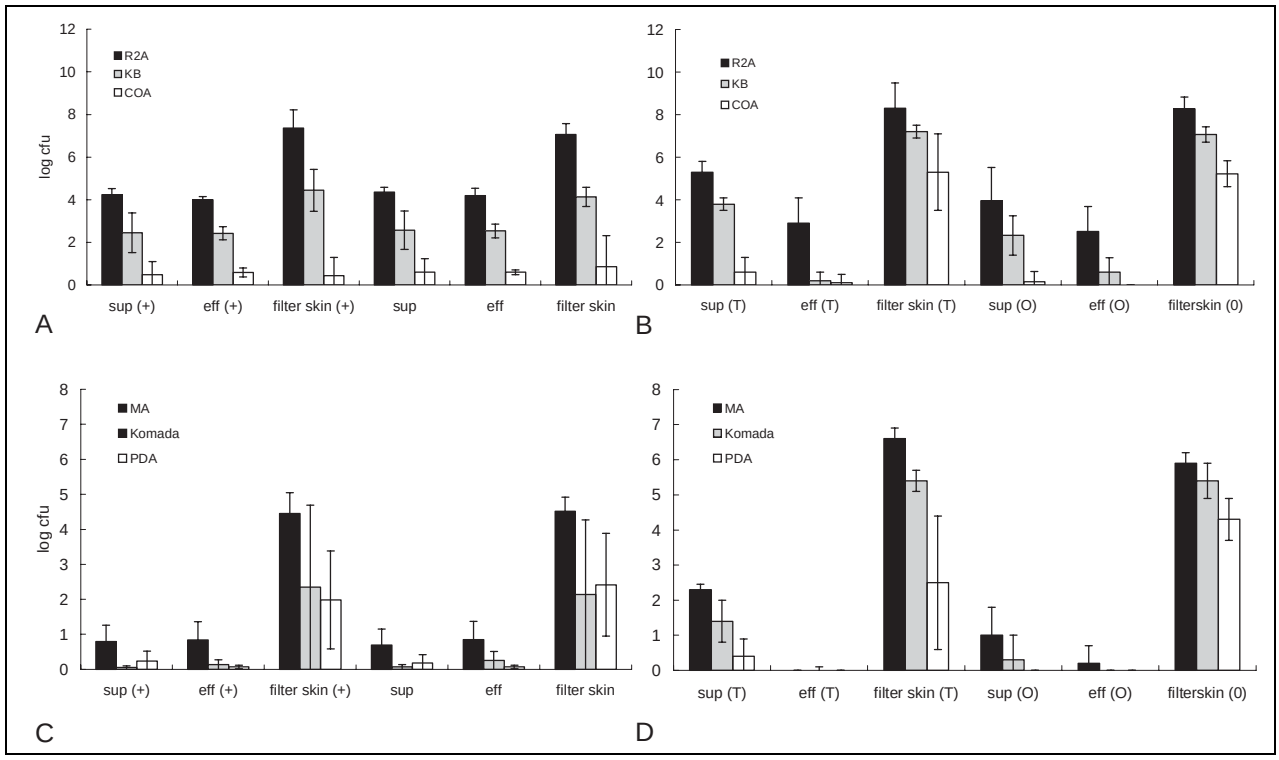


Fig. 3. Characterization of the nutrient solution (cfu ml^{-1}) before and after passage through horticultural slow filters and of the filter skin (cfu g^{-1}) in experimental (A, C) and commercial (B, D) slow filters by the general heterotrophic bacterial (R2A) and fungal (MA) flora, fluorescent pseudomonads (KB), filamentous actinomycetes (COA) as well as *Fusarium oxysporum* (Komada) and *Pythium aphanidermatum* (PDA). Six experimental slow filters were treated with a fungal cell wall preparation (+) while six slow filters served as a control. Tomato was used as a crop in the experimental systems and in one of the commercial ones (T). In the second commercial system (O), ornamentals were grown. Bars display mean values±SD as expressed as log cfu ml^{-1} (sup: supernatant; eff: effluent) and log cfu g^{-1} mineral wool (filter skin).

served. However, the differences between untreated and treated filters were not as expressed as in 2004 and only in week five and eleven significant differences were found.

