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Potential of Utilization of Selected Organic Compounds by the Microflora Inhabiting the Effluent Nutrient Solution of Closed Greenhouse Irrigation Systems

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Summary

The potential for break-down of organic substrates by the microflora inhabiting the nutrient solution effluent from closed systems was studied in a fruit-bearing tomato crop grown in small scale systems adopting nutrient film technique with respect to selected phenolic acids (benzoic acid, ferulic acid, p-hydroxybenzoic acid, salicylic acid, vanillic acid) and eleven substrate groups expected in rhizodeposition (carbohydrates, amino acids, carboxylic acids, polymers, amines, amides, alcohols, aromatic compounds, brominated compounds, esters, phosphorylated compounds). Factors essential for mediating the break-down (microflora, oxygen content and demand, total organic carbon, pH, electrical conductivity) were described for the studied growing system. Phenolic acids added to the nutrient solution or tap water samples and followed during 54 h, disappeared after a short lag phase when incubated in the colonized effluent, whereas when incubated in non-sterile tap water, the tested phenolic acids were significantly more persistent. Of the substrate groups assessed using Biolog GN-panel, carbohydrates, amino acids, carboxylic acids, polymers and amines were the most consistently utilized substrates during an observation period of 96 h. However, not all of the compounds on the GN-panel included in these classes were metabolized. Remaining six substrate groups were utilized inconsistently and beyond the threshold of 0.5 absorbance units. Viable count of all scrutinized microbial groups (general bacterial and fungal flora, fluorescent pseudomonads, actinomycetes, *Trichoderma*) was higher during the second out of two years of observation. Passage through the growing system indicated an increase in pH and electrical conductivity as compared to set values. Oxygen saturation and –content decreased from the first to the last sampling whereas the total organic carbon content (TOC), the biochemical oxygen demand (BOD_5) and the chemical oxygen demand (COD) remained unaffected.

Zusammenfassung

Mikrobielle Verwendung von organischer Substanz im Drain von geschlossenen Kultursystemen im Gewächshaus. Das Umsatzpotential von organischen Substanzen im Drain von geschlossenen Systemen wurde in einer früchtetragenden Tomatenkultur (NFT) in Hinblick auf Phenolsäuren (Benzoë-, Ferula-, p-hydroxybenzoë-, Salicyl-, Vanillinsäure) und elf Gruppen von organischen Substraten (Kohlenhydrate, Amino-, Karboxylsäuren, Polymere, Amine, Amide, Alkohole, aromatische Verbindungen, bromierte Verbindungen, Ester, phosphorylierte Verbindungen), die in Wurzelexsudaten erwartet werden, untersucht. Für den Umsatz organischer Substanz determinierende Faktoren (Mikroflora, O_2-Gehalt und –Verbrauch, totaler organischer Kohlenstoffgehalt, pH und elektrische Leitfähigkeit) werden für das untersuchte Kultursystem beschrieben. In belebten Drain wurden die ausgewählten Phenolsäuren in vitro nach kurzer lag-Phase während des Beobachtungszeitraumes von 54 h umgesetzt. In nicht sterilem Leitungswasser hingegen verhielten sich die Phenolsäuren bedeutend persistenter und Vanillinsäure wurde nicht umgesetzt. Substratverwertung wurde mit Hilfe von Microlog GN-Panels untersucht. Von den elf Substratgruppen wurden Kohlenhydrate, Amino- und Karboxylsäuren, Polymere und Amine während des 96-stündigen Untersuchungszeitraumes am konsequentesten verwertet. Allerdings wurden nicht sämtliche einzelne Kohlenstoffquellen in den verschiedenen Klassen metabolisiert. Die Verwertung der übrigen sechs Klassen lag unter dem Schwellenwert von 0.5 Absorptionseinheiten. Die Gesamtkeimzahl für die Bakterien- und Pilzflora sowie für einzelne Gruppen (fluoreszierende Pseudomonaden, Aktinomyzeten und *Trichoderma*) war im zweiten Versuchsjahr höher als im ersten. Nach Passage der Nährlösung durch das Kultursystem stiegen sowohl pH als auch Leitfähigkeit im Vergleich zu den Richtwerten. Sauerstoffgehalt und –sättigungsgrad nahmen von der ersten zur dritten Probenentnahme ab, der biochemische und chemische Sauerstoffverbrauch sowie der organische Gesamtkohlenstoffgehalt blieben unbeeinflusst.

Key words. Biolog GN-panel – *Lycopersicon esculentum* L. – oxygen demand – oxygen content – phenolic acids – tomato – total organic carbon content

Introduction

Closed hydroponic cultivation systems are considered to be conducive to poor plant growth and concomitantly decreased yield and/or quality due to an increased risk for spread of pathogens, in particular pythiaceous fungi (EHRET et al. 2001) and to accumulation of organic compounds (JENSÉN and ADALSTEINSSON 1993). Dissemination of root infecting pathogens may be controlled by various active or passive disinfection methods as reviewed by EHRET et al. (2001). However, control measures should be chosen in relation to the grown crop, its economic value, the spectrum of pathogens in questions as well as the costs of potential crop losses.

