Development of hydroculture plants for the improvement of indoor air quality

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Student Declaration

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Abstract

Tissue-cultured plants (*Syngonium podophyllum*) planted into conventional potting mix and hydroculture were investigated for their capacities to bring about reductions of the two major types of indoor air pollution; volatile organic compounds (VOCs) and CO_2.

The results confirm that, with a moderate increase in indoor light intensity, the species used could be developed and used to remove significant amounts of indoor CO_2. The results also indicate that hydroculture as a growth medium makes for greater efficacy of CO_2 removal than potting mix. Furthermore, the VOC removing potential of hydroculture plants was demonstrated. Whilst the rate of VOC removal was somewhat slower than plants grown in traditional potting mix, the simultaneous capacity of the system for effective CO_2 removal is evidence that hydroculture is a more effective system for functional indoor plants than the potting mix systems that are used now.

An examination was also made of the possibility of bioaugmentation with both rhizosphere bacteria and arbuscular mycorrhizal fungi to improve the performance of the hydroculture plants to improve growth and remove more VOCs, while maintaining a lower microbial load than potting mix, so as to reduce soil CO_2 emissions, however, the efforts trialled here in this species were unsuccessful.

The effect of benzene on the community level physiological profiles of rhizospheric bacteria of hydroculture plants was assessed. Whilst the bacterial community present in hydroculture was reduced in diversity compared to potting mix, the species present encompassed at least some of those involved with VOC removal, thus indicating that hydroculture plants should still be an effective means of reducing indoor VOC concentrations.

A qualitative screen for pathogenic fungal spores from plants grown both in hydroculture and potting mix showed the nutrient solution and supporting media did not harbour any pathogenic fungi and are thus unlikely to pose a major health risk.

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List of Abbreviations

ANOSIM	Analysis of Similarity
ANOVA	Analysis of Variation
AMF	Arbuscular Mycorrhizal Fungi
CFU	Colony Forming Units
CO_2	Carbon Dioxide
CO	Carbon Monoxide
CLPP	Community Level Physiological Profiles
GC	Gas Chromatography
HP	Hypersensitivity Pneumonitis
HVAC	Heating, Ventilation and Air Conditioning
IAQ	Indoor Air Quality
LCP	Light Compensation Point
LRC	Light Response Curve
NO_x	Oxides of Nitrogen
OD	Optical Density
PAH	Polycyclic aromatic Hydrocarbons
PAR	Photosynthetically Active Radiation
PCA	Principal Components Analysis
ppmv	Parts Per Million by volume
RBC	Rose Bengal Chloramphenicol Agar
SBS	Sick Building Syndrome

SO_x	Oxides of Sulfur
SDX	Sabouraud Dextrose Agar
TSB	Tryptic Soy Broth
TVOC	Total Volatile Organic Compound
UAP	Urban Air Pollution
UTS	University of Technology, Sydney
VOC	Volatile Organic Compound
± SE	Standard Error of the Mean

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1. INTRODUCTION

1.1 General aims

The goal of this project was to evaluate the efficacy and feasibility of developing a soil-less potted-plant growth system that could be used to improve urban indoor air quality. A comparative assessment was made of tissue-cultured plants planted into either conventional potting mix or hydroculture, to reduce levels of both CO_2 and volatile organic compounds (VOCs), both significant components of indoor air pollution (Anderson 2009). An examination was also made of the possibility of bioaugmentation to improve the performance of the hydroculture plants. Tests included the addition of a bacterial culture derived from potting mix, which could possibly enhance the capacity of the hydroculture plants for VOC reduction; and a preparation of arbuscular mycorrhizal fungi (AMF) as a possible promoter of tissue hardening and plant growth which could enhance CO_2 uptake capacity, and which might also act as a source of VOC removing bacteria. The information obtained from this project contributes to the development of plant-based air quality improvement systems which have the potential to play a major role in future building design, by ameliorating growing concerns over poor indoor air quality and the associated health problems, whist producing far lower greenhouse gas emissions than current engineering-based systems.

1.2 Health impacts of air pollution

Throughout history it has been known that substances in the air can have adverse effects on human health (Anderson 2009; Hopke 2009). Urban air pollution (UAP) is a world-wide health problem, causing between 1.2 and 2 million deaths globally each year (Kenworthy and Laube 2002). Most UAP comes from fossil fuel emissions, which are comprised of a mixture of airborne lead, oxides of sulfur (SO_x), oxides of nitrogen (NO_x), carbon monoxide (CO), carbon dioxide (CO_2), volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs), and ozone (Hedge 2009).

In Australia it is estimated that UAP causes over 1,400 deaths per annum in Sydney alone (Department of Health 2009), with national health costs estimated to be as high as 1 per cent of gross domestic product (Brindle *et al.* 1999) or $AUD 12 billion a year, from lost productivity and medical expenses (Environment Australia 2003). Australians are known to

spend up to 96 per cent of their time indoors (Newton 2001), and thus indoor air quality has a major impact on the health of most Australians. Pollution levels in indoor air are generally two to five times higher and sometimes as much as 100 times more concentrated than outside air (Environment Australia 2003). This is a result of contaminated outdoor air entering buildings which is then further augmented with indoor-sourced pollutants, in particular more VOCs from building materials, furnishings and equipment (Hedge 2009), and more CO_2, produced by human respiration (Gurjar *et al.* 2010).

The accumulation of and continued exposure to indoor air pollution may result in a condition known as *Sick Building Syndrome* (SBS) (Hedge 2009). The term 'SBS' is used to describe situations in which building occupants experience acute or subacute health and discomfort effects that appear to be linked to the duration of time spent in a building (Engvall *et al.* 2005; Wang *et al.* 2008). Typical SBS symptoms include headache; eye, nose and throat irritation; dry cough; dry or itchy skin; dizziness and nausea; difficulty concentrating and fatigue. The direct cause of the symptoms are seemingly unknown, but symptoms are rapidly relieved after leaving the building (Fisk *et al.* 2009; Simoni *et al.* 2010). The research reported here investigated the capacities of potted-plants to bring about reductions of the two major types of urban air pollution usually found in higher concentrations indoors, VOCs and CO_2.

