PREVENTION OF ROOT DISEASES IN CLOSED SOILLESS GROWING SYSTEMS BY MICROBIAL OPTIMISATION AND SLOW SAND FILTRATION

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Abstract

Closed hydroponic systems are good alternatives for soil grown crops using methyl bromide in protected cultivation. Root-infecting pathogens may be dispersed over the nursery by the circulating nutrient solution, which was reason to disinfect the nutrient solution. The natural microflora in the nutrient solution will be destroyed by sterilisation and possibly loosing its suppressiveness against certain pathogens, while the use of slow filtration does not destroy the microflora. In the present study it is the aim characterise the microflora and to investigate if a shift occurs after passing a slow sand filter.
Four sand filters were placed in four independent closed growing systems growing cucumber. First influent and effluent of the sand filter were analysed, in later trials also the nutrient solution in stone wool slabs were analysed for microorganisms with plate counts, BIOLOG and DGGE.
Characterisation of the microflora with plate counts showed that numbers of total aerobic bacteria were similar or slightly lower after slow sand filtration. Fluorescent pseudomonads, filamentous actinomycetes and fungi gave large differences between influent and effluent of the slow sand filters. This is an indication for a shift in the natural microflora. Also with BIOLOG and DGGE, such a shift in the microbial population was detected.

1. Introduction

The phase-out of the soil fumigant methyl bromide started in the Netherlands after its detection in tap water after passing poly-vinyl-chloride pipelines, in the early eighties. Hydroponics or soilless growing systems can be a viable alternative to soil growing with the use of methyl bromide. Advantages of hydroponics are a higher production, energy saving, a better control of growth and independence of the quality of soil (Van Os, 1999). Open systems, where the nutrient solution is discharged after use, were popular in the beginning. After getting aware of polluting effects of fertilisers in ground and surface water closed systems were introduced, at which the nutrient solution is recycled. The use was strongly promoted by governmental regulations.

Root diseases did not disappear after the change to hydroponics, at most a shift occurred. In closed systems the risk of dispersal of root-infecting pathogens increased and the need for disinfecting the nutrient solution arose. Heat treatment appeared on the market in 1987 (Van Os *et al.*, 1988; Runia *et al.*, 1988) followed by a great number of techniques, which all had advantages and disadvantages (Runia, 1996; Van Os, 1999). All techniques were based on the elimination of all micro-organisms (sterilisation). The use of slow sand filtration (Wohanka, 1995; Van Os *et al.*, 1997; Runia *et al.*, 1997) proved that it was possible to eliminate pathogens but to keep alive (part of) the natural microflora. The importance of the natural microflora was shown by Postma *et al.*, (2000), indicating that a system is less susceptible for (certain) diseases if the original microflora is still present as compared to

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sterilised systems. Until now it is unclear which organisms play that role in the nutrent solution and which are responsible for the suppression of diseases.

The present study has the aims:

- to characterise the natural microflora in a recirculating nutrient solution growing cucumbers at which slow sand filtration is used as disinfection technique;
- to investigate if a shift in the microflora occurs due to the slow sand filtration.

The study is part of a larger series of experiments within the EU-FAIR programme and the Dutch Israel Agricultural research Programme (DIARP) to develop a sustainable system for the prevention of root diseases in closed soilless growing systems by optimising microbial suppression in the root environment.

2. Material and methods

In a 300 m^2 greenhouse compartment four independent rows of 20 m^2 each were connected to four slow sand filters to test the elimination of pathogens and to characterise microbial groups in the circulating nutrient solution. The sand is characterised as fine (0.15-0.3 mm; D_{10}= 0.23 mm; D_{60}=0.30mm; UC=1.33) and has a bulk density of 1600 kg/m^3 and a porosity of 35%. Drain water of each row was collected and pumped to the corresponding filter, where it trickles through a 80 cm sand layer. The effluent was collected in a container and mixed with fresh nutrient solution and pumped to the plants.

In the first experiment (autumn 1998) the influent and effluent of the sand filters were assessed just before, and 1, 2, 3, 4 and 6 weeks after planting. In a second experiment samples of the nutrient solution were taken from just before, and 1, 2, 4, 8, and 12 weeks after planting during spring 1999. Samples were taken from different locations within the cropping system: from the nutrient solution in the rockwool slabs, from the drain water of a row of plants, flowing into the filter (influent), and from the solution just below the sand filter (effluent).

The microflora in the samples was characterised by the following methods:

- Plate counts

The following groups of micro-organisms were quantified using semi-selective media: total aerobic bacteria (R2A), fluorescent pseudomonads (KB), filamentous actinomycetes (COA with filter) and fungi (1/4 PDA).

- BIOLOG

The ability of all samples to use the 95 substrates in GN plates has been measured after 48 h incubation time. The data were summarised by calculating the average well colony development and by counting the number of substrates that showed a reaction (i.e. optical density larger than in the control).