In 2004, no significant differences for protease activity were found between the two treatments, except from week one and thirteen. In contrast to 2004, protease activities were enhanced in both treatments in 2005. Average protease activity (mU) in treated filters (2004: 0.7±0.4; 2005: 1.4±0.4) was significantly higher only 2005 ($p=0.07$) compared to untreated filters (2004: 0.3±0.2; 2005: 0.8±0.2).

Xylanase activity (mU) was detected only at one sampling date in 2004 in May. In 2005, activities were detectable at five sampling dates in at least one of the filter

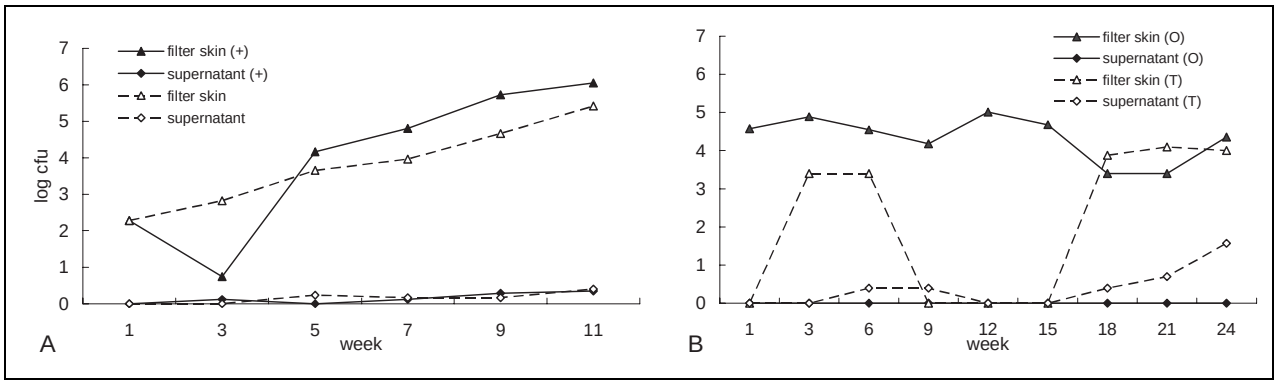


Fig. 4. Mean values of *Pythium aphanidermatum* expressed as log cfu ml^{-1} in (A) experimental (+: treated with 1.75 g FCWP weekly) and (B) commercial (O: ornamental; T: tomato) systems in supernatant and filter skin of slow filters in 2005. Measurements started in April (experimental system) and May (commercial systems) and were conducted every two weeks or three weeks, respectively.

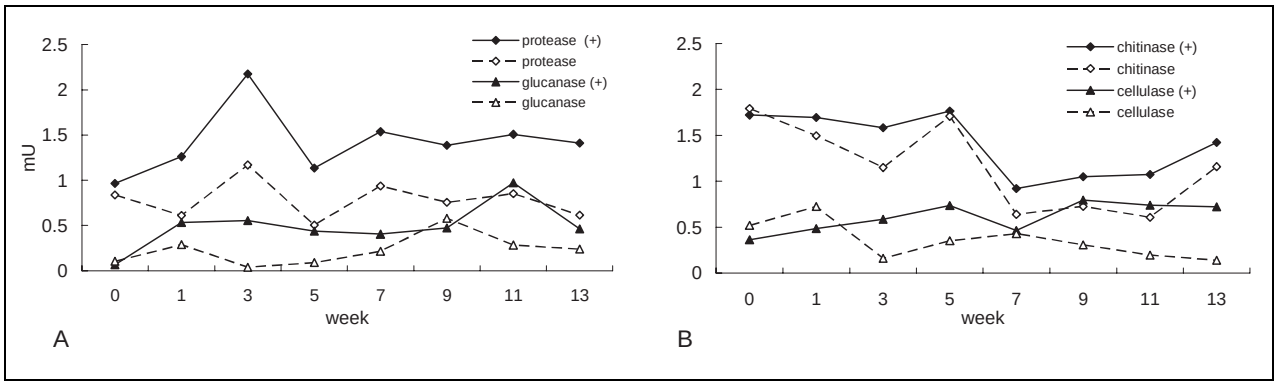


Fig. 5. Dynamics in (A): protease and glucanase as well as in (B): chitinase and cellulase activities (mU) observed in filter skins of slow filters with (+) or without administration of fungal cell wall preparation (1.75 g FCWP per week) in 2005. Treatment started early April (week 1) and was conducted till end of June. Week 0 served as a control (n=6).