1.3 Association between VOC & CO_2 concentration and Sick Building Syndrome

VOCs consist of various classes of carbon-containing low boiling-point compounds that are therefore often gaseous at room temperature. Much of the VOC load associated with indoor air quality (IAQ) comes from sources inside the building, such as adhesives, paints, varnishes, carpeting, building materials or cleaning products (Sakai *et al.* 2004; USEPA 2011), as well as outdoor fossil fuel emissions. The combination of these VOC sources within buildings can result in the occupant being exposed to anywhere from 50 to 300 different individual VOCs, at levels generally in the $\mu g/m^{-3}$ concentration range (Bernstein *et al.* 2008). VOCs have been linked with an array of adverse health responses. Even at levels well below human perception, VOCs can contribute to symptoms that resemble those of SBS, and at high levels have the potential to be hematotoxic, neurotoxic, leukemogenic, and in the case of some compounds, such as the chosen VOC for this study (benzene), also carcinogenic (Vaughan *et al.* 1986; Wallace 2001; Wolkoff and Nielsen 2001).

Indoors, excess CO_2 is produced mainly by human respiration. It is not normally considered to be a toxic air contaminant, but it can be a simple narcotic, and when present at very high levels can cause asphyxiation (Milton *et al.* 2000). It has been found that elevated CO_2 concentrations in office buildings are associated with increased illness symptoms among occupants (Milton *et al.* 2000; Erdmann and Apte 2004; Seppänen and Fisk 2004). With increasing levels above the outdoor ambient concentration (approximately 390 ppm in 2012 (Conway *et al.* 2012)), CO_2 has been associated with adverse symptoms relating to the mucous membranes (dry eyes, sore throat, nose congestion, sneezing) and to the lower respiratory tract (tight chest, short breath, cough and wheezing) (Erdmann and Apte 2004). At concentrations between 2500 and 5000 ppmv, CO_2 can cause headache. Exposure to high concentrations (50,000 ppmv) may cause high CO_2 concentration in the blood resulting in hypercapnia, while at extremely high levels (70,000 ppmv) loss of consciousness may occur (Harris and Moore 2009) (Hedge 2009). At 200,000 ppmv, CO_2 causes partial or complete closure of the glottis results (Gurjar *et al.* 2010).

Student academic performance and workplace productivity have both been shown to decline with increased CO_2 levels (Bakó-Biró *et al.* 2004; Seppänen *et al.* 2006; Shaughnessy *et al.* 2006). In most cases, the building ventilation system is found to be at the heart of the problem (Redlich *et al.* 1997). The American Society of Heating, Refrigeration and Air-Conditioning Engineers (ASHRAE) recommends that the maximum concentration of CO_2 should not exceed 1,000 ppm (ASHRAE 2011), and this standard is also used generally in Australia (Environment Australia 2001).

1.4 Ventilation and indoor air quality

In an effort to reduce energy wastage, buildings designed in the past two decades tend to be air-tight and have lower outdoor air exchange rates and an almost complete reliance on contained heating, ventilating and air conditioning systems (HVACs) (Wargocki *et al.* 2004). The design and use of HVACs in modern buildings is thus integral to establishing a healthy indoor environment. Most HVAC systems achieve this goal by diluting indoor air with outdoor air that is generally less polluted (Jacobson 2002).

The ventilation air flow rate of outdoor air entering into the building is primarily what determines the IAQ. Automated monitoring of indoor CO_2 is commonly used to regulate ventilation air flow rates. Airflow rates below 10 L/s per person have been associated with

significantly increased health symptoms (Hoge *et al.* 1994; Kajtar *et al.* 2006; Hummelgaard *et al.* 2007). With ventilation rates higher than 25 L/s per person, these health symptoms are significantly reduced (Wargocki *et al.* 2004). Of course, these rates are dependent on the quality of the outdoor air entering the HVAC systems: outdoor air with CO_2 levels greater than normal ambient will clearly increase the necessity for higher ventilation rates.

Although CO_2 concentration alone may not indicate health risks from specific pollutant emissions inside a building, it is commonly used as a surrogate for the accumulation of total indoor air pollutants including VOCs (Gurjar *et al.* 2010), and a number of studies have found that buildings with high indoor CO_2 concentrations have an increased prevalence of SBS (Seppänen and Fisk 2004). It thus follows that by increasing the ventilation air flow rate, reductions in the accumulated indoor air pollutant load can be made; which should reduce the prevalence rates of SBS symptoms. However, given the costs of energy use, and the contribution of anthropogenic climate change through the emission of greenhouse gases, it is important to balance the benefits and costs of increased ventilation. Since increases in ventilation will increase building energy consumption, research is needed to identify practical, passive methods of decreasing ventilation requirements by reducing pollutant concentrations from within buildings.

1.5 Indoor plants can improve indoor air quality

Pioneer studies supported by the US National Aeronautics and Space Administration (NASA) indicated that indoor plants could be used to reduce indoor concentrations of VOCs (Wolverton *et al.* 1984). Reductions of formaldehyde by several plant species were robust and subsequent studies showed promising reductions in benzene and trichloroethylene (Wolverton *et al.* 1989). The species tested could remove benzene by 47–90% in 24 h, and it was hypothesised that the root zone of the plant was the most effective part of the potted plant system for VOC removal.

Research carried out at the University of Technology, Sydney (UTS) by the Plants and Indoor Environmental Quality Group have expanded on the early NASA work, demonstrating reductions of high airborne benzene and *n*-hexane levels within 24 hours by 10 plant species (Wood *et al.* 2002). The group also produced evidence that a component of the plant's rhizospheric bacterial community was responsible for almost all of benzene removal. However it is likely that the plant was crucial for the maintenance of the root-zone microbial community through the release of organic exudates from the root system (Orwell *et al.* 2004).