Although frequently presented as a fact in practical production situations, the theory on accumulation of organic compounds, especially phenolic acids, as causal agents for decreased crop yield and quality, still is of hypothetical character. For accumulation to occur, non-mineralisation of organic compounds originated from the incoming water and/or released by roots, substrate or technical devices (WAECHTER-KRISTENSEN et al. 1999) is a precondition. Mineralisation is mediated by microorganisms. Several studies indicate the presence of microorganisms in the nutrient solution and different parts of closed hydroponic growing systems (STRAYER 1994) and the crop's impact on microbial counts as well on the composition with respect to function and taxonomy (BERKELMANN 1992; KHALIL and ALSANIUS 2001; ALSANIUS and JUNG 2003). Other conditions for accumulation of an organic compound in the nutrient solution might be

– non-solubility of the compound in the nutrient solution,
– no match between the site where the compound is present with the site where microorganisms able to degrade this compound are present,
– inability of the resident microflora to produce enzymes to degrade the compound in question and/or incapacity to induce such enzyme complexes.

The objective of the present paper was to describe the potential to degrade organic compounds, with special focus on phenolic acids, in the nutrient solution of closed hydroponic greenhouse systems by the resident microflora.

Material and Methods

Tomato seeds (*Lycopersicon esculentum* cv. 'Gitana') were pre-cultivated in a cultivation tent in the greenhouse for 4 weeks (r.h.: 75 %, light intensity: 200 μmol m^{-2} s^{-1}). The temperature was reduced successively during the first week under greenhouse conditions to 19 °C. Five-week-old tomato plants were then transplanted with three replicates per treatment at a density of 15 plants per gutter and 2.3 plants m^{-2} into independent closed small-scale NFT-growing systems. Standard values (in mM) for the composition of the nutrient solution were for macronutrients: 6.0 $Ca(NO_3)_2$, 8.0 KNO_3, 2.0 $MgSO_4$, 1.0 NH_4NO_3, 2.3 KH_2PO_4 and for micronutrients: 0.4 Fe-EDTA, 0.01 $MnSO_4$, 0.025 H_3BO_3, 0.005 $ZnSO_4$, 0.00075 $CuCl_2*2H_2O$, 0.0005 Na_2MoO_4 (pH 5.8±0.1; EC 3.2 mS cm^{-1}). The nutrient solution (flow rate: 0.9 L min^{-1}) was continuously replenished to an EC-level of 3.2 mS cm^{-1}. Analysis of the nutrient solution with respect to inorganic compounds was performed once a week and values adjusted in accordance with the result. During the cultivation period, temperature was kept at 19 °C and 18 °C during day and night time, respectively. From transplanting and six weeks on, artificial light (400 W m^{-2}) was supplied to compensate for natural low light conditions.

Sampling of nutrient solution

Effluent nutrient solution (2 L) was sampled from the mature, fruit-bearing tomato crop thrice with three independent replicates during a six-week-period. The background composition of phenolic compounds was analysed using supported liquid membrane extraction followed by liquid chromatography (HPCL) as further described by JUNG et al. (2002). The collected nutrient solution was immediately transferred to the laboratory for assessment of microorganisms and degradation of organic compounds.

Substrate utilization

Studies on substrate utilization were performed *in vitro*. For production of stock solutions (1000 μM), benzoic acid (BA), ferulic acid (FA), *p*-hydroxybenzoic acid (pOHBA), salicylic acid (SA) and vanillic acid (VA) dissolved in 4 ml of methanol were added to 96 ml of water and membrane filtered (0.22 μm). Aliquots of 10 ml of the supplemented nutrient solutions (final concentration: 30 μM) were then kept on a rotary shaker (200 rpm) in the dark at 20 °C. Non-sterile tap water was used as a control. After 7, 31 and 54 hours aliquots of 1 ml were filtered (0.22 μm) and analysed using HPLC ((2690 Separation Module, Waters) connected to a diode array detector (996 Photodiode Array Detector) and Techsphere C_8-column (0.003 mm x 150 mm x 4.6 mm; HPLC Technology, Welwyn Garden City, UK; software: Millenium 32, Chromatography Manager V4.0, Waters); injection volume: 80 μl; flow rate: 0.7 ml min^{-1}; storage temperature prior to injection: 4 °C). The mobile phase consisted of acetonitril and 0.085 % phosphoric acid according to each compounds requirements (Table 1).

Utilization of organic compounds was studied on Microlog GN-panel (Biolog, Haywood, CA, USA). The 96 wells of the microtitre plates contain a low concentrated, buffered nutrient medium, a carbon source (except the control well) and the redox dye tetrazolium. According to GARLAND and MILLS (1991) the carbon compounds in the GN panel may be divided into 11 groups: alcohols, amides, amines, amino acids, aromatic compounds, brominated compounds, carbohydrates, carboxylic acids, esters, phosphorylated compounds and polymers.

The redox dye tetrazolium is soluble in water in its oxidized state and appears colourless. By microbial respiration tetrazolium is reduced to the insoluble violet formazan complex, irreversibly deposited inside the bacterial cell (BOCHNER and SAUVAGEAU 1977).

The density of the nutrient solution was adjusted to an optical density of OD_{590} 0.010 using 0.85 % NaCl. Aliquots of 150 μl of the solution were inoculated into

Table 1. Conditions for HPLC-analysis

Compound	Wave length (nm)	Mobile phase	% Acetonitril
Benzoic acid	230	Isocratic	35
Ferulic acid	324	Isocratic	25
p-hydroxybenzoic acid	256	Isocratic	25
Salicylic acid	237	Gradient	0 min: 10, 15 min: 70
Vanillic acid	262	Isocratic	15

the microtitre plates. The plates were incubated at room temperature and analysed spectrophotometrically (590 nm; DigiScan, Asys, Linz, Austria; software: Digiwin) by colour change of the redox dye tetrazolium to formazan, directly after inoculation and after 4, 18, 24, 30, 36, 48, 60, 72 and 96 h.