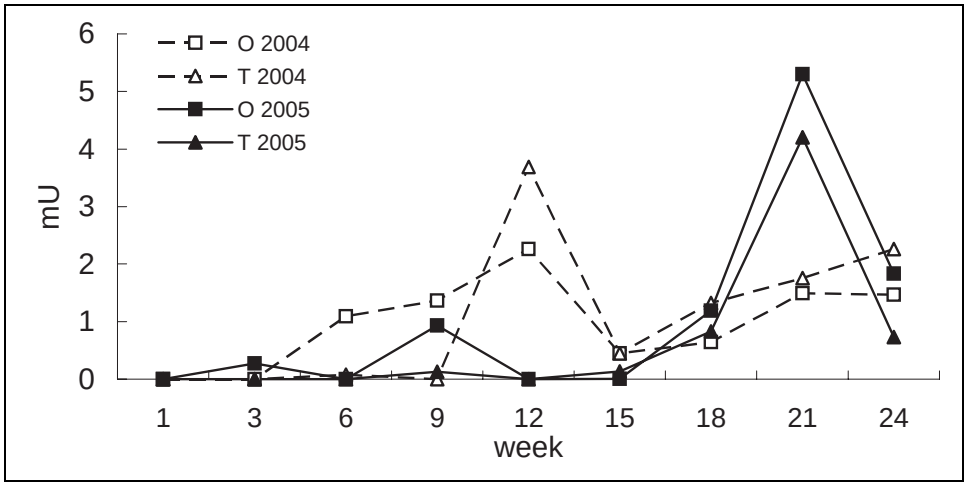


Fig. 6. Average xylanase activities of microorganisms colonizing commercial filter skins (O: ornamental; T: tomato) in 2004 and 2004. Sampling period was from May until October and n=2 for each data point. Regression analysis showed that xylanase activities of 2004 and 2004 together (n=18) were influenced by filter operating time.

skins and no differences between treated and untreated filters were visible. It was only at one sampling date in April that all twelve filters showed xylanase activities ranging between 0.8±0.3 and 0.6±0.1 for treated and untreated filters respectively.

In the commercial slow filters substantial standard deviations resulted from varying enzymes activities levels in the filter skin. Statistical comparisons between enzymatic activities of commercial and experimental systems were not calculated due to differences in number of observations and sampling periods. In general, enzyme activities in the filter skin of the ornamental and tomato growing systems were lower in the treated filters of the experimental set up. When compared to the untreated experimental systems, the commercial systems showed similar enzyme activities in 2004 but not in 2005. Apart from xylanase activities, no seasonal influences on enzyme activities were stated. Long-term observations of commercial systems revealed a highly significant linear increase of xylanase activities with time (Fig. 6). Regression equation (2004 and 2005) for tomato system was: (mU)=0.09 (mU week^{-1})* week (r^2=0.537, p<0.001) and for the ornamental system: (mU)=0.086 (mU week^{-1})* week (r^2=0.581, p<0.001).

Efficacy tests

Efficacy tests performed in the experimental systems with *Fusarium oxysporum* f. sp. *cyclaminis* as a test pathogen did not show any significant differences between the treatments and years. In 2004, there was a trend towards higher efficacies of treated filters. However, in 2005 a reversed trend was observed (Table 1). Filter efficacy was never significantly correlated to one of the observed parameters.

Discussion

Indirect methods for assessment of efficacy of slow filters for horticultural purposes are a novel approach. Brand and Alsanius (2004) found that enzyme activities might be part of such tool. As enzyme activities only explained part of the filter efficacy, further state variables should be identified. For potable water purposes, Weber-Shirk and Dick (1997a, b) pointed out biological as well as physical and chemical mechanisms for retention of particles and *Escherichia coli*. They pointed out particle size as a dominant factor. This finding was also supported by observations for other filter types for horticultural industries (Alsanius and Bergstrand 2004). Calvo-Baldo et al. (2003) characterized the microbial communities active in slow sand filters, but do not display tools for efficacy assessment. The present paper characterized a broad spectrum of physical, chemical and biological state variables in the nutrient solution in closed growing systems and of the filter skin that might be of interest for slow filters in horticulture including both experimental and commercial slow filters.