- DGGE

All samples were concentrated on filters with a pore diameter of 0.2 µm and stored at –20 °C. PCR-DGGE gels of the bacterial population were prepared following the method described in Postma *et al.* (2000). Profiles of the bacterial populations were obtained from the 4 replicate samples of the influent, the effluent, and the nutrient solution in the slab, 4 and 8 weeks after planting. In addition, drain and effluent were compared for each sand filter, during all sampling dates.

3. Results

In the experiment of autumn 1998 plate counts of total aerobic bacteria did not show significant differences (fig. 1) between influent and effluent. However, it could be seen that the total concentration decreased slowly during the sampling period of 6 weeks. In the following experiment in spring 1999 there were siginificant differences between influent and effluent of the slow filters (fig. 2). Besides, the slabs are sampled too. The numbers of aerobic bacteria, fluorescent pseudomonads and actinomycetes were often highest in the rockwool slab. The influence of the sand filter was different per group of micro-organism. Largest differences between drain and effluent were present for fluorescent pseudomonads, filamentous actinomycetes and fungi, i.e. most of the sampling times numbers of these

organisms were significantly lower in the effluent than in the influent. Numbers of bacteria were only slightly lower in the effluent than in the influent (significant at week 1 and 12). These results showed that a shift in the microbial communities can be determined with the executed plate counts.

Using BIOLOG an interesting shift in the microflora of the effluent occurred around 4 weeks after planting. The potential of the microflora to use the different substrates was lower in the effluent than in the influent, especially after the sand filters had been used for more than 4 weeks (fig. 3). The potential of the microflora in the nutrient solution from the slabs and the drain to use substrates remained more or less stable during the experiment. A similar shift could be seen using DGGE. Bacterial communities differed between the type of sample (influent, effluent and solution in slab) and sampling time (fig. 4). There was a clear shift during the cropping period. Changes due to the slow sand filtration were most pronounced at week 4 and thereafter.

4. Discussion

Hydroponic growing systems are often seen as a sterile growing method without natural ingredients. Berkelmann (1994) showed a fast increase in numbers of micro-organisms within 20 hours after starting watering. Our data show starting values of 10^5 cfu/ml in the nutrient solution just before planting, however, microbial diversity might be low. In this study a start has been made to characterise the microflora and to investigate if the microflora is influenced by the disinfection method, slow sand filtration in this case, and if the microflora has a certain role in suppressing root-infecting pathogens.

To get a good view of the microflora the place of sampling is of great importance. In our 1998 experiment it was realized that sampling of the effluent was too far away (about 1 m) from the filter outlet. Besides, sampling of slabs has also to be executed to get a better picture of the changes around the plant roots. Therefore the sampling methods are adapted in the 1999 experiment.

With the three detection techniques, plate counts, BIOLOG and DGGE, a shift in the microflora could be detected during the sampling period. This phenomena becomes even more interesting if the microflora is not only characterized using slow filtration, where it was known that the effluent was not sterile (Van Os *et al.*, 1997), but especially if a sterilization technique is used, such as UV or heat treatment. In the latter case a recolonisation takes place which may influence the suppressing potential of the natural microflora against certain root-infecting pathogens, such as *Pythium* spp. and *Phytophthora* spp.. These mentioned aspects are subject of further investigations.

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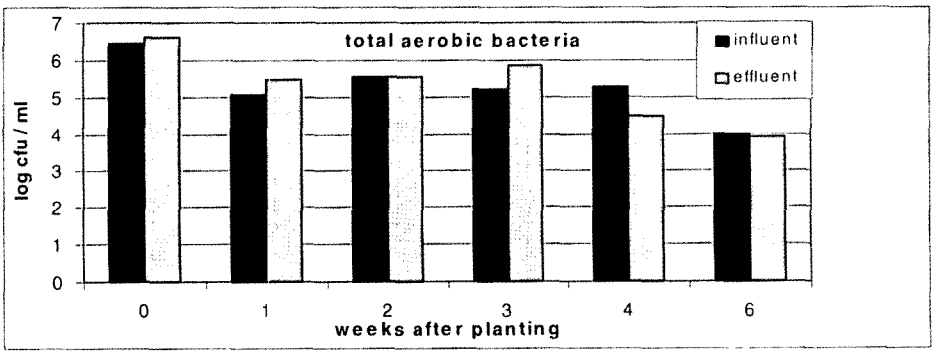
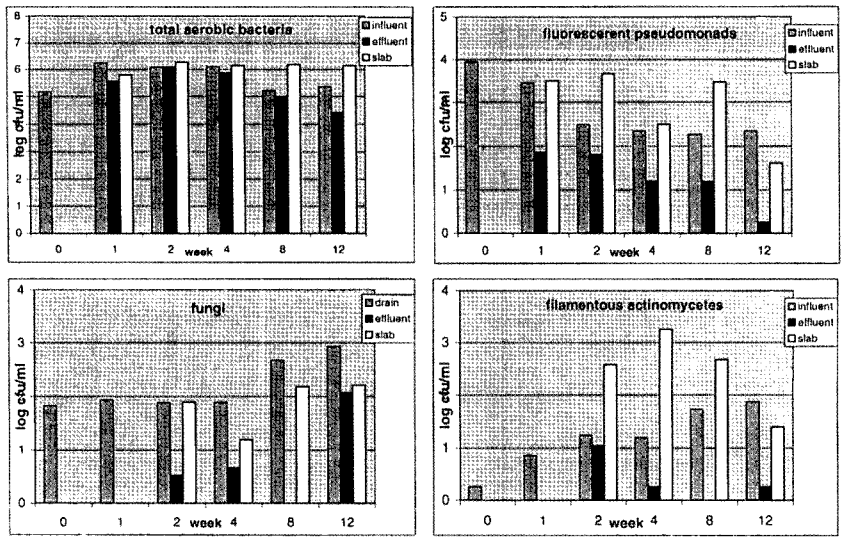
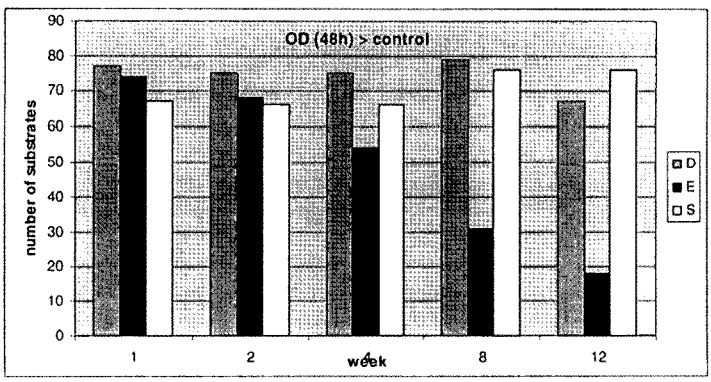
Figures