Microbial analyses

Aliquots of 1 ml of the collected effluent nutrient solution were serially diluted and volumes of 100 µl spread, in duplicates, on the following media:
- R2A (DIFCO 1826-17-1) complemented with cycloheximide (100 µl ml^{-1}) for enumeration of the general bacterial flora.
- King Agar B (KB, KING et al. 1954) with cycloheximide (100 µl ml^{-1}) for enumeration of fluorescent pseudomonads,
- modified 0.25 Potato Dextrose Agar (PDA, DIFCO 013-01-4) for enumeration of the general fungal flora as well as selective enumeration of *Trichoderma* (POSTMA et al. 1999) and
- chitin oatmeal agar (COA) complemented with cycloheximide (100 µl ml^{-1}) for filamentous actinomycetes (POSTMA et al. 1999).

For the latter one, the diluted nutrient solution was inoculated on sterile membrane filters (Optitran BA-S 83, 0.2 µm; Ø82 mm; Schuell & Schleicher, Dassel Germany). The filters were removed and discarded after 5 days of incubation. Plate counts were done after additional 5 days of incubation. Apart from KB plates, all the plates were incubated at room temperature. KB plates were incubated for 24 h at 27 °C, R2A plates for 72 h whereas PDA plates were incubated for 7 days. The number of fluorescent pseudomonads was counted under UV-light (Blak-Ray Lamp, 366 nm, UVL-56).

Physical and chemical analyses of the effluent solution

The effluent nutrient solution was characterized with respect to electrical conductivity (EC; TD Scan 4, Eutech Instruments) and pH, oxygen content and saturation (mg L^{-1} and %, respectively, Oxygard), chemical oxygen demand (COD; mg L^{-1}; LCK 314; Dr. Lange, Düsseldorf, Germany). The biochemical oxygen demand was measured as BOD_5 (mg L^{-1}) after five days of incubation in an incubation block at 20 °C (LCK 555; Dr. Lange, Düsseldorf, Germany). Analyses of total organic carbon content (TOC, mg L^{-1}) were performed by a commercial analytical laboratory (Lennart Månsson International, Helsingborg, Sweden).

The study was conducted during two years. Assessment of substrate utilization and microbial analyses were performed both during the first and second year. The phenolic acid background in the effluent as well as the decline in concentration of selected phenolic acids was characterized during the first experimental year whereas analyses of physical and chemical factors potentially affecting utilization processes in the effluent were executed during the second experimental year.

Calculation and statistical analyses

The results from viable counts are expressed as mean ± SD after log transformation (ANGLE et al. 1996) and representative results are shown. For analysis of the break-down of carbon sources using the Biolog GN-panel, no adjustment was done for negative values. The measured values from carbon utilization may be separated into different hierarchical levels: utilization performance on the level of the whole plate, utilization of groups of compounds and utilization performance of the single C source. The "average well colour development" of the total per groups of substrates ($AWCD_G$), expressing the sum of absorbance units of individual substrate sets divided by the number of compounds belonging to the substrate group, and the "number of positive wells" with an absorbance level >0.5 per substrate group were calculated. These results as well as the results on disappearance of phenolic acids, viable count and intrinsic factors in the effluent were analysed using one-way ANOVA and Tukey-test with $p < 0.05$ considered significant.

Results

Significant differences were obtained regarding all five groups of microorganisms (general bacterial and fungal flora, fluorescent pseudomonads, filamentous actinomycetes, *Trichoderma*) between the two studied seasons (Fig. 1). They were in general substantially lower in the second than in the first year. Also the ratio of fluorescent pseudomonads to the general bacterial flora was considerably higher during the first year compared to the second one. Further, viable counts for the general bacterial flora and for fluorescent pseudomonads increased both years from the first to the third sampling incident. However, the ratio of fluorescent pseudomonads to general bacterial flora remained unaffected.

No differences were obtained with respect to electrical conductivity (5.13 mS cm^{-1}±0.21) and pH