Despite the findings of Brand and Alsanius (2004) the data in the present experiment do not fully support the interaction between filter efficacy and enzyme activity in the filter skin. This implies difficulties in drawing conclusions on the load of the different evaluated state variables on prediction of efficacy of slow filters. The decreased efficacy of slow filters supplemented with FCWP might depend on different facts. The most noticeable difference between the experiments carried out by Brand and Alsanius (2004) as compared to the here presented one is the occurrence of larvae of *Chironomidae* sp. Apart from microorganisms, prokaryotes, arthropods, larvae of *Chironomidae* sp. and nematodes are ubiquitous on the filter skin, feeding on particulate material and contributing to the self-regeneration process of the upper filter layer (Rumm 1999; Röske and Uhlmann 2005). Larvae of *Chironomidae* sp. may be found to a depth of 5 cm in the filter. Irrespective the administration of FCWP, the filter skin in all of the experimental slow filters was thin. Larval activity may thereby lead to a continuous disturbance of the upper filter layer in the supplemented filters and subsequently counteracted filter head loss and clogging processes. As the filters operating in the experimental set up were suboptimal in height (58 cm of filter column) the combination of these factors might have reduced efficacy towards *Fusarium oxysporum* f. sp. *cyclaminis*. This might also explain the occurrence of *F. oxysporum* in the effluent of the experimental filters. However, the potential caused by the higher colonization and enzyme activity rate of supplemented filters as opposed to non-treated ones should be followed closer in future experiments.

Quantitative (Brand and Wohanka 2001) and qualitative data (Alsanius et al. 2001; Calvo-Baldo et al. 2003) on microbial communities in slow filters for horticultural purposes are scarce. The profile and number of microorganisms in the supernatant and on the filter skin were comparable to previous work (Khalil and Alsanius 2001; Koohakan et al. 2004). The dynamics of *Pythium aphanidermatum* in the nutrient solution before filtration is supported by Tu et al. (1999). Both quantitative and qualitative references are on a descriptive level. As previous data (Van Os and Postma 2000), our results indicate a reduction of viable counts of both the general fungal and bac-

Table 1. Average filter efficacies towards *Fusarium oxysporum* f. sp. *cyclaminis* (%) of filters treated with 1.75 g FCWP per week (treated) and untreated filters (untreated) at different sampling dates in 2004 and 2005. (n=6). Mean values of all tests (Test 1, 2, 3 and 4, n=24) are shown.

	Test 1 (2004)	Test 2 (2004)	Test 3 (2005)	Test 4 (2005)	Mean value
Treated (%)	98.76^a±0.73	97.71^a±1.12	98.74^a±0.63	98.07^a±1.02	98.32^a±0.96
Untreated (%)	98.27^a±0.65	95.70^a±2.25	99.27^a±0.52	98.44^a±0.88	97.92^a±1.81
P-value	0.24 *	0.08 *	0.39 *	0.58 *	0.33 **

Mean values within columns sharing the same letter are not significantly different (p-value <0.05).
*ANOVA
**Wald-Wolfowitz Sequenz Test

terial flora as well as of selected naturally inoculated fungal pathogens in either experimental or commercial slow filters. The reduction in the treated experimental filters compared to the non-treated ones might appear confusing. However, the load of organic material was changed after the sampling point in the supernatant by the addition of FCWP. As the relationship between COD and DOC was affected by slow filtration, the differences in fungal and actinomycetal colonization increased in commercial filters and fungal pathogens in the effluent solutions from commercial filters were scarcely detected, these factors should be further considered as potential supportive state variables for assessment of filter efficacy.

Oxygen availability is a key factor for aerobic turn-over of organic material (MADIGAN et al. 2003). In the present approach, oxygen in the nutrient solution and its fate affected by passage through the slow filters was determined both directly by measuring of oxygen saturation and content and indirectly by assessment of the biochemical and chemical oxygen demand. All variables are closely linked to occurrence of organic matter. Although dissolved organic carbon was detected in the nutrient solution both before and after passage through the slow filter the BOD was not substantially affected. Compared to norm values given for the values for the two different sampling sites in the growing systems would correspond to good quality river water (LESTER and BIRKETT 1999). As the content of dissolved oxygen always exceeded the BOD, there was no risk for intrinsic induction of anoxic conditions in the nutrient solution. In contrast to the BOD, the COD displays the total quantitative of oxygen demand required to oxidize organic compounds to carbon dioxide and water. As expected its value was substantially greater than for any of the BOD measurements. Found values lay in the range of the norm values for unpolluted surface water to effluent rich water (LESTER and BIRKETT 1999) and appear reasonable for closed hydroponic greenhouse systems (LOSCHENKOHL and PORTING 1995; BAR-YOUSEF et al. 2001; ALSANIUS and JUNG 2004). For neither BOD, COD nor for dissolved organic carbon content, no interactions with microorganisms have been displayed. Therefore it is difficult to draw any conclusion beyond a descriptive level.

