Approaches for Mitigating the Environmental Impact of Greenhouse Horticulture

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Abstract

Greenhouse horticulture creates environmental impacts of different kinds. Use of energy for heating and lighting and pollution by pesticides and nutrients are major known problems, but impacts on the landscape and light emissions from supplementary lighting have also attracted attention. However, increased productivity and new technologies provide possibilities for reducing the impact of greenhouse horticulture on the surrounding environment. This thesis presents various approaches for moving greenhouse horticulture towards sustainability. Three main directions are examined: use of closed hydroponic systems and slow filters for reducing losses of nutrients and water; use of biosurfactants to control root disease and reduce the need for chemical pesticides; and use of light-emitting diode (LED) technology to save energy and reduce the use of chemical growth retardants. Closed systems with slow filters functioned satisfactorily with respect to phytosanitary issues. Closed systems eliminate the losses of nutrients and water and save 25-40% on the use of fertilizers and water. The resident microflora in these systems possessed the ability to produce biosurfactants that can be used to control zoosporic pathogens. LED lighting proved useful for growth control of flowering plants. The LED-technology did also affect plant growth strongly, which opens possibilities for plant growth regulation with different light regimes, thus eliminating the needs for chemical growth regulators. These three technologies (closed systems with slow filters, biosurfactants and LED) represent good examples of measures to create the greenhouse of the future.

Keywords: Biosurfactants, enzyme activity, filter skin, hydroponics, light-emitting diode (LED), microflora, *Pythium,* slow filter

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Populärvetenskaplig sammanfattning

Växthusodling innebär olika typer av miljöbelastning. Växthusodlingens miljöpåverkan har varit föremål för en långvarig debatt både i Sverige och utomlands, och växthusodling har pekats ut som ineffektiv och miljöfarlig. Huvudpunkterna i kritiken har varit energianvändning, användning av bekämpningsmedel och kemiska tillväxtregulatorer samt utsläpp av näringsberikat dräneringsvatten, men också påverkan på landskapsbilden och ljusnedsmutsning från assimilationsbelysning nattetid har debatterats i växthustäta områden. Ökning av produktiviteten och införande av ny teknik innebär stora möjligheter att minska växthusodlingens miljöbelastning och har också lett till en avsevärd sänkning av miljöbelastningen per producerad enhet. Ytterligare insatser behöver dock göras för att uppnå helt uthålliga produktionssystem. Denna avhandling behandlar olika aspekter på anpassningen växthusodlingen i en mer uthållig riktning. Tre huvudinriktningar är i fokus för denna avhandling; slutna hydroponiska system med långsamfilter för att minska utsläpp av näringsberikat spillvatten, biotensider för motverkande av rotpatogener i hydroponiska system och minskad användning av kemiska växtskyddsmedel, samt LED-teknik som innebär möjligheter både till energibesparing och till minskad användning av kemiska tillväxtregulatorer. Slutna hydroponiska system med långsamfilter visade sig ha goda egenskaper ur ett växtpatologiskt perspektiv. Användning av slutna odlingssystem innebär en total reduktion av spillvattenutsläpp, med åtföljande minskning av vatten och gödselmedelsförbrukning med 25-40%. Den naturliga mikrofloran i de hydroponiska systemen producerade biotensider som kan vara effektiva mot oomyceter. LED-baserad belysningsteknik visade sig användbar för styrning av blommande krukväxter. LED-tekniken hade också stark inverkan på växternas utveckling, vilket öppnar möjligheter för styrning av tillväxten med ljusprogram, med reduktion av användningen av kemiska tillväxtregulatorer som följd. Dessa tre teknologier (slutna odlingssystem med långsamfilter, biotensider och LED) är goda exempel på vilken typ av åtgärder som kan införas för att minska växthusodlingens miljöbelastning.

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

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 inoculation in closed hydroponic growing systems. *Accepted for publication in the Open Horticulture Journal.*

IV Hultberg, M., Bergstrand, K.-J., Khalil, S. & Alsanius, B.W. (2008). Production of biosurfactants and antibiotics by fluorescent pseudomonads isolated from a closed hydroponic systems equipped with a slow filter. *Antonie van Leeuwenhoek* 93, 373-380.

V Hultberg, M., Bergstrand, K.-J., Khalil, S. & Alsanius, B.W. (2008). Characterization of biosurfactant-producing strains of fluorescent pseudomonads in a soilless cultivation system. *Antonie van Leeuwenhoek* 94, 329-334.

VI Bergstrand, K-J., Schüssler, H.K. Recent progress on the application of LEDs in horticultural production. *Acta Horticulturae, in Press.*

The contribution of Karl-Johan Bergstrand to the papers included in this thesis was as follows:

I Greenhouse experiment, laboratory analyses, co-author in writing the paper

II Greenhouse experiment, laboratory analysis, co-author in writing the paper

III Planning and performing experiments, laboratory analyses, treatment of data, main author

IV Greenhouse experiment, laboratory analysis, co-author in writing the paper

V Greenhouse experiment, laboratory analysis, co-author in writing the paper

VI Planning and performing experiments, analysis, treatment of data, main author

Abbreviations

BOD	Biological oxygen demand
bPs	Biofilm polysaccharides
CU	Capillary uptake
cfu	Colony forming units
CO_2-eq	CO_2-equivalent
COD	Chemical oxygen demand
DIT	Drip irrigation technique
DOC	Dissolved organic carbon
EC	Electric conductivity
f. sp.	*forma specialis*
HPS	High pressure sodium
IPM	Integrated pest management
KB	King´s agar B
LED	Light emitting diode
OM	Organic matter
MA	Malt extract agar
mU	Milliunit
NB	Nutrient broth
NFT	Nutrient film technique
PDA	Potato dextrose agar
SF	Slow filtration
TI	Temperature integration
TOC	Total organic carbon
tPs	Total polysaccharides

1 Introduction

1.1 Greenhouse production of today

Greenhouse production involves a wide range of produce, such as vegetables, fruits, flowers, bedding plants and ornamental plants. The use of greenhouses has several advantages, which led to a dramatic increase in greenhouse-covered production areas worldwide during the 20th century (Wittwer & Castilla, 1995). Off-season production, increased land and water use efficiency, improved pest control, better produce quality and increased yield due to optimisation of growth factors such as temperature and CO_2 concentration are advantages achieved by greenhouse production (Wittwer & Castilla, 1995; Bjelland, 1972). Nowadays, more than 500 000 ha of agricultural land are covered with greenhouses globally. The Swedish greenhouse production area is around 260 ha (Jordbruksverket, 2010a). The economic importance of greenhouse production is strongly regionally dependent. In the Netherlands, the total greenhouse area is around 10 000 ha (tuinbouw.nl, 2010), which comprises about 0.5% of the agricultural land area in that country (EU, 2010), but contributes 39% of total turnover in the agricultural sector (tuinbouw.nl, 2010). Swedish greenhouse horticulture had an annual turnover of 1.6 billion SEK in 2008, with a distribution of 42% for vegetables and berries and 58% for ornamental crops (Jordbruksverket, 2010a).

The greenhouse production systems of today can be divided into two distinct types: 'northern' type, typically glasshouses with heating and generally a high content of technology, and 'southern' type, plastic-covered houses with simple or no heating systems and a low level of technical complexity (Castilla & Hernández, 2007; Wittwer & Castilla, 1995; Castilla, 1994). In Europe, the production areas with southern-type greenhouses are located in the Mediterranean region, while northern-type greenhouses are

used in countries such as the Netherlands, the UK and the Scandinavian countries (Wittwer & Castilla, 1995). This thesis focuses on northern-type greenhouse production, but some of the findings may be partly applicable to southern-type greenhouses.