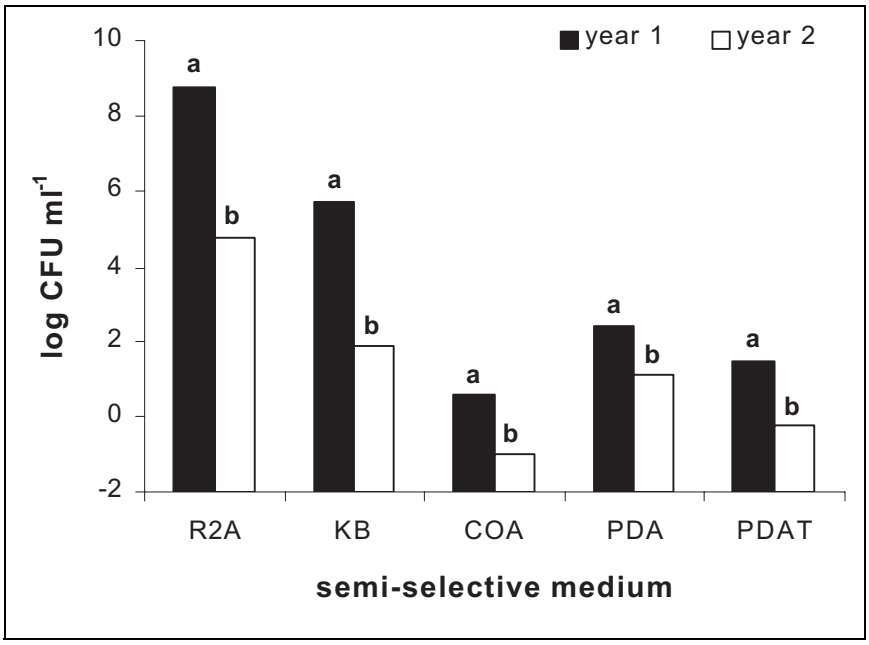


Fig. 1. Comparison of microbial colonization of effluent nutrient solution of a closed hydroponic system with tomato adopting NFT between two seasons. Viable count (log CFU ml^{-1}) colonies of the general bacterial (R2A) and fungal flora (PDA), of fluorescent pseudomonads (KB), filamentous actinomycetes (COA) as well as *Trichoderma* were enumerated on semi-selective media using R2A, Potato Dextrose Agar (PDA), King Agar B (KB) and chitin-oatmeal agar (COA), respectively. Differential enumeration of *Trichoderma* (PDAT) was performed on PDA. (Year 1:■; year 2: ❑). Bars within the same group of organisms labelled with different letters were significantly different at $p<0.05$ (Tukey-Test) (n=9).

Table 2. Oxygen saturation (%) and content (mg L^{-1}), chemical (COD, mg L^{-1}) and biochemical (BOD, mg L^{-1}) oxygen demand as well as total organic carbon content (TOC, mg L^{-1}) in the effluent nutrient solution of a closed hydroponic greenhouse system with tomato using NFT. Samples were taken thrice during a six weeks period in three individual systems.

Sampling	O_2 Saturation	content	COD	BOD	TOC
1	59.33 a[1]	5.07 a	67.73 a	0.00 a	21.23 a
2	32.33 b	2.83 b	52.00 a	1.33 a	17.70 a
3	35.33 b	3.07 b	57.27 a	2.67 a	16.90 a

[1]Values within the same column followed by the same label were not significantly different at $p<0.05$ (Tukey-test). (n=9).

(6.71±0.18) in the effluent nutrient solution between the replicates or sampling incidents. However, both electrical conductivity and pH were considerably higher as compared to set values. Mean values obtained for BOD, COD and TOC (Table 2) did not vary between the sampling incidents, but the monitored values for all three parameters were highest for one of the replicate systems compared to the two other ones. However, oxygen saturation and content, monitored during the second run of the experiment, decreased from the start to the end of the experiment, resulting in significant differences between the first and the two later samplings (Table 2).

In the effluent, VA (68 nM±9.7) was verified in seventeen of 21 extractions. Of the other scrutinized compounds, FA, pOHBA and phenazine-1-carboxylic acid were verified sporadically – FA (82 nM) and pOHBA (18 nM) at one of the samplings and in one of the growing systems, whereas phenazine-1-carboxylic acid (7 nM±1) was found in all three replicates when sampling effluent for the second time in four of nine extractions.

As the sampling incident did not interact with the decline in concentration of the selected phenolic compounds, data from all three samplings were pooled. Almost all of the five selected phenolic acids showed a similar pattern in decline during the 54 h of observation (Fig. 2) when added to colonized nutrient solution. Apart from SA, none of the compounds could be verified after 54 h of incubation (detection limit: <1 nM). Of the five selected compounds, SA occurred to be the most persistent compound. At all of the sampling incidents, 0.5 to 15 % of SA could still be detected after 54 h of incubation. No trend towards an accelerated pace of disappearance (ALEXANDER 1999) at later sampling incidents was observed for any of the tested compounds, although very low concentrations of almost all assayed compounds were still found at the last sampling incident even after 54 h of incubation.

When added to nonsterile tap water, significant reductions in substrate concentration were found in solutions enriched with BA sampled after 54 h. Significant differences were, however, found between the compounds. Already after 7 h of incubation the concentration of FA and pOHBA were lower than the ones of BA, SA and VA. This pattern was also valid after 31 h. At the end of the observation period, SA and VA accounted for the strongest persistence of all five tested acids. Significant differences between compounds incubated in the colonized nutrient solution effluent and non-sterile tap water were found after 31 and 54 h of incubation.