BOURGEOIS et al. (2001) studying waste water treatment suggested the COD/BOD ratio to estimate the biodegradability of solutions. A COD/BOD ratio close to 1 indicates high biodegradability. For all of the present sampling sites, this would mean that biodegradability was low. This is supported by the fact that the reduction in dissolved carbon content by slow filtration was about 10 %. However, in contrast to other waste water sources the load of inorganic compounds, especially of Cu^{2+} and Zn^{2+}, in closed growing systems with vegetables is high. The compounds may have counteracted BOD evolution (WÜRTZ and MERGEAY 1997).

Before and after passage through the slow filter, COD and DOC in the nutrient solution were positively correlated. This means (i) that the DOC constituted a constant portion of the COD and (ii) that the four growing systems were comparable. However, the interaction was stronger before the filtration step. The weaker interaction after filtration might be an interesting fact to follow up for appointment of supporting state variables for prediction of filter efficacy of slow filters used for horticultural purposes.

Oxygen consumption in commercial filters was up to three times higher than in the experimental system during the season of 2005. However, our results on the highest oxygen consumption as expressed as ppm h^{-1} in experimental slow filters might partly be explained by the fact that microbial respiratory is decreasing with increasing filter depth (MERMILLOD-BLONDIN et al. 2005).

The enhanced activity of the cell wall degrading enzymes (CWDE) by addition of FCWP to the filter skin of slow filters has previously been shown by BRAND and ALSANIUS (2004). However, as no difference in efficacy was found between treated and non-treated filter in the present study, reports that enzyme activity, especially chitinase and protease, might partly predict filter efficacy (BRAND and ALSANIUS 2004), could not be supported by our findings. This may be due to different environmental conditions or due to colonization of the filters by different microbial communities. This point is not clear to answer and would need further investigations. Despite slight variations in the protocol for enzyme analysis between the commercial and experimental systems, measured enzyme activities in commercial systems were comparable to untreated filters in experimental systems. Enzyme activities were successfully linked to microbial biomass (LE BIHAN et al. 1998; HENDEL et al. 2001; BUTTERFIELD et al. 2002) and results of our survey are similar. In treated experimental systems the highest amounts of cfu of the general bacterial and fungal flora were found in the filter skin and enzyme activities were highest of all systems, too. Furthermore, results for xylanase activities in commercial filters demonstrated the usefulness of enzymatic tests to monitor changes of microbial enzymatic activities within the filter skin. As enzymes are inducible or constitutive (MADIGAN et al. 2003), this might display environmental shifts within the microbial communities. This is the first report of strongly and repeatedly enhanced xylanase activities in the filter skin of commercial filters. However, cause and influence on filter efficacies remain uncertain and demand further research.

As the efficacy tests did not reveal substantial differences between treated and untreated filters in their ability to retain *F. oxysporum* f. sp. *cyclaminis*, it appears difficult to draw any conclusions on the value of the measured state variables with respect to their support for prediction of filter efficacy. For future experiments, factors adventuring filter efficacy, such as perturbation of the filter skin, should be avoided. Changes in the relationship between dissolved organic carbon and chemical oxygen demand before and after slow filtration should be further followed up. However, it appears doubtful, if other abiotic parameters in the nutrient solution are solid enough to act as variables supporting prediction of slow filters. Attention should be drawn to variables constitutive for the filter skin instead.

Acknowledgments

The authors want to thank Eva Olsson, Nelia Varelia, Michaela Klaisle, Torunn Jorde and Micael Wendell for helping hands in greenhouse and laboratory. We are grateful to Johnny Nilsson, Ingelstorp trädg rd, Ystad, Joergen Jensen, Lundby, Magnus Hedström, Poppelg rden, Ängelholm, for opening their greeneries for the present study. The study was supported by Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (FORMAS) and by the Swedish Farmers' Foundation for Agricultural Research (SLF).

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Received February 8, 2006 / Accepted October 13, 2006

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