1.2 Environmental implications of greenhouse production

Northern European greenhouse horticulture is resource-intensive, with high inputs of fossil fuels, nutrients, growing media and chemical pesticides. This has implications for both the external and the occupational environment (Fig. 1). Possible impacts on the external environment include pollution of air, groundwater/surface water and the land on which the greenhouse is built and wastes from the operation. A poor occupational environment can have a negative effect on workers' health, and possibly produce quality.

Greenhouse production has been widely criticised for its high demand for resources and its CO_2 emissions (Carlsson-Kanyama, 1998; Wada, 1993). Greenhouses used for production in the cold season require substantial amounts of energy for maintaining the desired temperature. Traditionally, fossil fuels such as oil, coal or natural gas have been used (Jordbruksverket, 2010b; Taragola, 1996; van der Velden & Verhaegh, 1992). Conventional greenhouses heated by natural gas are estimated to generate 800 ton of CO_2 ha^{-1} a^{-1} (in Dutch conditions). Carlsson-Kanyama (1998) reported emissions of around 4 kg of CO_2-eq per kg Dutch or Swedish tomatoes, including emissions from transportation to the retailer and production of fertilisers. However, the increasing cost of fossil energy and the debate on climate issues associated with the use of fossil fuels has intensified the search for alternative ways of heating (Lagerberg & Brown, 1999). Electric energy is also used in substantial amounts in greenhouse horticulture, the two major uses being: i) electricity needed for the operation of technical systems, and ii) electricity used for artificial lighting. Artificial light, in turn, is used for three major purposes: i) supplementary light for driving photosynthesis during periods of insufficient natural light, ii) photoperiodic light for regulation of flower initiation in long- or short-day plants, and iii) the sole light source for growing plants in rooms with no intromission of natural light (Wittwer & Castilla, 1995; Gray, 1971; Canham, 1966).

Leaching of pesticides (Kreuger *et al.*, 2010; Teunissen, 2005) and release of nutrient-enriched wastewater (Bres, 2009; Hansson, 2003; Van Os, 1994) are problems associated with greenhouse horticulture. At commercial

production sites, excessive nutrient solution is often discarded in a process referred to as an *open* system. An alternative is to collect the excessive nutrient solution, mix it with fresh water and readjust the nutrient content, and return it to the crop, a process referred to as a *closed* system (Jensen & Collins, 1985). There are diverging reports on the losses of nutrients from open hydroponic systems; annual losses of 850 kg N, 80 kg P and 850 kg K ha^{-1} have been reported for Swedish tomato nurseries (Hansson, 2003), whereas Marcelis *et al.* (2000) estimated that in Mediterranean conditions, 300-350 kg N and 125-300 kg P are lost per ha and year. Depending on the greenhouse construction, the excess nutrient solution can end up in the groundwater, in surface water or at a municipal wastewater treatment plant. Release to the groundwater or surface water causes severe environmental problems with eutrophication (Göhler & Molitor, 2002). Also delivery to municipal wastewater plants causes problems with disturbed nitrogen removal. Open hydroponic systems are inefficient in their use of water. The greenhouse system as such is considered water-efficient compared with open field cultivation, as evaporation is decreased by higher humidity, restriction of irradiance by shading and, for traditional greenhouse systems, lower air movements (Stanghellini *et al.*, 2003). The water consumption for a hydroponic greenhouse tomato crop ranges from the theoretical lower limit of 1.5 L (van Kooten *et al.*, 2004) up to 24 L (Munoz *et al.*, 2008) per kg of tomatoes. Water consumption and the associated risk of depletion of groundwater resources is a major problem in regions with large areas of greenhouse cultivation (Stanghellini *et al.*, 2003).
Other problems such as emissions of light (van't Ooster *et al.*, 2008; Hänel, 2006), disposal of solid wastes such as used growing media (Alsanius *et al.*, 2010), plastic mulches and plant residues and impact on the landscape have also been mentioned in connection with greenhouse horticulture.

In order to reduce the inputs and outputs depicted in Figure 1, it will be necessary to close the loops. The concept of 'the closed greenhouse' needs to be implemented to meet the demands for environmentally sound greenhouse horticulture.

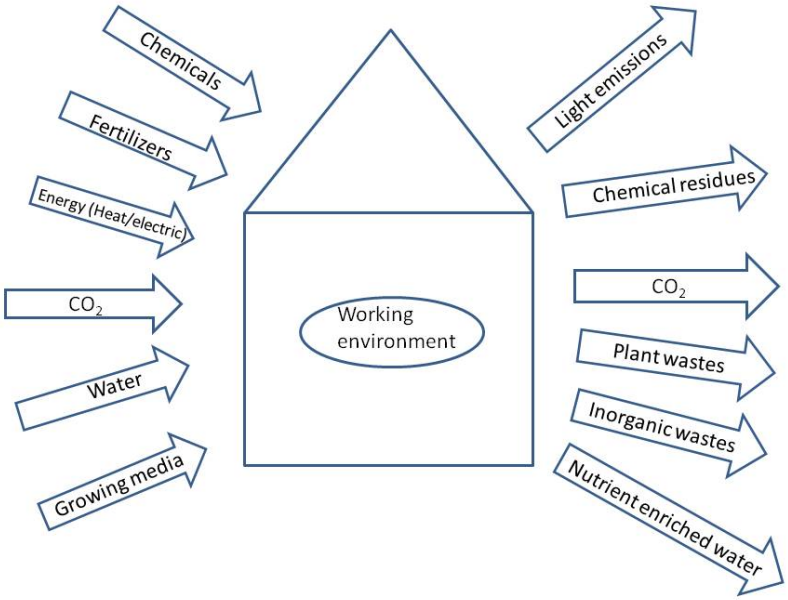

Figure 1. A greenhouse industry affects the environment by inputs and outputs in a number of ways.

1.2.1 The closed water and nutrient cycle

Closed hydroponic systems are the obvious solution to eliminate leaching of nutrients, as well as reducing the leakage of pesticides and optimizing water use. Closed fertigation systems are also recommended from a legislative point of view, as any release of pollutants to the groundwater or marine environments is prohibited by law in both Europe (WFD, 2000) and Sweden (Miljöbalken, 1998). However, closed systems involve some possible problems for the crop. Unevenness in the nutrient distribution and plants' uptake of nutrients will cause imbalances in the nutrient solution which passed through the root zone (Schröder & Lieth, 2002). However, with current knowledge on nutrient composition and the availability of computer programmes (van Kooten *et al.*, 2004; Savvas, 2002), this should no longer cause any problems for the commercial-scale grower.

Accumulation of Na and Cl can occur in a closed system (Sonneveld, 2000) and can lead to salinisation of the nutrient solution, causing poor plant growth, although increased salinity is not necessarily detrimental to the crop (Dorais *et al.*, 2000). In general, closed systems are considered to place

higher demands on raw water quality (Göhler & Molitor, 2002) compared with open systems. Collecting rainwater is a recommended approach that yields water with low ionic concentrations (Schwarz *et al.* 2005). Organic compounds deriving from root exudation can also accumulate in the nutrient solution and this was often reported to have caused early failures of closed systems during the 1980s. However, recent research (Jung *et al.* 2002; Jung, 2003; Alsanius & Jung 2004; Jung *et al.* 2004) has shown that this problem is less significant and that occasional crop failures are attributable mainly to infections of the roots.