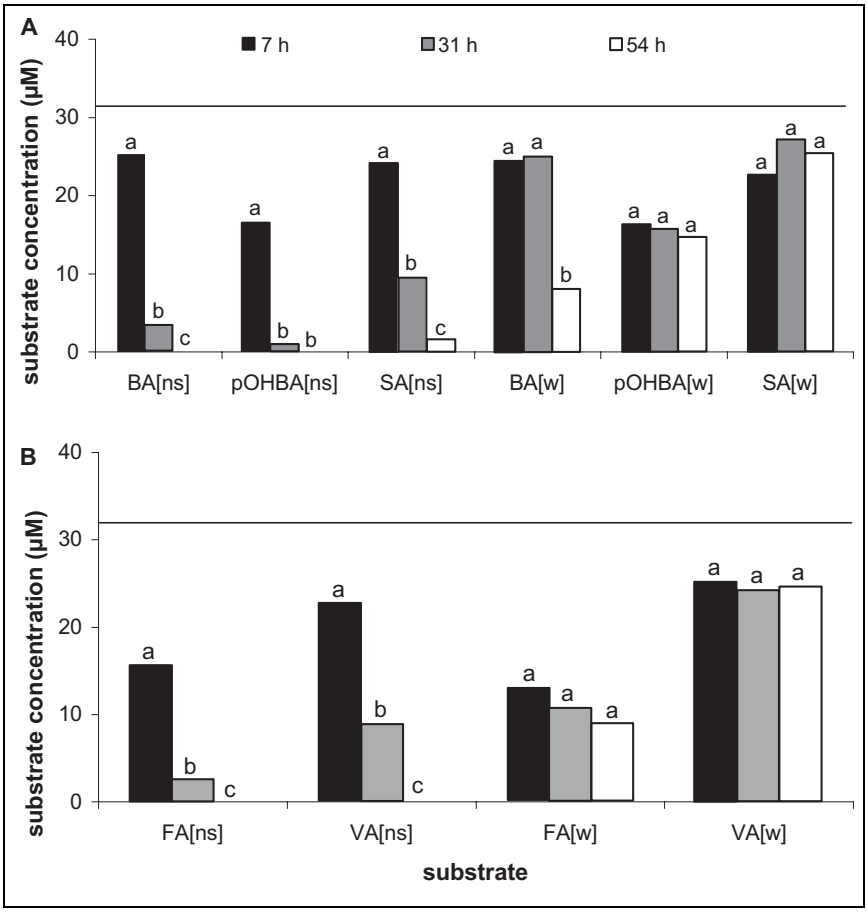


Fig. 2. Disappearance in substrate concentration (µM) of five selected phenolic acids (BA=benzoic acid, FA=ferulic acid, pOHBA=*p*-hydroxybenzoic acid, SA=salicylic acid (*o*-hydroxybenzoic acid), VA=vanillic acid) when added to the colonized effluent nutrient solution from a closed NFT-system with tomato (ns) or to non-sterile tap water (w). Replicate samples were taken after 7, 31 and 54 h of incubation. The horizontal line represents the start concentration of the added compounds (30 µM). Bars within the same treatment labelled with different letters were significantly different (Tukey-test; $p<0.05$). Displayed values are based on results obtained during the first experimental year (n=27).

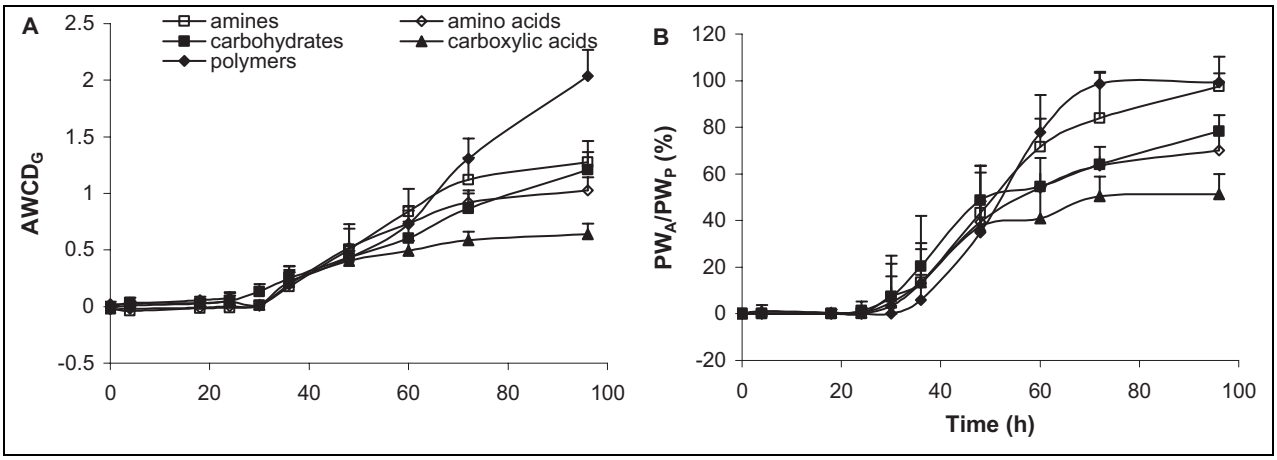


Fig. 3. Utilization of selected groups of substrates by the microflora inhabiting the effluent nutrient solution in closed NFT systems. The analysis was performed on the basis of the Micrologg GN-panel directly after inoculation and 4, 18, 24, 30, 36, 48, 60, 72 and 96 h after inoculation. $AWCD_G$ (A) expressing the sum of absorbance units of individual substrate sets divided by the number of compounds belonging to the substrate group at 590 nm by colour change of the redox dye tetrazolium blue and PW_A/PW_P (B) expressing the "number of positive wells" with an absorbance level >0.5 per substrate group divided by the number of compounds belonging to the substrate group were calculated. Displayed values are based on results obtained during the second experimental year (n=27±sd).