These tests were carried out at rather high VOC concentrations (25 ppmv – 50 ppmv (81,000 163,000 μg m^{-3})), which introduced the possibility that the potted plant microcosm may not become efficiently 'induced' to remove VOCs at the lower concentrations typically found in indoor situations. However, Orwell et al (2006) subsequently showed induction of the VOC removal response at concentrations as low as 0.20 ppmv using toluene and *m*-xylene, while simultaneously showing that the system is as effective in the dark as it is in light providing further evidence that the substrate microorganisms, rather than the plant itself, are the predominant degraders of VOCs.

This led to the first experimental investigation of the effectiveness of the potted-plant microcosm in 'real-world' indoor environments to remove VOCs (Wood *et al.* 2006)**.** Among sixty offices sampled, within both air-conditioned and non air-conditioned buildings, total VOC loads (TVOCs) were reduced by up to 75% when indoor concentrations exceeded 100 ppbv units. Furthermore, offices with three or more plants, recorded reductions in CO_2 levels by 10% in an air-conditioned building, and by 25% in a non air-conditioned buildings (Tarran *et al.* 2007)**.**

1.6 Potential of indoor plants to reduce air-conditioning costs

More recently, a second UTS office field study was conducted to determine the minimum number of plants and/or pot sizes required to reduce quantifiably loads of TVOCs and CO_2 (Burchett *et al.* 2010)**.** Both VOC and CO_2 removal were much weaker than found in the previous office study although slight reductions were still recorded. However, the study's control treatments (ie offices with no plants) had TVOC and CO_2 levels considerably lower than the previous study which was attributed to more technologically–advanced HVAC systems in the latter study compared to those of the earlier study, where the buildings were more than 20 years old. It was concluded that the low removal rates were due to the more efficient HVAC systems masking the potential contribution of plants to improve IAQ, due to their higher ventilation rates (Brennan 2011). Comparing the results of the two studies, it was hypothesised that indoor plants could be deployed to decrease indoor concentrations of VOCs and CO_2 which would allow for lower air-conditioning ventilation requirements in urban buildings, hence reducing not only energy costs but also reduce the building's contribution to green-house gas emission and carbon footprint. It has been estimated that the use of appropriate green plant design could reduce HVAC energy loads by 10–20% (Afrin 2009).

1.7 CO_2 reduction by indoor plants

A laboratory study by Burchett et al. (2011) investigated the photosynthetic response profiles of nine common indoor plant species and their potential contribution to reducing indoor CO_2. Using a range of plants acclimated to an indoor light regime; whole-potted-plant net CO_2 reductions were assessed across a range of photosynthetically active radiation (PAR) intensities. The authors found that at the lower light intensities usually encountered within offices (~10 PAR μmol m^{-2} s^{-1}), only 4 out of the 9 test species showed net CO_2 removal, and the rates were insufficient for a feasible number of plants to make a significant improvement to indoor air quality in a sealed building with an HVAC system. This low CO_2 removal efficiency was due to the respiration of the non-green plant tissues and the potting mix microorganisms producing CO_2 emissions equal to the uptake by the leaves, so that no net CO_2 reduction was achieved by the mircocosm. It was proposed that, for potted-plants to be a viable instrument in reducing indoor CO_2 loads, moderate increases in light intensity, potentially coupled with the reduction of potting mix microorganisms would be needed (Burchett *et al.* 2010; Brennan 2011), indicating that soil-less growth systems such as hydroculture might be a viable component of such an improved indoor plant system. Conversely, however, the potential reduction in VOC-removal capacity resulting from lower microbial numbers could possibly negate any improvement in IAQ that might be achieved by improved CO_2 reduction, depending on the capacity of the soil-less medium to support a sufficiently vigorous VOC-degrading microbial consortium. It has previously been shown that the potting mix microflora that degrade benzene are culturable in artificial media (Wood *et al.* 2002), thus the theoretical potential for a low-respiration / high VOC-degrading growing medium exists. However testing was clearly necessary to investigate whether this potential could be realised with a reasonably low-cost and low-maintenance system, and without microbial growth that could negatively affect indoor air quality or occupant health.

1.8 Hydroculture for ornamental plants

Hydroculture is a method of growing plants without any organic material like soil, but rather an inert support such as perlite, vermiculite or expanded clay, with a controlled nutrient solution. Having the roots growing in an inert medium allows the roots to absorb oxygen and receive water and nutrients from a reservoir. The roots' improved access to oxygen, nutrient and water accelerates plant growth and leads to increased yields (Loewer 1981). It has previously been hypothesised that hydroculture plants could be more effective in removing

airborne pollutants than plants growing in potting mix (F Torpy P Irga 2011, pers. Comm 3rd June).

In recent years there has been an increasing interest in replacing soil-based growing systems for indoor ornamentals with hydroculture, with the perceived advantages being reductions in mess, avoidance of over watering, lowered risk of hazardous fungal growth, and lower maintenance compared to that of plants in conventional potting mixes.

Such benefits notwithstanding, in recent years, questions have been raised as to whether hydroponic cultivation should be considered as a potential source of fungal contaminants and thus a health concern, especially for immunocompromised people (Adan and Samson 2011). A case of childhood hypersensitivity pneumonitis (HP) that was serologically linked to be the result of fungal contamination of hydroponically grown indoor plants was presented by Engelhart et al., (2009). In this case-study investigation, a water sample from the hydroponic reservoir suspected to be the source of infection revealed that the most dominant fungal microorganism was *Aureobasidium pullulans*, an opportunistic pathogen that has been implicated in HP (Hawkes *et al.* 2005). The possible presence of pathogenic fungal spores potentially jeopardises the suitability of hydroculture plants to be used to improve air quality (Burchett *et al.* 2011). In this project, therefore, both potting-mix and hydroponic media were examined and compared for the possible presence of potentially dangerous numbers or species of mould fungi.