The possible dispersal of water-borne pathogens with the nutrient solution is a major problem with closed hydroponic systems (Ehret *et al.*, 2001; Stanghellini & Rasmussen, 1994). To prevent this, a disinfection step has to be included in the system to disinfect the collected nutrient solution before reuse in the crop. A number of disinfection methods are commercially available today: UV treatment (Zhang & Tu, 2000; Runia, 1994; Buyanovsky & Gale, 1981), pasteurisation (Runia & Amsing, 2001), slow filtration (Wohanka, 1993) (papers I-III), microbial stabilization (van Os & Postma, 2000; Waechter-Kristensen *et al.* 1994) and chemical treatment (Alsanius & Brand, 2000). The chemical treatments include synthetically produced surfactants used to eliminate zoosporic pathogens in hydroponic systems (Ehret *et al.*, 2001). However, surfactants require consecutive additions and considering them a chemical treatment makes them questionable from a legislative and health perspective. In contrast, internal production of biosurfactants within the system is regarded as a biocontrol measure and is therefore more attractive from an economic and environmental perspective (Papers IV-V). Slow filtration and biosurfactants were chosen for further studies within this thesis (Papers I-V).

1.2.2 The closed air cycle

Closing the air cycle of the greenhouse (e.g. reducing ventilation) provides several benefits from an environmental point of view. Two major advantages are apparent: reduced resource use and increased yield (Lee *et al.*, 2009). The closed greenhouse concept is described by several authors (van´t Ooster *et al.*, 2008; Zaragoza *et al.*, 2007; Bakker *et al.*, 2006; Opdam *et al.*, 2005). The concept involves collection of redundant heat from the greenhouse during summer days and storing it in tanks or in underground aquifers for use during night time or winter time, respectively. In its original form (Opdam *et al.*, 2005), the closed greenhouse concept provided no possibilities for natural ventilation, thus requiring a cooling capacity of

around 700 Wm^{-2}. Due to high investment costs for fully closed greenhouses, the semi-closed greenhouse concept was introduced. This retained the vents for enabling reduced natural ventilation around midday in summer (Heuvelink *et al.*, 2008). Systems for removing redundant heat without collecting it have also been suggested (Särkkä *et al.*, 2006).

Reduced ventilation allows the CO_2 concentration to be increased to 1000 ppm (compared with around 500 ppm in an open greenhouse) (Opdam *et al.*, 2005). This can increase crop yield by 22% (De Gelder *et al.*, 2005). The occupational exposure limit for CO_2 is 5000 ppm (AFS, 2005). The reduction in water use due to recovery of condensed water can be up to 50% (Opdam *et al.*, 2005). The need for an external supply of CO_2 is also reduced with less ventilation (van´t Ooster *et al.*, 2008), and there is less need for chemical pest control due to the reduced risk of contamination from outside. Van Os *et al.* (1994) calculated that 30-50% of the pesticides applied leave the greenhouse via ventilation. By closing the air cycle and reducing ventilation, the risk of pollution from chemicals to the surrounding environment is expected to be decreased. Regarding the occupational environment, closed greenhouses provide benefits through reduced use of chemicals in the production system, as exposure to chemicals is a major problem for workers' health within greenhouse horticulture (Hjollund *et al.*, 2004; Jaga & Dharmani, 2003; Illing, 1997).

A challenge with the closed greenhouse concept, as described by Opdam *et al.* (2005), is the high dependence on electric energy. The environmental impact of electricity is largely dependent on the production method (Gagnon *et al.*, 2001). In order to reduce the reliance on externally supplied electric energy, various systems for integration of photovoltaic panels into the greenhouse structure have been suggested (Sonneveld *et al.*, 2006). The closed greenhouse concept is associated with high investment costs, which may compromise the economic feasibility (Bakker, 2009; Heuvelink *et al.*, 2008), in particular for small businesses.

1.2.3 The closed energy cycle

The greenhouse itself is by definition a sun collector, which annually collects from the sun two to three times the energy needed for heating during winter time (Heuvelink *et al.*, 2008; Bot, 1994). However, radiation is highest in summer months when ambient temperatures are high, thus leading to low heating requirements, and low in winter, when the demand for heat supply is high. The normal case involves ventilating away a vast amount of excess energy during the summer months, and using external

fuels for heating the greenhouse during winter. To increase the sustainability of greenhouse horticulture, the proportion of solar energy used will have to increase and the use of externally supplied fuels will have to decrease. The first step in maximising the efficiency of the greenhouse with respect to solar energy utilisation is to decrease the need for externally supplied heat during winter (Bakker *et al.*, 2008). Technical improvements in greenhouse buildings, such as insulating covering materials (Bot *et al.*, 2005; Sonneveld & Swinkels, 2005) and the use of thermal screens (Tantau, 1998), have been implemented since the first oil crisis in the 1970s. The easiest way of conserving energy from the daylight hours to the night is passively. The ground below the greenhouse, but also the greenhouse construction itself and the crop, accumulate heat. Through the use of temperature integration (TI), this effect can be exploited (Tantau, 1998). Temperature integration is based on the fact that plants respond to diurnal or weekly mean temperatures, rather than momentary temperatures (Cockshull *et al.*, 1981). The application of model-based TI can save 5-15% on the energy required for heating (Elings *et al.*, 2005; Körner & Challa, 2003).

The second step in reducing the energy demand for heating is to actively collect heat by closing the air loop and recovering the redundant heat, as described in section 1.2.2. The redundant heat can be stored for use during night time, or during the winter season, depending on storage method. Suggested technologies for heat storage are water tanks, underground aquifers (Heuvelink *et al.*, 2008; Opdam *et al.*, 2005), the ground (Mavroyanopoulos & Kyritsis, 1986), or phase-change materials (Öztürk, 2005; Kürklü, 1998). As the annual solar radiation influx by far exceeds the heating demand, a fully closed greenhouse with seasonal storage will produce surplus heat, which can be used for other buildings (Bakker *et al.*, 2008). The total reduction in primary energy use for a closed greenhouse based on long-term storage is 20-35%, calculated per unit area (Opdam *et al.*, 2005). However, with respect to the increase in productivity, the reduction per kg produce should be greater.

The use of electricity for assimilation lighting can be reduced by more efficient light sources. The High Pressure Sodium (HPS) lamps traditionally used for greenhouse purposes have an electricity to light conversion of around 35% (Massa *et al.*, 2006) or 1.8 μmol PAR J^{-1} (van Ieperen & Trouwborst, 2008), which is regarded as efficient. However, the spectral output from HPS lamps is less favourable with respect to plant photosynthesis (Baevre & Gisleröd, 1999). The use of Light Emitting Diode (LED) technology enables a more precise composition of the spectral distribution and narrow-band lighting (Hogewoning *et al.*, 2007; Pinho *et*

al., 2007). With the application of narrow-band lighting, adjusted to the active spectrum for photosynthesis, less light will be required to obtain the same assimilate production, with lower energy consumption as a result. Pinho *et al.* (2007) reported 53% higher fresh weight in lettuce grown under LEDs than in those grown under HPS at the same photosynthesis photon flux. Furthermore, Hogewoning *et al.* (2007) indicated that the possibilities for narrow-band lighting, combined with the fact that LED equipment is improving extremely rapidly with respect to energy efficiency, makes LED technology highly interesting for the horticultural sector. In this thesis, LED lighting was studied in Paper VI.