Also for the eleven substrate groups on the GN-panel (alcohols, amides, amines, amino acids, aromatic compounds, brominated compounds, carbohydrates, carboxylic acids, phosphorylated compounds) high affinity between the sampling incidents was found. Utilization of these compounds started slowly and low utilization was observed for all compound groups during the first 36 h subsequent to incubation. As the overlay of the GN-panel is dominated by carbohydrates, amino acids and carboxylic acids, a domi-

nance of these groups was expected with respect to the number of corresponding wells. However, when standardizing the number of responding wells to the number of compounds incorporated into the overlay, only five of the eleven groups of compounds comprised in the GN-panel were utilized above the threshold level of 0.5 absorbance units (Fig. 3). Results for the other groups, registered beyond this level, were lacking in consistency. Carbohydrates displayed the shortest lag phase of all of the compound groups. Far from all of the carbohydrates (78 %) comprised by the panel were utilized; the same situation was observed for amino and carboxylic acids (70 and 51 %, respectively). Within 72 and 96 h, all polymers and amines were metabolised. The first named group accounted also for the most frequently utilized class. Within these five groups of compounds, high affinity respect to single compounds, that had not been utilized at all, were found. However, differences occurred between the replicate systems for single carbon sources, without affecting the general result for the group of compounds.

Discussion

The present results indicate that there is a potential for microbial utilization of organic compounds in the effluent solution of closed hydroponic systems (Fig. 2, 3). Previous reports (SUNDIN et al. 1995; DE KREIJ 203) support the finding that phenolic acids disappear from the nutrient solution after a short acclimation (lag) phase as opposed to tap water. In addition to these studies we conclude that disappearance applies to a broader range of phenolic acids and that it is consistent over time. SUNDIN et al. (1995) indicated that disappearance of phenolic acids in the nutrient solution of closed hydroponic greenhouse systems was mediated by the resident microflora. ALEXANDER (1999) outlined that individual species often act on several structurally similar substrates and that the microbially mediated disappearance of related substrates is favoured by the initial compound. This might explain the rapid acclimation phase, which was observed for the assayed compounds in combination with the comparably high viable counts enumerated both for the general bacterial flora and fluorescent pseudomonads during the first year (Fig. 1). Interestingly, the pattern in disappearance of structurally very closely related compounds, such as BA, *p*OHBA and SA (*o*-hydroxybenzoic acid), differed. As no data on microbial proliferation in the nutrient solution during incubation were added to the present study, it is difficult to present a model applying to the processes of the here assayed compounds linked to growth. Such information as well as information on disappearance of different levels of added phenolic acids would be very valuable.

It might occur surprising that utilization of other groups of compounds using the GN-panel occurred initially much slower as compared to the results obtained for disappearance of phenolic acids and that similar results were displayed during both years of evaluation (Fig. 2 and 3). The high affinity towards both factors might be a result of the dilution step performed before inoculation to the GN-panel, which inhabits a standardization of cell density. As the cell number in the nutrient solution was reduced, microbial proliferation (ALEXANDER 1999) had to take place in order to detect a substantial utilization of the compound in terms of colour change of the redox dye. In contrast to the original purpose of the GN-panel, namely identification of pure cultures of gram-negative bacteria, we adopted the panel to study the general utilization pattern of a composite microflora. The substrate groups on the GN-panel display compounds expected in the rhizodesposition (LYNCH and WHIPPS 1990). The classification of the resident microflora in the effluent solution performed on the semi-selective media is coarse and comprises a lot of different species with different nutritional preferences. As in all complex systems, an ecosystem occurs in each single well of the panel favouring the species, which have the highest substrate affinity. This might explain the differences in utilization of some single compounds between the systems. It is though surprising that the general utilization of the substrate group was not affected.

The Biolog GN-panel has not been used for description of substrate affinity of composite microflora. The present results do not allow any conclusion on the quality in microbial composition of the effluent nutrient solution, but reflect the groups of substrates that the resident microflora is well adapted to and allows a grouping with respect to the length of acclimatization for individual substrate groups. BERKELMANN (1992) characterized the bacterial flora of the nutrient solution in closed hydroponic systems to be heterotrophic, dominated by *Pseudomonas*–p a nutritionally versatile group. This might explain the efficient utilization of substrates based on carbohydrates, amino acids, carboxylic acids and amines by the microflora inhabiting the effluent solution. The high utilization frequency and rate of polymers was less anticipated (MADIGAN et al. 1997). In this context, it should be emphasized that the sources on the GN-panel previously classified as polymers (GARLAND and MILLS 1991) is a diverse group of compounds. Three of them are storage polymers (glycogen, dextrin, cyclodextrin). Storage polymers occur in almost all microorganisms and serve as reserve material under nutrient-deprived conditions (MADIGAN et al. 1997).

Both microorganisms and oxygen availability are key factors for biodegradation of organic compounds (PATRICK 1971; ALEXANDER 1999). In closed hydroponic systems, both are strongly interrelated. Variation in intrinsic factors of the nutrient solution is expected to cause qualitative and quantitative shifts in microbial communities. BERKELMANN (1992) found differences in microbial colonization in closed cultivation systems with or without plants. KHALIL and ALSANIUS (2001) following the microflora inhabiting the nutrient solution of a closed commercial greenhouse system with tomato found considerable differences in viable count for the general fungal and bacterial flora as well as number of fluorescent pseudomonads, filamentous actinomycetes and *Trichoderma* during different stages in the vegetation period. In the light of the survey performed by KHALIL and ALSANIUS (2001) the observed differences in viable count between the two years might be judged as two extremes but not as unusual for closed hydroponic systems with tomato (Fig. 1).