1.9 Hydroculture plants' capacity to improve air quality.

Only two published studies have assessed the capacity of plants, under hydroculture conditions, to degrade VOCs. The first was a pilot study that examined benzene and *n*-hexane removal by three plant species using vermiculite as the supporting growing medium (Wood *et al.* 2002) and showed reductions in VOCs at comparable rates to plants using potting mix as the growth medium**.** The second study analysed a further four plant species using growstone as the hydroponic support and focused on their ability to remove formaldehyde (Aydogan and Montoya 2011)**.** The species tested demonstrated formaldehyde removal; however, the rate at which they achieved this was significantly different for each species.

Both studies failed to demonstrate whether a plant that is fully acclimated to hydroponic conditions is capable of VOC removal, since in both investigations the plants had been placed in hydroponic conditions for only 48 hours. The full adaptation of soil or potting-mix grown

plants to hydroponic conditions takes approximately 90 to 120 days (Sundstrom 1989). Furthermore, the 48-hour adjustment period would have been insufficient to allow the plants to become fully adapted to the low light levels present indoors, a plant trait that is crucial for CO_2 reduction capabilities (Burchett *et al.* 2011). No previous study has assessed the ability of indoor hydroponic plants to reduce indoor CO_2.

1.10 Bioaugmentation of benzene degrading rhizospheric organisms

Previous efforts have been made to identify the main bacteria in indoor plant growing media involved in the aerobic degradation of VOCs (Wood *et al.* 2002). A total of forty nine culturable species of bacteria were isolated from the benzene-degrading potting mix of *Dracaena deremensis* indoor plants and were individually tested for their ability to remove benzene versus a mixed culture extracted from the same potting mix. None of the axenic cultures displayed the ability to remove substantial amounts of benzene and thus no individual culture was considered likely to be the sole cause of the observed benzene biodegradation in the potting mix. The inability of the pure cultures to degrade benzene at rates demonstrated by the potting mix suggests that it is a community or microbial consortium that is involved in VOC removal, each member of which either metabolises the VOC itself or secondary products released from initial stages of the biodegradation process (Moldovan 2006). Supporting this hypothesis, the mixed culture tested by Wood et al. (2002) removed benzene with equal efficiency as potting mix. These observations initiated a study in which biostimulant solutions were formulated and applied to fresh potted-plants in an effort to specifically augment the rhizospheric organisms involved in benzene removal (Moldovan 2006). *Biostimulation* involves changing the conditions of the substrate in order to provide bacterial communities with a more favourable environment to effectively degrade contaminants, whereas *bioaugmentation* involves the direct addition to the medium of putatively performance enhancing organisms (Tyagi *et al.* 2011). In Moldovan (2006), biostimulation of benzene removal was observed; with increases in removal reaching 15% greater than unstimulated treatments.

1.11 Tissue culture acclimatization and the potential for AMF to assist in hardening.

AMF are fungi that biotrophically colonise the cortex of the roots of approximately 70% of all vascular plants and develop extra-radical mycelia that help the plant to acquire resources. They produce coils, highly branched structures called arbuscules and in some cases terminal swellings called vesicles within host root cells, these structures being involved with plant–fungus nutrient transfer (Gosling *et al.* 2006). There is a wealth of data to indicate that if successful colonisation takes place; AMF can increase the vigour of plants by increasing absorption of water and mineral nutrients (Krishna *et al.* 2005; Kapoor *et al.* 2008). Moreover, AMF can protect host plants from root pathogens and lessen the effects of extreme variations in temperature, pH and water stress (Vestberg and Cassells 2009). Since AMF symbiosis can benefit plant growth and health, there is an increasing interest in ascertaining their effectiveness in different plant growth situations (Azcón-Aguilar and Barea 1997).

In recent years, there has been a lot of attention devoted to the use of AMF inoculation during *ex vitro* acclimitization of tissue cultured plant stock to reduce hardening duration and reduce losses of micropropagated plants, including the indoor plant species *Syngonium podophyllum* (Azcón-Aguilar and Barea 1997; Gaur and Adholeya 1999; Rai 2001). This process is called *biopriming* (Nowak and Shulaev 2003) and is achieved by minimizing the sudden impact and shock of environmental stresses, detailed above, while the plant is in transfer from *in vitro* to *ex vitro* conditions.

1.12 EXPERIMENTAL AIMS

The specific aims of the study were to conduct a first-ever integrated investigation of cultivation and function in a tissue culture derived indoor plant species. This project investigated:

a) whether a commonly used indoor species, *Syngonium podophyllum* (Schott), propagated in tissue-culture, would be as robust in hydroculture as in standard potting mix in terms of survival and plant growth;
b) the effects of bacterial co-culture with hydroculture plants, so as to test the value of bioaugmention of the rhizosphere to enhance VOC degradation;
c) the effects of bioaugmentation with AMF on survival, growth and air-cleansing capacities of hydroculture plants;
d) the comparative capacities of plants in potting mix and in the three hydroculture treatments, to remove CO_2;
e) the comparative capacity of the four treatments for the removal of benzene as test VOC;
f) any differences between, and changes in bacterial community level physiological profiles (CLPPs) (Biolog Ecoplates) for hydroculture and potting mix based plants, before and after exposure to benzene.
g) A comparison of the presence, abundance and diversity of moulds in both plant growth media, from which to formulate a hazardous mould risk analysis.

For the experiments into the possible benefits of bioaugmentation with hydrocultured plants, two methods were used. The first involved the inoculation of pots with a culturable bacterial community (in tryptic soy broth) derived from root zone potting mix of an indoor plant (*Spathiphyllum wallisii* (Schott)), which had previously been shown to remove benzene. This procedure was used to determine whether adding a known biodegradative bacterial community could enhance the capacity of the hydroculture system to remove VOCs.