1.2.4 The closed raw material cycle

Greenhouse horticulture demands inputs of various raw materials; the greenhouse building itself needs concrete, steel and glass or plastic for its construction, while the crop requires fertilisers, plastic mulches, irrigation pipes and growing medium. Significant amounts of plastic mulches are used in greenhouse horticulture. According to Stanghellini *et al.* (2003), around 1 t ha^{-1} of plastic is used and has to be disposed of after terminating the crop. The same authors estimated that a rockwool-based hydroponic tomato crop uses 2 t of growing media ha^{-1}. Keskitalo (2009) calculated that 1.8 kg of 'non re-usable' waste are produced per ton of tomatoes and that the CO_2 emissions from 'marginal' sources (growing medium, plastic mulch, packaging material and production of N fertilisers) are around 80 kg per ton of tomatoes.

Greenhouse production also gives rise to significant amounts of biomass waste, such as leaves, shoots, non-marketable produce and spent organic growing medium (Cheuk *et al.*, 2003). Solid non-produce biomass can amount to some 250 ton per hectare and year for a tomato crop (Stanghellini *et al.*, 2003).

In order to reduce the amounts of waste to be removed from the greenhouse, on-site composting is recommended (Cheuk *et al.*, 2003). More advanced composting procedures allow harvesting of both heat for heating and CO_2 for enrichment of the greenhouse atmosphere (Fulford, 1986; Fulford, 1983). Greenhouse wastes are also valuable as raw material for biogas production (Bouallagui *et al.*, 2003).

In order to reduce plastic waste, Stanghellini *et al.* (2003) suggest the use of glass or long-life plastics for greenhouse covering, and biodegradable plastics for mulching. Recycling of plastic for raw material or power production is the obvious alternative today, due to EU restrictions on

landfill disposal. For growing media used in sustainable closed growing systems, van Os (1994) suggests certain standards to be met, such as resistance to steam sterilisation, no decrease in physical properties during use, a lifespan of at least three years and low costs. Growing media that meet these conditions include perlite, pumice, rockwool and polyurethane foam (Van Os, 1994). The same author suggests Nutrient Film Technique (NFT) or aeroponics as sustainable culture practices, as no growing medium at all is used in these methods. The use of composted wastes as growing medium has been suggested by several authors (Surrage & Carlile, 2009; Benito *et al.*, 2004; Cull, 1982), mainly as a way to reduce the exploitation of peat bogs.

2 Objectives and hypotheses

2.1.1 Main objectives

The greenhouse sector in Sweden needs to improve its sustainability and decrease its environmental impact. Three key areas for improvement were identified here: i) Use of fertiliser and discarding of spent nutrient solution, ii) use of chemical pesticides and growth regulators, and iii) use of energy and CO_2 emissions.

Within the framework of this thesis, these three areas were studied with respect to closed water and nutrient cycling and alternative technologies for illumination. Both these options can lead to reduced use of pesticides and/or growth retardants.

Implementation of closed growing systems within greenhouse horticulture is central in order to decrease the use of fertilisers and water and the discarding of nutrient solution.

Two options for reducing the use of chemicals (pesticides and chemical growth retardants) were investigated in this thesis: hydroponic systems with slow filter and biosurfactants as a way of controlling root pathogens; and LED technology for controlling growth by management of the light spectrum.

In terms of energy use, the work within this thesis was mainly aimed at reducing electric energy for driving photosynthesis through assimilation light. Here too, our expectations were centred on LED technology in order to optimise spectral distribution and control light intensity and composition, and thus increase efficiency in the use of electric energy.

2.1.2 Hypotheses

The general hypotheses for the studies included in this thesis were:

I: Closed hydroponic systems with integrated slow filters are robust and safe from environmental and phytosanitary aspects (Papers I, II and III)

II: The resident microflora in a closed hydroponic system possesses abilities for producing biosurfactants, possibly controlling oomycete pathogens spread by the nutrient solution (Papers IV, V)

III: LED technology can replace HPS technology with respect to crop development (Paper VI)

IV: Monochromatic LED-light can be useful for flowering and growth regulation of ornamental pot plants (Paper VI).

3 Materials & Methods

3.1 Experimental setup

3.1.1 Greenhouse experiments-tomatoes (Papers I-V)

The experiments were performed in a research greenhouse at SLU Alnarp, Sweden (55°39N, 013°04E). In Paper I, the treatments were addition or no addition of fungal cell wall preparations to the filter skin of the slow filter. In Paper II, three treatments with different EC levels (EC 2, 3 and 5 mS cm^{-1}, respectively) were compared, while Paper III compared treatments with or without addition of mycelium of *Pythium aphanidermatum* to the filter skin.

The experiments were performed in a 90 m^2 greenhouse chamber. Tomato plants (*Solanum lycopersicum* cv. Aromata) were grown in NFT gutters in six independent systems. Two slow filter units were connected to each system. Each slow filter consisted of a 100 L cylindrical plastic container filled with compressed rockwool granulates (Granulaat, Grodan BV., Roermond, the Netherlands) at a density of 136 kg m^{-3} (Papers I, II) or 190 kg m^{-3} (Papers III-V). Filter surface area was 0.19 m^2 and filter depth was 0.4 m + drainage layer 0.1 m. The flow rate was set to 300 l m^{-2} h^{-1}. 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 to 5.8±0.1 (in Paper II, different EC regimes were used with respect to treatment). The temperature of the greenhouse chamber was set to 19°C/18°C (Papers I, II) or 22°C /21°C (Papers III-V) with vents opening when the temperature exceeded setpoints by 2°C. Artificial lighting was used to compensate for low natural light conditions (HPS, 200 W m^{-2}, 11 h day^{-1}).

The upper layer of the filter skin was enriched weekly with fungal cell wall preparation of *Fusarium oxysporum* f.sp. *cyclaminis* as described by Sivan & Chet (1989), modified by Brand & Alsanius (2004a) (in Paper I, only one treatment). The fungal cell wall preparation was added at a density of 9.2 g m^{-2} (Papers I, II) or 10.5 g m^{-2} (Papers III-V). In brief, aliquots (20 mL) of yeast medium (per L distilled water: 10 g glucose (Fluka 49150), 5 g yeast extract (Merck 1.03753.0500), 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 25°C in the dark, mycelium was harvested and washed four times in deionised water with intermediate resuspension and centrifugation. Mycelium was autoclaved and 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 passage through the filter was restarted.

The overall objective of these studies was to identify factors possibly impairing the efficacy of slow filters.

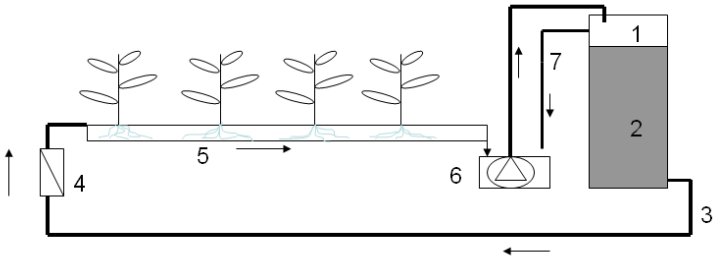

Figure 2. Schematic view of the NFT systems with integrated slow filter used for the greenhouse experiments in Papers I-V. 1: Water supernatant, 2: Filter medium, 3: Filter outflow, 4: Flow meter, 5: NFT gutter, 6: Reservoir with plunger pump, 7: Filter overflow.