Although closed hydroponic systems, in particular those adopting liquid hydroponics (nutrient film technique, deep flow culture), appear to be simple and straight forward, dramatic changes in the environmental conditions occur within the growing system and within the gutter (GISLERØD and KEMPTON 1983; GRAVES 1983). At the start of the unit fed with freshly recompensated nutrient solution, the pH and nutrient content as well as EC are optimised with respect to the crop's demand, whereas a concentration of nutrients may have taken place due to the plants uptake of nutrients and water until the end of the gutter, albeit a change of pH and EC as compared to the set values. Further, organic carbon conditions are enriched during the passage through the root zone of the crop and concomitantly, oxygen depleted. From the perspective of organic carbon situation, nutrient solution in a closed hydroponic greenhouse system should be considered as a continuous flow culture. However, the prevailing conditions are expected to change over time.

The O_2-content and -saturation in the effluent nutrient solution decreased with progressive growing period, which is also supported by the increasing BOD (Table 2). At the set-point temperature of 19 °C, an oxygen content of 9.0 mg O_2 L^{-1} should be expected (KRÜSSMANN 1999). This means that the oxygen content in the effluent nutrient solution of the present study was considerably reduced. However, the change in O_2-content and -saturation should be expected as the rhizosphere displays a zone of competition for oxygen (LYNCH 1990), in particular in NFT where a stratification within the root mat compiling different stages of active and decaying roots from the top to the bottom of the root mat, which interacts both with the organic carbon and O_2-situation. Different reports indicate a reduction in O_2-content concomittantly to addition of organic compounds to the nutrient solution of growing systems with or without plants (WOHANKA and LINDEMANN 2003; ALSANIUS and BRAND 2003).

Total organic carbon content as well as oxygen demand (COD and BOD) is randomly integrated into studies on closed hydroponic greenhouse systems and no threshold values are at hand for these particular systems. The values registered in the present study are regarded as moderate. Commercial analytical laboratories adopt a critical threshold value of 40 mg L^{-1} for TOC (Lennart Månsson International, pers. communication), but considerably higher levels have been observed (WAECHTER-KRISTENSEN et al. 1999) and the specific growing conditions, especially with respect to choice of growing medium, have to be taken into account. With respect to the grouping conducted by LESTER and BIRCKETT (1999) the biochemical oxygen demand should be considered as low. Measured values for COD are in the range of values measured in the effluent of other closed systems (LOSCHENKOHL and PORTING 1996; BAR-YOSEF et al. 2001). However, as both plants and microorganisms compete for O_2 in the root zone, an optimisation of the chemical composition of the nutrient solution should be considered.

All of the carbon sources integrated in the present study were soluble in nutrient solution. This takes off the first statement of the initial list of conditions predisposing for accumulation. The two other statements have not been rigorously examined with the framework of the present study. However, match in production site and utilization site might be seen indirectly as the phenolic acid background composition in the sampled effluent solutions was very low and the concentration of the selected phenolic acids declined within days after addition. Furthermore, presence of adequate enzyme complexes for utilization of the studied compounds should be presumed as the selected compounds disappeared.

From the present study we conclude that there is a potential for efficient utilization of phenolic acids as well as carbohydrates, amino acids, carboxylic acids, amines and polymers by the microflora inhabiting the effluent nutrient solution of closed hydroponic systems. It is tempting to conclude that the risk is low for these groups to accumulate in the nutrient solution of such growing systems as indicated by the low background content of phenolic compounds. Laboratory conditions and the displayed O_2-supply by agitation, however, do not mirror the conditions in the nutrient solution of a closed growing system. Further as disappearance or utilization was monitored in the present study we do not have any clue on the metabolic pathway, i.e. if they are degraded to CO_2 or subject to bioconversion. This latter fact might be of special interest with respect to phenolic compounds and their involvement in disease suppressive compounds (WELLER et al. 2002). In order to find a final answer to the hypothesis on accumulation of organic compounds in the nutrient solution of closed hydroponic growing systems, disappearance of organic compounds should be studied with special emphasis on O_2-availability.

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References

ALEXANDER, M. 1999: Biodegradation and bioremediation. 2nd ed. San Diego: Acad. Press.

ALSANIUS, B.W., S. KHALIL, M. HULTBERG, L. HAGTORN and T. BRAND 2001: Comparative study of plant performance and root microbial communities of tomato grown in closed NFT greenhouse systems as affected by seed inoculation with Pseudomonas fluorescens 5.014 and 5-2/4. In: KHALIL, S.: Microflora in the root environment of hydroponically grown tomato. Agraria **263**.

ALSANIUS, B.W. and T. BRAND 2003: Test of BIOVENTA sedimentation filter B600 in a plantless system – organic compounds and plant pathogens. Confidential contract report.

ALSANIUS, B.W. and V. JUNG 2003: Dynamics in functional diversity of heterotrophic microorganisms in a closed hydroponic commercial nursery stock plant production system. Europ. J. Hort. Sci. **68**, 67–76.

ANGLE, J.S., J.V. GAGLIARDI, M.S. MCINTOSH and M.A LEVIN 1996: Enumeration and expression of bacterial counts in the rhizosphere. In: STOTZKY, G. and J.-M. BOLLAG (eds.). Soil Biochemistry. New York: Marcel Dekker, Inc.. p.233–251.