For the second method, the root mass of hydroculture plants were inoculated with living root material excised from *S. wallisii*, which had first been tested and shown to be harbouring both rhizospheric bacteria and arbuscular mycorrhiza. The transfer colonisation rates of the arbuscular mycorrhiza was analysed to determine whether colonisation was successful. Plants from both co-culture methods were also tested for their ability to remove both VOCs and CO_2, and rates compared to those in uninoculated treatments and potting mix plants.

1.13 Outcomes

The results of the project provide a quantitative comparison of the efficacy of cultivation of the selected indoor plant species under potting mix and hydroculture conditions, and advance the understanding of the role of plant root-zone microbial communities in improving indoor air quality. The results also throw new light on optimising ornamental plant culture conditions, and in comparisons of maintenance costs of cultivation. The findings contribute further evidence to the scientific foundation required to advance the horticultural development of indoor plants as routine instalments in city buildings for the improvement of urban indoor air quality.

2. MATERIAL AND METHODS

2.1 Hydroculture pot design and concept.

Individual 130 mm self-watering pots (Décor Corporation Pty Ltd, Sydney NSW) were used in this study (Figure 2.1). The pot design was chosen because it was light-weight, aesthetically acceptable and would allow for the plant to grow and be effectively displayed without a bulky structure or mechanics. The water holding reservoir was such that the nutrient solution could be changed as needed, and was designed so that roots had access to the reservoir.

Figure 2.1 Cross section of the 'self-watering' hydroculture pots.

As the solution was taken up from the roots, and water transpired from the leaves, the level in the reservoir was lowered. This has two benefits in terms of air cleaning potential. Firstly it exposes more roots and thus VOC degrading bacteria to the indoor gaseous atmosphere. Secondly, the water draining away creates an air space vacuum pulling contaminated air from the surrounding atmosphere to the roots. The transfer of VOC-laden air to the rhizosphere is further enhanced by the degradation of VOCs creating a diffusion gradient and hence pulls more of their molecules into the soil from the surrounding air.

2.2 Plant Materials

Tissue cultured plantlets of *Syngonium podophyllum* (Schott) were obtained from Jacksons Nursery (The Gap QLD) from the same stock of plants used for many indoor plant nurseries. The plantlets were genetically homogenous and grown in sterile conditions, having been cultivated in tissue culture *en masse* through vegetative propagation.

The species was chosen for the following reasons:

- *Syngonium podophyllum* is a common indoor plant species that is known to grow well in low light conditions (Wolverton *et al.* 1989).
- The species has previously been shown to reduce VOCs (Wolverton *et al.* 1984; Yoo *et al.* 2006).
- The species was available as genetic clones cultivated in tissue culture.
- The species is a likely candidate for further development for use in 'vertical gardens', 'plant walls' or similar multi-plant arrangements, which have been proposed to be a promising method of utilising plants to improve air quality (Burchett *et al.* 2011), and are therefore expected to constitute part of the work following on from the current project.
- The species is closely related (family Araceae) to the existing source of AMF and rhizospheric bacteria (*S. wallsii*), thus increasing the chances of host compatibility. Please note that *S. wallsii* itself was not available as tissue cultured plantlets

2.3 Substrate media and growth conditions

Plantlets were planted to a depth of 1.5 cm. Each pot was planted with two plantlets to allow for mortalities, as is common practice in ornamental plant culture. Plantlets were grown in two arrangements. A potting mix based system and a hydroponic based system.

A commercial potting mix was used for the 'standard' indoor plant treatment, with pots containing 500 mL Hortico all-purpose blend potting mix consisting of composted hardwood sawdust, composted bark fines, and coarse river sand (2:2:1) (bulk density ~0.6 gL^{-1}; air-filled porosity ~30%), with 1.25 g of slow release Osmocote Total all-purpose fertiliser (Scotts Australia) per pot. The potting mix was pasteurised at 60^{o}C for 24 hours prior to planting to reduce the initial microbial contamination, so as to reduce transplant mortality due to disesase. Potting mix plants were watered with 300mL of deionised water; any volume

above field capacity draining into the self-watering reservoir. A total of 16 potting mix samples were included in the study, with 2 plants per pot.

2.4 Selection of hydroculture growth medium.

A suitable growing medium must have most of the physical properties of good soil, in terms of providing support, adequate water-holding and aeration capacities (Sundstrom 1989). A mixture of perlite and vermiculite in a ratio of 2:1 was used as the support medium for the hydrocultured plants, as is commonly utilised in the horticulture industry. Perlite is a light-weight processed mineral with a high capacity for water retention. Grade 3 vermiculite has good water holding capacity and is very porous (Sevilkilinc *et al.* 2007). The ratio of 2 parts perlite to 1 part vermiculite was determined based on a balance of weight and holding capabilities that are ideal for growth of many indoor species (See Figure 1, Appendix).

The nutrient solution for the hydroponic system was Cyco Grow Platinum Series A and B formulation (S J Enterprises Pty Ltd, for composition see Appendix Table 2). Solutions were maintained between 0.8–1 microsiemens (µS) as was recommended by the manufacturer. Nutrient solution (300 mL) was poured over the perlite / vermiculite support and allowed to collect in the self-watering pot reservoir. A total of 52 hydroculture pots were included in the current experiment, consisting of 18 hydroculture control plants, and 36 bioaugmented samples.

Figure 2.2 Growth chambers and experimental treatments

Plants were randomly allocated to the treatments, and placed and maintained in eight perspex growth chambers, where temperature was controlled at 23.0 ± 0.1°C; relative humidity at 45 ± 10% and lit with an overhead light box (air gap 50 mm) with five 18 W fluorescent tubes (Wotan L 18/11 Maxilux daylight) providing 50 ± 20 PAR (μmol m^{-2} s^{-1}) with a 16/8 h (day/night) photoperiod. Plants were randomly rotated weekly among and within chambers in accordance with a randomised plot design.