3.1.2 Greenhouse experiments-pot plants (Paper VI)

Three experiments with LED light were carried out: i) LED as sole light source in a growth chamber; ii) LED used as day extension for photoperiod purposes; and iii) LED used for supplementary assimilation light. All LED experiments were performed with three model plants: *Chrysanthemum* x *morifolium* 'Token', *Kalanchoë blossfeldiana* 'Simone', and *Euphorbia pulcherrima* 'Novia'. Plants were potted in mid-August in a peat-based growing medium and were hand-irrigated with respect to depletion. The fertilisers Calcinit ($Ca(NO_3)_2$) and Superba brun (NPK 11-4-21+micronutrients, Yara AB, Landskrona, Sweden) were supplied weekly in a solution at EC 1.9±0.1, pH 6.7±0.2.

For the growth chamber experiments, four monochromatic lights blue (469 nm), green (525 nm), yellow (585 nm) and red (620 nm) and polychromatic white light (430-730 nm) were used. The light intensity was 25 μmol s^{-1} m^{-2} at a photoperiod of 18 h day^{-1}. The temperature was kept at 19°C. Each treatment had three replicate plants per species.

For the experiments on photoperiodic light, a greenhouse with day-length chambers that enabled exclusion of natural daylight was used (Fig. 3). LED equipment comprising 22W LED devices (TRG components, Arboga, Sweden) in four monochromatic colours and polychromatic white light was installed inside these chambers (Table 1). Greenhouse temperature was set to 18°C d/n with vents opening when the temperature exceeded 20°C.

The day was divided into three sectors of 8 h each: 8 h natural light (screens open), 8 h LED light (under closed screens) and 8 h dark (screens closed). Each treatment had seven (*Chrysanthemum, Kalanchoë*) or ten (*Euphorbia*) replicate plants per species.

Table 1. *Properties of the different light sources used for photoperiodic light in Paper VI. Light intensity was measured at a distance of 0.7 m from the light source*

	Blue	Green	Red	Yellow	White
λ (nm)	460	525	620	585	430-730
Lux	239	1230	240	380	2000
μmol s^{-1} m^{-2}	30.3	13.3	11.6	6.2	40
Stated effect (w)	22	22	22	22	22
Measured effect (w)	16	16	11	11	18.5

LED as complementary assimilation light was compared with conventional HPS technology. The experiment was carried out in a 90 m^2 greenhouse chamber in natural day length conditions (decreasing from 16 to 8.5 h day^{-1} during the experiment). The temperature setpoint was 18°C, with vents opening at 20°C. Additional light was given for 8 h day^{-1}. Two treatments were used; white LED (2 x 100W, ParkWay LED, Ruud Lighting Inc., Racine, USA) and HPS light (400W, Philips Master). Each treatment had 10 replicate plants per species.

Figure 3. Experimental setup used for the photoperiodic LED experiments in Paper VI.

3.1.3 Commercial greenhouses (Paper I)

In Paper I, two commercial systems equipped with closed hydroponic systems with integrated slow filters were studied. The first system produced ornamental pot plants in a capillary sub-irrigation system on a production area of 1 ha. The recirculating nutrient solution was disinfected using a slow filter with mineral wool (filter depth 1 m, filter surface 5.9 m^2). The second commercial greenhouse was a tomato producing unit with an area of 0.4 ha. Plants were grown in plastic containers with pumice, and fertigated by a drip irrigation technique (DIT) system. Drainage nutrient solution was collected in a gutter system and was filtered through a slow filter (depth of filter bed 1 m, surface area 10 m^2). Mineral wool (Granulaat, Grodan BV., Roermond, the Netherlands) was used as filter medium. The filtered solution was resupplied to the crop after mixing with fresh water and adjustment of EC and pH. Samples from the commercial systems were taken every second week from May to October, as described in section 3.2.1.

3.2 Analyses

3.2.1 Performance of slow filters

Sample collection (Papers I-III)

Samples were taken from the supernatant nutrient solution, the nutrient solution effluent after the slow filters and the filter skin every 2 weeks during the experimental period. Nutrient solution samples were taken in sterile 150 mL laboratory flasks and immediately transported to the laboratory for microbial analysis. Samples for TOC analysis were frozen at -20°C during storage. Filter skin samples were taken aseptically with a pair of tweezers from the top 5 mm of the filter skin. Samples for microbial analysis were analysed at once, whereas samples for enzyme activity analysis and TOC analysis were frozen at -80°C for 30 minutes. After lyophilisation, the samples were stored at -20°C. Samples for polysaccharide analysis and DNA analysis were stored in wet condition at -20°C.

Microbial analysis (Papers I-III)

The microflora of the nutrient solution and filter skin was analysed with respect to selected microbial groups using a plate-count technique. Different agar media were used (Table 1): R2A (Difco 218263, prepared according to the manufacturer's instructions) for general bacterial flora; Malt extract Agar (MA, per L distilled water: 10 g malt extract (Difco 218630, 20 g Bacto agar (Difco 0140-01)) for general fungal flora, Kings agar B (KB, (King *et al.*, 1954)) for fluorescent pseudomonads; Potato Dextrose Agar (PDA, Difco 213400, prepared according to the manufacturer's instructions and supplemented with rifampicin (Duchefa, Haarlem, the Netherlands, 100 μg ml^{-1}, Paper I) or rifampicin and kanamycin (100 μg mL^{-1}, 30 μg mL^{-1}, respectively, Paper III) for *Pythium aphanidermatum*; Komada medium (Komada, 1975) for *Fusarium oxysporum* (Paper I) and Chitin Oatmeal Agar (COA, (Postma *et al.*, 1999)) (Paper I). For all analyses, duplicate samples with two parallels were analysed and plates containing 30-300 colonies were enumerated.

Table 2. *Semi-selective media and incubation conditions used in this thesis*

	Media	Inoculation volume	Incubation time	Note
General bacterial flora	R2A	100 µL	3 days at 25°C	
General fungal flora	MA	100 µL	4 days at 25°C	
Fluorescent pseudomonads	KB⋆	100 µL	2 days at 25°C	1
Pythium aphanidermatum	PDA with antibiotics	200 µL	18 h at 35°C	2
Filamentous actinomycetes	COA	100 µL	5+5 days at 25°C	3
Fusarium oxysporum	Komada	100 µL	5 days at 25°C	

1: Reading under ultraviolet light (366 nm UV-lamp, Blak ray UVL-56, Ultraviolet Products Inc. USA).

2: PDA supplemented with rifampicin (100 µg mL^{-1}) and kanamycin (30 µg mL^{-1})

3: Membrane filter removed after 5 days, followed by a further 5 days of incubation
⋆ King et al., 1954

Efficacy tests (Papers I,II,III)

The experimental systems were subjected to efficacy tests three times during each experiment. *Fusarium oxysporum* f.sp. *cyclaminis* was used as model organism for the efficacy tests. *F. oxysporum* was inoculated on PDA agar plates (PDA, Difco 213400, prepared according to the manufacturer's instructions). After a two-week incubation period, plates were supplied with

10 mL of sterile NaCl solution (0.85% v/w) and the mycelia overgrowth on the plates was rubbed off using a sterile spatula. The conidial suspension obtained was filtered through cheesecloth and reached a final concentration of approximately 10^7 cfu mL^{-1}. The flow through the filters was stopped and the conidial suspension was inoculated into the system in the reservoir (item 6 in Fig. 2) at a final concentration of approximately 10^4 cfu mL^{-1}. Filters were started and samples were taken before and after filtration every 30 minutes for the next 2 h (before filtration) or 4.5 h (after filtration), respectively. Samples were analysed using Komada medium (Komada, 1975). The numbers of colonies were counted after five days of incubation at 25°C and efficacy was calculated as:

$$\text{Efficacy (\%)} = 1 - (cfu_{postfiltration}) \times (cfu_{prefiltration})^{-1} \times 100 \quad (1)$$

Enzyme activity (Papers I-III)