BAR-YOSEF, B., T. MARKOVICH and I. LEVKOVICH 2001: Pepper response to leachate recycling in a greenhouse in Israel. Acta Hortic. **548**, 357–364.

BERKELMANN, B. 1992: Charakterisierung der Bakterienflora und des antagonistischen Potentials in der zirkulierenden Nährlösung einer Tomatenkultur (*Lycopersicon esculentum* Mill.) in Steinwolle. Geisenheimer Berichte **10**.

BOCHNER, B.R. and M. A. SAVAGEAU 1977: Generalized indicator plate for genetic, metabolic and taxanomic studies of microorganisms. Appl. Environ. Microbiol. **33**, 434–444.

DE KREIJ, C., W.T. RUNIA and A.M.M. VAN DER BURG 2004: Metabolites, their decomposition, production of tomato and bioassays from open and closed rockwool systems. Acta Hortic., in press.

EHRET, D.S., B.W. ALSANIUS, W. WOHANKA, J. G. MENZIES and R. S. UTKHEDE 2001: Disease control in closed nutrient systems. Agronomie **21**, 323–339.

GARLAND, J. L. and A. L. MILLS 1991: Classification and characterization of heterotrophic microbial communities in the basis of patterns of community-level sole-carbon-source utilization. Appl. Environ. Microbiol. **57**, 2351–2359.

GISLERØD, H. R. and KEMPTON, R. J. 1983: The oxygen content of flowing nutrient solutions used for cucumber and tomato culture. Scientia Horticulturae **20**, 23–33.

GRAVES, C. J. 1983: The nutrient film technique. Horticultural Reviews **5**, 1–44.

JENSÉN, P. and S. ADALSTEINSSON 1993: Root dynamics in lettuce as affected by externally supplied phenolic acids in the nutrient solution. Proceedings of the 8th International Congress on Soilless Culture, Hunter's Rest, Rustenburg, South Africa, 2–9 October 1992, pp. 185–198.

JUNG, V., L., CHIMUKA, J. Å. JØNSSON, N. NIEDACK, P. BOWENS and B.W. ALSANIUS 2002: Supported liquid membrane extraction for identification of phenolic compounds in the nutrient solution of closed hydroponic growing systems for tomato. Anal. Chim. Acta **474**, 49–57.

KHALIL, S. and B. W. ALSANIUS 2001: Dynamics of the indigenous microflora inhabiting the root zone and the nutrient solution of tomato in a commercial closed greenhouse system. Gartenbauwissenschaften **66**, 188–198.

KING, E.O., M.K. WARD and D.E. RANEY 1954: Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. and Clin. Med. **44**, 301–307.

KRÜSSMANN, G. 1997: Die Baumschule. 6th ed. Berlin: Paul Parey.

LESTER, J. N. and J.W. BIRKETT 1999: Microbiology and chemistry for environmental scientists and engineers. 2nd edition. London, New York: E. & F.N. Spon.

LOSCHENKOHL, B. and P. PORTING 1996: Concentration of oxygen and organic matter in a recirculating watering system. IOBC wprs Bulletin **19** (6), 158.

LYNCH, J. M. 1990: The Rhizosphere. Chichester: J.Wiley & Sons.

LYNCH, J. M. and J. M. WHIPPS 1990: Substrate flow in the rhizosphere. Plant Soil **129**, 1–10.

MADIGAN, M.T., J. M. MARTINKO and J. PARKER 1997: Brock's Biology of Microorganisms. 8th ed. Upper Saddle River, N.J.: Prentice Hall.

PATRICK, Z. A. 1971: Phytotoxic substances associated with the decomposition in soil of plant residues. Soil Sci. **111**, 13–18.

POSTMA, J., E.A VAN OS and G. KRITZMAN 1999: Prevention of root diseases in closed soilless growing systems by microbial optimization. Med. Fac. Landbouww. Univ. Gent **64** (3b): 431–440.

STRAYER, R.F. 1994: Dynamics of microorganism populations in recirculating nutrient solutions. Adv. Space Res. **14**, 357–36.

SUNDIN, P., B. WAECHTER-KRISTSENSEN and P. JENSÉN 1995: Effects of saprophytic bacteria in the closed hydroponic culture of greenhouse crops. Acta Hortic. **396**, 243–250.

WAECHTER-KRISTSENSEN, B., P. SUNDIN and P. JENSÉN 1994: Degradation of phenolic acids by bacteria isolated from hydroponic tomato culture with circulating nutrient solution. Acta Hortic. **381**, 611–614.

WAECHTER-KRISTSENSEN, B., S. CASPERSEN, S. ADALSTEINSSON, P. SUNDIN and P. JENSÉN 1999: Organic compounds and microorganisms in closed hydronic culture: occurrence and effects on plant growth and mineral nutrition. Acta Hortic. **481**, 197–204.

WELLER, D. M., J. M. RAAIJMAKERS, B. B. MCSPADDEN GARDENER and L.S. THOMASHOW 2002: Microbial populations responsible for specific soil suppressiveness to plant pathogens. Ann. Rev. Phytopathol. **40**, 309–348.

WOHANKA, W. and S. LINDEMANN 2003: Menno-Florades (9 % benzoic acid) as an additive to nutrient solution of a hydroponically grown tomato crop. Acta Hortic. (submitted).

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