2.5 Measurement of physiological health on transplanting from tissue culture

In order to compare the transplant success of tissue cultured plantlets between potting mix and hydroculture, several plant morphological characteristics were measured. On a weekly basis, mortality rates, plant height and leaf number were measured from the commencement of the experiment. The total growing period was 133 d, at which stage the plants were equivalent in size to commercially available specimens at the pot size used. At the conclusion of all experiments, for all treatments and pots, the substrate medium was gently washed from roots, and the plants were divided into shoots (stems, leaves) and roots, and fresh weights determined. Final total leaf areas were measured using a leaf area meter (Licor LI-3000-A,Nebraska, USA.). The plant tissues were then oven dried at 70 °C until they reached a constant mass, to measure estimate dry weights.

2.6. Inoculation to enhance VOC degrading capabilities.

At 47 days after plantlets had been potted, it was considered that all plants had completed the 'weaning' stage of acclimatisation and were hardened to *ex vitro* under laboratory conditions; having developed a fully established root system. This stage of maturity was judged likely to be able to withstand the possible pathogenicity/shock from the inoculation manipulation. The experiment involved introducing into 36 hydroculture pots, controlled applications of rhizospheric organisms which, as detailed below, were derived from a donor potted plant that had been shown in previous experiments to reduce benzene, and whose presence was confirmed prior to their use in inoculations. The aim of this component of the project was to test whether pots thus inoculated would be able to reduce VOCs at rates comparable to potting mix plants, but which would have a lower microbial load than found in the potting mix treatment, so as to reduce respiratory CO_2 emissions from the substrate biotic community. The experiment involved the inoculation of pots with a tryptic soy broth culturable bacterial

community derived from root zone potting mix of a specimen of *Spathiphyllm wallisii* (Schott), which had previously been shown to remove benzene (Brennan 2011).

To do this; 2 inoculation methods were employed. Both methods aimed to introduce a broad microbial consortia associated with not only the plant rhizosphere, but VOC degradation. This was done in contrast to inoculating with a pure culture of a single micro-organism as benzene biodegradation has been shown to be a community process (section 1.9). Furthermore, inoculation with a single species culture may not be a successful due to issues concerning the long term survival of the inoculum, reduced capacity of the microorganism to proliferate and reduced possibility of a symbiotic relationship occurring with the plant (Lebeau *et al.* 2008). The two inoculation methods are outlined first, followed by a description of the preliminary tests for the presence of the relevant microorgansims in the preparations.

2.6.1 Hydroponic inoculation – METHOD 1

Samples of 1 g dry weight of potting mix was removed from a healthy *Spathiphyllum wallsii* (Schott) 'Petite' potted plant that had been grown in a shaded glasshouse for 12 months prior to sampling. The plant had previously been exposed to an experimental regime of benzene treatments ranging from 5 ppmv to 25 ppmv four months prior to use in the experiment (Brennan 2011). Potting mix samples were taken from between 5 and 15 mm depth in the potting mix, and pieces of bark >3 mm in any dimension and any residual Osmocote granules were removed manually. Each sample was added to 10 mL phosphate buffer (1.236 g Na_2HPO_4 (BDH); 0.18g NaH_2PO_4 (Sigma); 8.5g NaCl (Caledon) per litre of deionised water and sterilised by autoclave at 121ºC for 15 min) as per Grove et al. (2004). The phosphate buffer was used in preference to water to reduce bacterial cytolysis. Samples were shaken for 1 h at approximately 300 rpm using a Griffin Flask Shaker (Griffin & George Ltd, London, U.K.). The resulting suspensions were then coarse filtered to obtain 2.5 mL of fine suspension that was made up to 25 mL with water, 100 µL of which was used to inoculate 50 mL aliquots of Tryptic Soy Broth (TSB) (Oxoid). Cultures were incubated for 48 h at 23°C, at which time they had reached approximately McFarland number 2 turbidity, equivalent to approximately $6x10^8$ CFU mL^{-1}. After incubation, 1500 µL aliquots were taken from the broth cultures, centrifuged at 1500 rpm for 3 min, washed in deionised water and resuspended in 1 mL of deionised water. 2 mL of the suspension was used to inoculate the base of the stem of the hydroculture plants (ie. 2 aliquots per pot).

2.6.2 Hydroponic inoculation – METHOD 2

Secondary or lateral roots < 1 mm in diameter were carefully excised from the same *S. wallsii* 'Petite' plant used in inoculation method 1. Samples were taken from between 5 and 15 mm depth in the potting mix. The excised roots were washed with several changes of sterilised (autoclaved) deionised water to remove visible remains of the potting mix, with no further effort made to sterilise the roots. The material was examined to confirm that they were harbouring both rhizospheric bacteria and AMF. One gram (fresh weight) of root material was used as the inoculum per pot, the material being placed at the base of the established root ball of the recipient plant (n=18).

The application of 1 g of root material would introduce a significant amount of decaying organic matter that would support a contingent of saprophytic microorganisms; which could potentially augment the rate at which benzene was degraded whilst also affecting the recipient plant's physiological health. In order to control for this, an equivalent mass of autoclaved roots from the same donor plant was added to 8 plants in hydroculture (n=8).

Figure 2.3 One gram fresh weight samples of *Spathiphyllum wallsii* roots used for inoculation method 2

It should be noted that an *a priori* decision was made not to include a procedural control for these inoculation procedures. The general aim of this work was to produce practical outcomes for the indoor plant production industry, thus both inoculation methods needed to include both the microbial bioaugmentation materials and the physical processes associated with inoculating the plants. Thus these effects cannot be separated in the findings, and conclusions can only be made on the effects of the 'inoculation process' as a whole.