The extracellular enzyme activity of five different enzymes (protease, chitinase, cellulase, β-1,3-glucanase and xylanase) in the filter skin was analysed using the colorimetric method of Brand & Alsanius (2003). A 0.5 mL portion 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, glucanase, cellulase and xylanase, respectively) was added to Eppendorf tubes with 0.02 g of the lyophilised sample. Following 2 h of incubation at 37°C, 150 μL of the enzyme substrate were transferred to a microtitre plate. To prevent clogging of pipette tips by organic materials, the samples from the commercial systems were centrifuged in Eppendorf vials for 5 min using a desk centrifuge (Millipore, Billerica, USA) before transferring the enzyme substrate. After the 2 h incubation period, 150 μL of sulphuric acid (2 M) were added to lower the pH, causing degraded molecules of enzyme substrate to precipitate, while unaffected molecules remained in solution. After centrifugation (3250xg, 20 min.), the supernatant solution was transferred to an additional microtitre plate. Extinction of the solution was measured spectrophotometrically with a microplate reader (Asys Hitec, Austria, software Digiwin), at 590 nm (chitinase) or 550 nm (cellulase, β-1,3-glucanase, protease, xylanase). The controls were non-incubated samples prepared as described above. Enzyme activity was calculated as:

$$\text{Milliunit} = E_s - E_c \times 1000 \times t^{-1} \quad (2)$$

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

Polysaccharide analysis (Paper III)

Total polysaccharide and biofilm polysaccharide content (mg g^{-1}) of the filter skin were analysed using the modified Dubois method (Furtner *et al.*, 2007; Dubois *et al.*, 1956). Total polysaccharide (tPs) was analysed by incubating lyophilised filter skin samples with 5% phenol solution and concentrated sulphuric acid in test-tubes at 25°C. After 25 min., incubation tubes were centrifuged and the supernatant solution was transferred to plastic cuvettes. Light absorbance was measured at 488 nm with a spectrophotometer (Cary 50, Varian Inc. Palo Alto, USA). The tPs content was calculated on the basis of a glucose standard curve.

Biofilm polysaccharide (bPs) content was analysed as described by Furtner *et al.* (2007). In brief, lyophilised samples (0.05 g) were incubated with 12.5 mL solution of 0.23% formaldehyde and 0.85% NaCl. After 1 hour, 5 mL of NaOH solution (1 M) were added. After centrifugation (20 000xg, 20 min.), the solution was membrane-filtered (Minisart, 0.2 μm, Sartorius, Göttingen, Germany). The polysaccharide content of the filtered solution was determined as described for tPs.

DNA analysis (Paper III)

To ensure that cell lysis was low during polysaccharide extraction, the DNA content of the filter skin was measured using the 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 DNA assay solution (90 mL dist. water, 10 mL TNE buffer (1000 mL dist. water, 12.11 g (trishydroximethyl)aminomethane, 3.72 g EDTA $Na_2 \times 2H_2O$) and 10 μL dye stock solution (10 mL dist. water, 10 mg Hoechst dye H 33258). The DNA content was measured photometrically (Hoefer DyNA Quant 200, Hoefer Scientific Instruments, San Francisco, USA) and expressed as mg g^{-1}.

Chemical/physical analyses (Papers I-III)

Total Organic Carbon (TOC, mg L^{-1}) was measured using the system of Hach-Lange (LCK 385, Hach-Lange AG, Düsseldorf, Germany). In

principle, organic compounds in the sample suspension were oxidised and the carbon dioxide produced caused a colour reaction in a reagent. This colour reaction was measured spectrophotometrically using a photometer (Xion 500, Hach-Lange AG, Düsseldorf, Germany). Chemical oxygen demand (mg L^{-1}) was determined (Papers I, II) using tests from Hach-Lange (LCK 314) in which 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, DOC (dissolved organic carbon) was determined as described for TOC, but the sample solution was membrane-filtered (Filtropur S 0.2, Sarstedt, Nümbrecht, Germany; pore size 0.2 μm) before analysis. In Paper I, biological oxygen demand (BOD_5, mg L^{-1}) was determined using Hach-Lange test LCK 555 with measurement after five days of incubation.

Organic matter (OM) content of the filter skin (%) was determined at the end of the experiment (Paper III). 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:

$$OM\ (\%) = 1 - (Wt\ sample_{postincineration}) \times (Wt\ sample_{preincineration})^{-1} \times 100 \quad (3)$$

Levels of dissolved oxygen (mg L^{-1}) in the nutrient solution before and after filtration were measured once a week (experimental systems) or in connection with sampling (commercial systems) with a hand-held 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).

3.2.2 Crop assessment (Paper III)

For the tomato crops, crop development was monitored weekly in the greenhouse or every second week in the commercial systems. The following state variables were monitored: plant height (cm), number of leaves, 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 tutorial string, after seven days of growth (14 days for commercial systems). The numbers of fully developed leaves (under flowering truss) were counted. Stem diameter was measured at two opposite 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.

3.3 Detection of biocontrol metabolites produced by resident microflora (Papers IV, V)

Samples were taken from the hydroponic growing systems and colonies of fluorescent pseudomonads were isolated on KB agar as described in section 3.2.1. For detection of biosurfactant production, selected colonies were transferred to two different liquid media, Nutrient Broth (NB) or mineral medium, with the same composition as the nutrient solution described in section 3.1.1., amended with 2% glucose. The culture was incubated at room temperature on a rotary shaker (180 rpm) for 48 hours. After incubation, the bacterial growth medium was subjected to drop-collapse tests as described by Youssef *et al.* (2004). A parafilm strip was covered with pennzoil oil (Pennzoil® 10W-40), a 15 µL droplet was placed on the parafilm and examined visually with respect to float out after 2 min. The drop-collapse was compared with growth medium in which sodium dodecyl sulphate (SDS, CAS No.:151-21-3) was dissolved.

For the detection of antibiotic production, dual-culture agar plate cultures were used. *Pythium ultimum*, *Phytophthora cryptogea* and *Fusarium oxysporum* f.sp. *radicis lycopersici* were tested by inoculating the pathogen in the centre of an agar plate with nutrient agar (Nutrient broth, Difco 0003-17, Bacto agar, Difco 0140-01, 1.5%). The bacterial strain was inoculated 1 cm from the edge of the agar plate. After three days of incubation at 25°C the inhibition zone was measured.

3.4 Evaluation of LED lighting (Paper VI)

3.4.1 Crop assessment

For the ornamental crops (Paper VI), the length of the apical shoot (for *Chrysanthemum* longest lateral shoot) was recorded weekly. The development of flower buds was observed weekly. At the end of the

experiment total plant height, plant width (two diametrically opposite measurements), number of flowers/buds and number of lateral shoots were counted and dry weight/fresh weight was determined. The internodal length was calculated as total shoot length of the main shoot divided by the number of nodes.

3.4.2 Climatic factors

The air temperature for each treatment was logged every 30 minutes using data loggers (HOBO H8, Onset computer corp., Bourne, USA).

3.5 Statistical analyses

Experiments were repeated twice with three independent replicates (papers I, II, IV, V) or once with three independent replicates (paper III). For Paper VI, the experiment was performed once with seven replicates. 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 (Papers I, II), $p<0.05$ considered significant, or the non-parametric Wald-Wolfowitz sequence test. For correlations, stepwise regression analysis was employed. To decide on differences for crop assessments and oxygen contents, two-sided t-tests were performed (Papers I-III, VI). In Papers IV and V, data were analysed by chi-square tests or analysis of variance followed by Tukey's multiple comparison test, $p<0.05$. Calculations were done using SPSS 11.5 (SPSS Software GmbH, München, Germany) (Paper I), Minitab software (ver. 15.1.20.0, Minitab inc., State College, USA) (Papers II, III, VI) or Minitab ver. 14 (Paper IV, V).