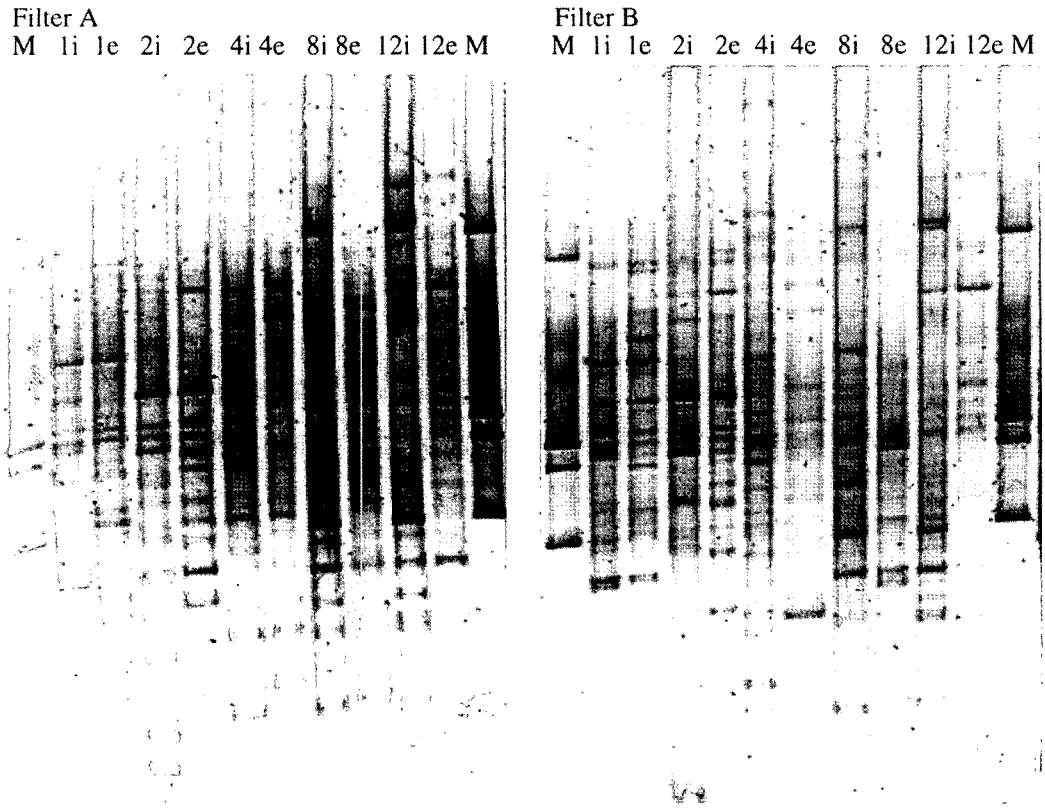
1. Total aerobic bacteria in influent and effluent of slow sand filters. $LSD_{0.05}$ = 0.3 (autumn 1998).



2. Plate counts of different microbial groups from rockwool slabs, the influent and the effluent of sand filters (spring 1999). $LSD_{0.05}$ values are: 0.46, 1.10, 0.78 and 0.75 for total aerobic bacteria, fluorescent pseudomonads, fungi, and filamentous actinomycetes, respectively



3. Number of substrates that reacted (i.e. optical density larger than in the control) using BIOLOG.



4. PCR-DGGE patterns of bacterial populations of the influent (I) and effluent (e) of two slow sand filter systems 1, 2, 4, 8, and 12 weeks after planting. M is a mix of four bacteria used as a marker.