2.6.3 Screening of inocula

2.6.3.1 Testing for VOC removal capacity

To determine whether the inocula derived from the potting mix were capable of degrading benzene in the absence of plants and substrate, preliminary tests on their efficiency was made. For Inoculation Method 1, 10/1 (w/w) potting mix suspensions were prepared using deionised water, and were used to inoculate tenth-strength tryptic soy broth (TSB) in quadruplicate. The suspensions were added to autoclaved, Grade 3 vermiculite as a support medium (50 mL broth to 20 g vermiculite), to increase the effective surface area of the liquid phase of the broth. For Inoculation Method 2; 50 g (fresh weight) of root material was placed in 20 g of vermiculite in quadruplicate. The samples were aseptically sealed in light-proof, 3 L air tight jars, the lids of which were fitted with silicone rubber septa (microbiological anaerobic jars). Jars containing uninoculated media were used as controls to correct for background loss of benzene. A dose of 5 ppmv benzene was introduced into the jars, this being equal to the Australian occupational allowable 8-h averaged maximum concentration (Environment Australia 2003), and succeeding concentrations remaining were monitored by GC analysis (Shimadzu; Sydney, Australia) of headspace gas samples taken by syringe through the septa. Treatments were incubated at room temperature (23 ± 1.5^{O}C) with the sampling pattern as follows: Day 1: benzene added to jars at 5 ppmv and sampled at 12 hours; Day 2: Jars topped up with a subsequent 5 ppmv dose of benzene and sampled twice; Day 3: Jars topped up with a third dose of benzene, this time at 25 ppmv and sampled at 2 hour intervals daily until the system no longer had any measurable benzene remaining.

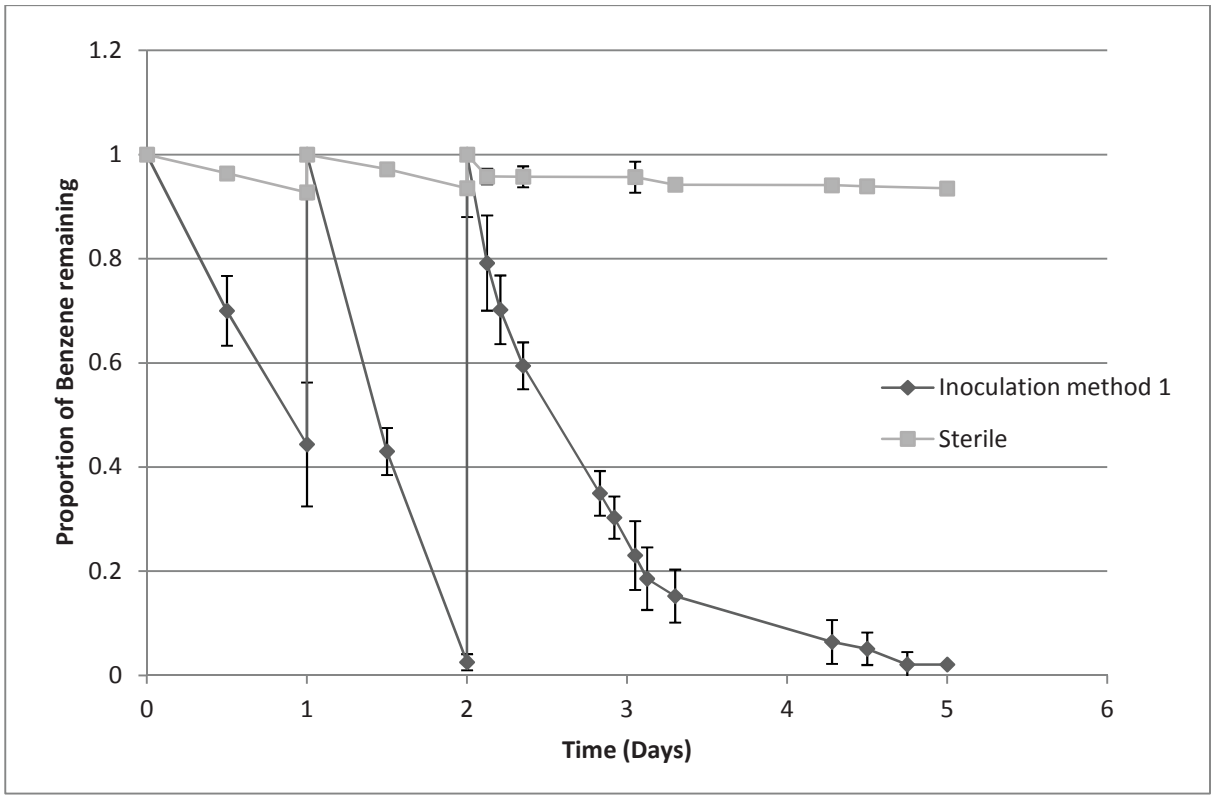

Figure 2.4 Removal from test-jar air of two doses of 5 ppm, and one dose of 25 ppm benzene, with TSB/vermiculite bacterial cultures developed from the potting mix of *Spathiphyllum wallisii* compared to sterile jars of the same contents (Data are means ± SE; n=4).

The two inocula were found to degrade benzene with the same pattern as found in previous indoor potted-plant experiments conducted in this laboratory (Figure 2.4, 2.5) (Wood *et al.* 2002; Orwell *et al.* 2004; Orwell *et al.* 2006; Wood *et al.* 2006; Burchett *et al.* 2010). That is, reduction of benzene was observed upon the initial 5 ppm dose, and the rate of degradation increased upon a receiving a repeated top-up dose of another 5 ppm of benzene. Furthermore, the system was able to remove benzene at a yet higher rate when a final, higher dose, of 25 ppm was applied.