4 Results & Discussion

4.1 Performance of slow filters

4.1.1 Microbial assessment of slow filters (Papers I-III)

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 in the microflora with respect to season or repetition were observed (Paper I). In the effluent nutrient solution from the commercial systems only low counts of fluorescent pseudomonads were found, in contrast to the supernatant and the filter skin, which were always densely colonised with fluorescent pseudomonads. *Fusarium oxysporum* was generally detected in the supernatant and effluent and *Pythium aphanidermatum* in the supernatant nutrient solution in the experimental systems with tomato. The effluent from the commercial systems was always free from both *F. oxysporum* and *P. aphanidermatum* except in one single sampling. Presence of *F. oxysporum* was verified on the filter skin in all systems (Paper I).

The addition of *P. aphanidermatum* to the filter skin did not alter the composition of the culturable microflora significantly (Paper III). Elevated EC levels generally did not affect the microbial colonisation in the systems (Paper II). As expected, there was more dense colonisation in the treatment with the lowest EC, as increasing salinity normally decreases microbial growth (Rietz & Haynes, 2003; Paper II).

4.1.2 Efficacy of slow filters (Papers I, II, III)

When the EC level was elevated to 5 mS cm^{-1}, the efficacy of the slow filters against *F. oxysporum* f.sp. *cyclaminis* was significantly reduced (Fig. 3 in Paper II). Many modern cropping practices involve elevated EC levels, for

example ornamental pot plant production where osmotic stress is used as a tool for growth regulation (van Leeuwen, 1993), or tomato production, where modern cultivars often require higher EC levels than have traditionally been employed (Sonneveld & Straver, 1989). Therefore, the findings in Paper II are worth considering before recommending installation of slow filters.

In the experiment with *Pythium* addition (Paper III), filter efficacy was high (99.9%) on all test occasions and due to these high and consistent efficacies, no differences with respect to treatment or relations with other parameters were observed. The higher filter efficacies generally obtained in Paper III, compared with Papers I and II, are probably due to higher packing density of the filter medium in Paper III. This indicates that the physical properties of the filter column are important for filter efficacy, in addition to the suggested state variables of enzyme activity (Brand & Alsanius, 2004b) and polysaccharide content (Furtner *et al.*, 2007).

4.1.3 Biochemical and physical assessment of slow filters (Papers I,II,III)

The activity of chitinase, cellulase, β-1,3-glucanase and protease was significantly enhanced by the addition of FCWP. This agrees with previous findings (Brand & Alsanius, 2004a). In Paper I, xylanase was only detected occasionally, whereas in Paper III xylanase was generally detected at activities up to 1.5 mU. The addition of *P. aphanidermatum* had no effect on enzyme activity (Paper III). Elevated EC level inhibited the activity of chitinase, cellulase and xylanase in the filter skin (Fig. 1 in Paper II), as previously reported by García & Hernández (1996). Chitinase activity combined with EC level was found to be a predictor of filter efficacy according to:

$$\text{Eff} = 102 - 1.2\ \text{chi} - 1.14\ \text{EC}\ (R^2 = 91.3\%,\ p < 0.001) \quad (4)$$

where Eff=filter efficacy, chi=chitinase activity and EC=EC level of the nutrient solution. These findings further support the theory that microbial metabolism rather than composition is changed by elevated salinity. The connection between EC level and chitinase activity was confirmed by the relationships found for the commercial system with ornamental crops, as described by the following equation:

$$\text{EC} = 2.42 - 0.804\ \text{chi} + 0.438\ \text{FO sup} + 0.081\ \text{PA sup} \quad (5)$$
$$(R^2 = 61.0\ \%,\ p = 0.008)$$

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

The EC value might be an important factor in fully explaining the filter function, in addition to chitinase, cellulase and protease activity, which according to Brand & Alsanius (2004a) can predict 50% of filter efficacy.

No correlations between viable count and TOC levels were found in these studies. This supports the theory that dissolved organic compounds and not microorganisms account for the majority of the organic compounds in the nutrient solution, as previously suggested by Waechter-Kristensen *et al.* (Waechter-Kristensen *et al.*, 1999). In systems with elevated EC values, TOC and COD levels were significantly higher. These results confirm previous findings, as slower degradation of organic compounds in saline environments was also reported by Rietz & Haynes (2003) (Paper II). Alteration of the microflora metabolism is most probably the reason, rather than a change in composition. TOC/DOC values were only slightly affected by the filtration. BOD_5 analysis is probably of minor interest for nutrient solutions, as only very low values, close to the detection limit for the chosen method, were found.

Results from the commercial system established a correlation between COD and DOC (Fig. 2 in Paper I). Similar observations (R^2 = 91.8; $p<0.001$) were made in Paper III.

Addition of FCWP had no effect on oxygen saturation, oxygen consumption of the filters or the tPs and bPs of the filter skin. The levels of dissolved oxygen decreased during filtration, more markedly in the (larger) commercial filters than in the experimental systems. Depletion of oxygen during the filtration process has also been demonstrated by Wohanka (1995). Addition of *P. aphanidermatum* to the filter skin lowered the level of dissolved oxygen in the supernatant and effluent significantly ($p=0.002$, $p=0.004$, respectively). The temperature of the nutrient solution was not different between treatments in any of the experiments. EC and pH were generally not affected by the filtration.

The general conclusion from the experiments on slow filters is that efficacy is high and consistent, provided that the filter column is properly prepared. Among the variables tested here, only elevating EC to a level of 5 mS cm^{-1} significantly affected filter efficacy negatively, and this effect was relatively moderate. Protease activity on the filter skin, together with EC of the incoming nutrient solution, was found to act as a good predictor of filter efficacy during filter operation.

4.1.4 Crop assessment in systems with slow filter (Paper III)

The plant height in the experimental system described a linear regression at R^2=0.99 (Fig. 4 in Paper III). The numbers of trusses and leaves increased in the first part of the experiment and then stabilised after harvesting started. Addition of *P. aphanidermatum* did not affect plant development.

4.2 Production of biosurfactants and antibiotics (Papers IV, V)

In the larger screening study (Paper V), it was observed that approximately 20% of the isolated strain possessed the ability to produce biosurfactants. Most of the biosurfactant-producing strains were found on the filter skin. Antibiotic production by the isolated strains was common and inhibition of *P. ultimum* was greater than inhibition of *F. oxysporum*. It should be noted that the initial screening study (Paper IV) clearly showed that the medium used for cultivation of the strains affected biosurfactant production. This was also the case for antibiotic production, as shown by Duffy & Défago (1999). Interestingly, none of the strains isolated possessed the ability to produce both biosurfactants and antibiotics. However, from these studies it can be concluded that fluorescent pseudomonads with high capability to produce secondary metabolites of importance for biocontrol of oomycetes are naturally present in the cultivation system.

The potential to use biocontrols against oomycete pathogens has been demonstrated by several authors (Perneel *et al.*, 2008; Tran, 2007; Nielsen *et al.*, 2006; De Jonghe *et al.*, 2005; De Souza *et al.*, 2003; Zheng *et al.*, 2000). Two possible ways of using a biocontrol in hydroponic systems are: i) external production of the biocontrol agent (McCullagh *et al.* 1996) and addition to the system; or ii) using the resident microflora of the system (Pagliaccia *et al.* 2007; Spadaro & Gullino, 2005, Van Os & Postma, 2000). Considering that hydroponic systems have the potential to be optimised in a number of ways, for example with respect to mineral composition and oxygen saturation, the second option is a feasible, sustainable and exciting alternative in future research. Developing the use of the resident microflora would solve the current problem of access to only a small range of different biocontrol agents. However, option (i) should not be overlooked and our studies suggest that inoculation onto the filter skin is a possible approach. Such an approach has been discussed by Déniel *et al.* (2004). It is also possible that manipulation of the filter skin could be the way forward.

4.3 Effects of LED on crop development

In the study with LED lighting (Paper VI), plant development was strongly affected by the different treatments. For the growth chamber experiments, plants were strongly elongated in the yellow, red and green light, whereas growth was more normal in the blue and white light. For all treatments, plants remained in the vegetative state throughout the experiment.

In the photoperiodic light experiment, the length of the main shoot was significantly increased by the blue light for both *Chrysanthemum* and *Euphorbia*. Yellow, red and green light resulted in lower total plant height compared with white or blue light. In *Euphorbia*, the fresh and dry weight was higher in the blue light than for all other treatments. Flower initiation was completely prevented by the white, yellow, green and red light, but for the blue light flower buds emerged when the light intensity was below 10 μmol s^{-1} m^{-2}. The white LEDs were the most effective in converting electricity (w) into light (μmol m^{-2} s^{-1} PAR), which makes them most suitable for photoperiodic lighting. Our expectations of reduced shoot elongation by the blue light, as has been suggested by several authors (Ménard *et al.*, 2006; Shimizu, 2006; Shimizu *et al.*, 2005; Rajapakse & Kelly, 1992; Appelgren, 1991), were not confirmed in these studies. One major difference in our study compared with previous studies was the use of LED technology instead of spectral filters. It has been suggested that spectral filters allow pollution with other spectral bands than the intended (Parker *et al.*, 1946), whereas the LED technology emits a sharp peak at the intended wavelength. Such cross-pollution has also been demonstrated for fluorescent tubes with spectral filters (Appelgren, 1991).

Instead, for the chosen cultivation method, yellow light seemed to be of interest for keeping stem elongation low, which is often desired in pot plant cultivation (Löfkvist, 2010). The measured air temperature for all treatments was within the ±0.35°C range of accuracy stated by the logger manufacturer (Fig. 4). Total dry weight was lower for *Chrysanthemum* in the treatment with LED for supplementary light compared with HPS lamps. Total plant height for *Kalanchoë* was lower in the LED treatment due to less elongated flower stalks. For *Euphorbia*, internodal length as well as stem diameter and number of cyathia were lower for LED-treated plants, whereas the number of bracts was higher ($p<0.05$). LED-treated *Kalanchoë* reached anthesis three days later than HPS-treated plants, probably due to lower leaf temperature, as leaf temperature was around 1°C lower for LED-treated plants.

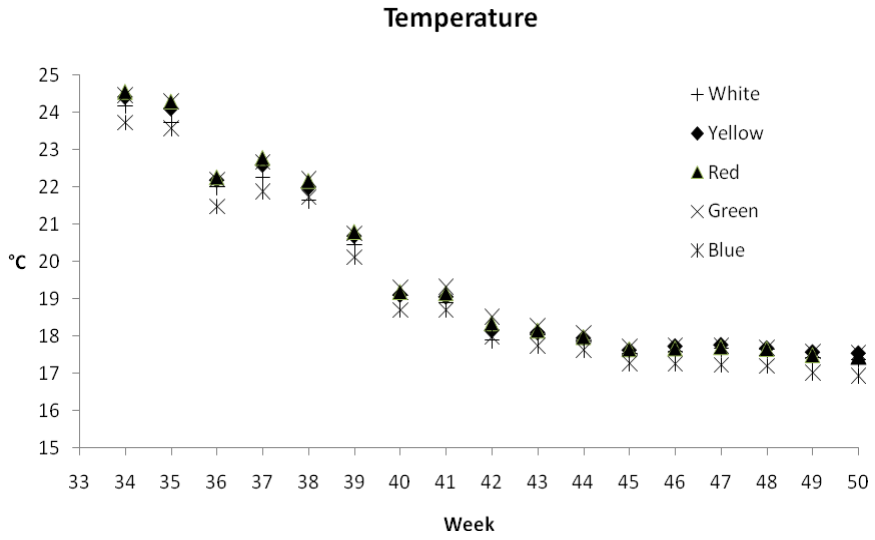

Figure 4: Weekly average temperatures for different LED treatments from mid-August to early December. Temperature was logged every 30 minutes with a logger (HOBO H 8). Temperature setpoint was 18°C.

5 Conclusions and outlook

The studies presented in this thesis are different approaches, each representing step towards high-efficiency greenhouse horticulture production with high sustainability and low impact on the surrounding environment. The three basic concepts presented here (closed irrigation systems with biological filtration, microbial optimisation and LED technology) are all typical solutions that can be combined to create the desired high-efficiency greenhouse. The biological filtration solutions represent typical low-tech solutions for the horticultural industry, especially in temperate regions, but can be used successfully in all regions of greenhouse horticulture. Slow filtration proved to be efficient in controlling some of the most important root pathogens, such as *Pythium* sp. and *Fusarium* sp. The reluctance to introduce slow filtration on a larger scale is mainly attributable to the uncertainty regarding efficiency during all operating conditions, and the lack of possibilities for quickly monitoring filter efficacy during operation. The studies in this thesis show that the fears of poor operation are generally unfounded. The filter withstood the tests of high EC values and high pathogen load. This, combined with the good experiences from slow filters in commercial applications, will question the need for monitoring filter efficacy during commercial operation. The search for one or a few state variables explaining most of the filter efficacy has not been completely successful, and it is doubtful whether any more efforts in this direction are meaningful.

The concept of using the resident microflora for biocontrol is an interesting approach within biocontrol. The work performed within the framework of this thesis shows the potential of the resident microflora for production of biocontrol agents in hydroponic systems, thus reducing the need for chemical fungicides and possibly for costly disinfection devices.

The work on LED technology presented here can be seen as a precursor for further developments within this area. LEDs only became commercially available in the 1960s and light intensities useful for plant production did not become available until around 2000. Thus, this technology is still in its infancy, with rapid improvements still occurring in terms of higher light intensity, higher efficiency and lower costs. This study shows promising results from using LED lights for flowering regulation in short-day plants, while having a strong influence on plant growth habit. Further applications for these findings have yet to be investigated. LED technology is claimed to be almost a general solution for greenhouse cultivation, with possibilities such as energy conservation, lowered use of chemical growth retardants and pesticides, higher internal and external quality of the produce and even entirely new production systems. Much research is still required within this area and the future will tell whether these expectations will be fulfilled.

Further solutions for the sustainable greenhouse need to be added to the current work. The general idea of closing the systems will have to be implemented to a large extent to meet future demands on sustainability.

Local production is considered positive from an environmental perspective. Reduced transport not only decreases CO_2 emissions but also emissions of nitrous oxides and particles, noise, traffic deaths and the need for society to invest in traffic infrastructure. Geographically disperse production will also decrease the risk of pathogen spread, thus decreasing the need for chemical pesticides. Modern greenhouse horticulture provides the possibilities for local production of high-quality foods and ornamental plants with low use of fossil resources and low impact on the surrounding environment. Local production also provides new ways of small-loop recycling of mineral nutrients, energy and CO_2. The greenhouses of the future will provide horticultural products that are locally produced in a sustainable way.

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