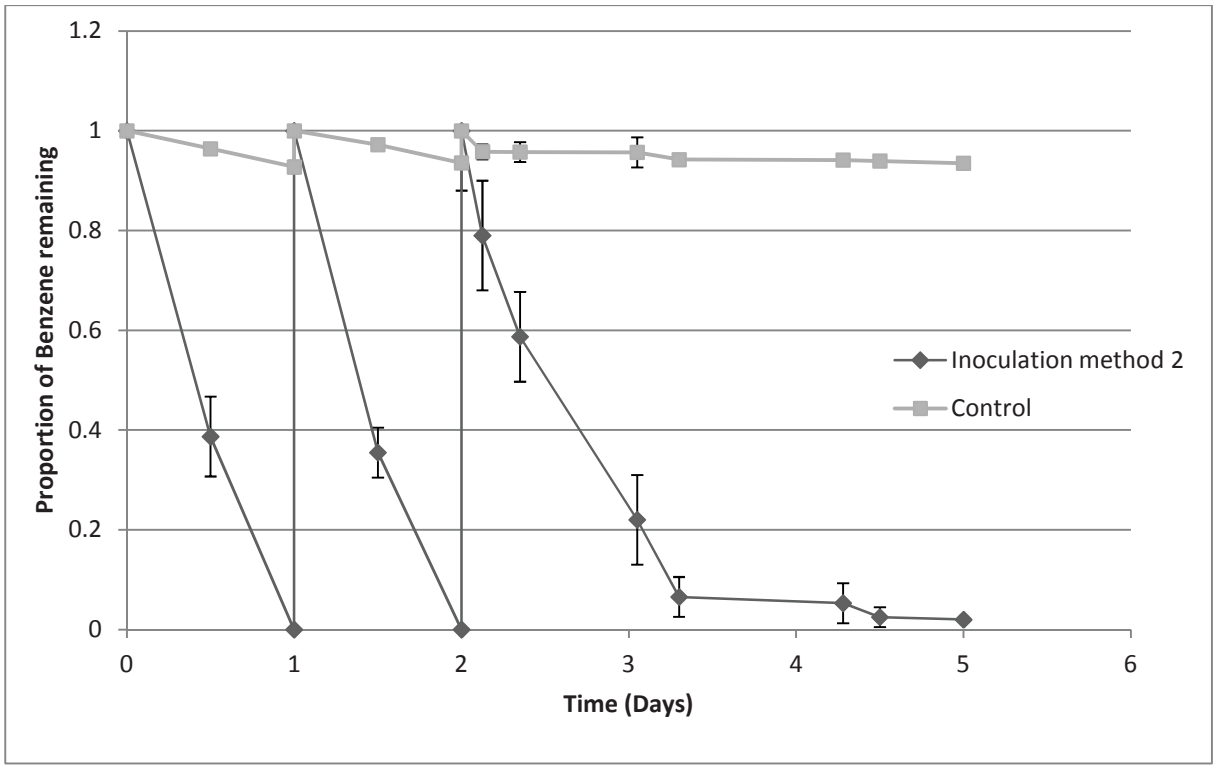

Figure 2.5 Removal from test-jar air of two doses of 5 ppm, and one dose of 25 ppm benzene, with 2 gram wet weight excised root suspended in moist vermiculite developed from the potting mix of *Spathiphyllum wallisii* compared to sterile jars of the same contents (Data are means ± SE; n=4).

2.6.3.2 Testing roots for arbuscular mycorrhizas

As well as their potential value in VOC removal, inoculation method 2 was also used to introduce viable AMF inoculum into the test pots to test whether AM colonisation of the hydrocultured plants was possible, and whether it had a growth enhancing effect on the plants. As AMF are obligate symbionts, axenic inoculum is hard to obtain and very expensive, thus reducing its appeal for commercial plant growing operations. Thus infected root lengths were used as an inoculation source in the current experiment, as have been successfully used in previous work (Krishna *et al.* 2005).

Quantification of AMF in the roots of the donor *S. wallissii* plant was undertaken to determine the level of fungal colonisation, and thus the likelihood of the inoculum having the potential to colonise experimental recipient plants. The donor plant was uniformly root-bound, so it could be assumed that the entire pot volume had been explored by the roots, and that any AMF propagules present would have the opportunity to form mycorrhizas. The roots were

examined for mycorrhizal colonisation after clearing and staining, using a method based on that of Torpy (2000). Roots were extracted from the potting mix by water washing, then excised from the shoots, and fixed in 95% ethanol. Clearing was then performed in 2.5% aqueous potassium hydroxide solution for 15 min at 90°C, and staining for 20 minutes in 0.05% tryptan blue in acidic glycerol (1% HCl) at 90°C. The samples were then left to de-stain overnight at room temperature in deionised water. Slides were examined microscopically and AMF structure types were recorded as being arbuscular, vesicular or hyphal (Table 2.1). The degree of colonisation by arbuscular mycorrhizal fungi was found to be 68 per cent of total roots analysed, which was regarded as sufficient for the root material to be used as an inoculum.

Table 2.1 Colonisation of *Spathiphyllum wallisii* roots by arbuscular mycorrhizal fungi, subsequently used as an inoculum.

Mycorrhizal type	**% Colonisation**
Arbuscular	9
Vesicular	22
Hyphal	37
Total	68

Treatments summary

A total of five growth media treatments were set up

-Potting mix (n=8)

-Hydroculture control (no inoculum) (n=15)

-Hydroculture + inoculum, Method 1 (n=18)

-Hydroculture + inoculum, Method 2 (n=18)

- Hydroculture + inoculum, Method 2 Control (Sterile roots) (n=8)

2.7 Indoor *S. podophyllum* CO_2 reduction capacity

2.7.1 Estimating leaf based light compensation points

In recent years, doubts have been expressed on the capacities of potted plants to reduce significantly indoor CO_2 (Llewellyn and Dixon 2011). As mentioned in chapter 1, this is mainly due to low light levels in the indoor environment, which are often at or very near the leaf photosynthetic compensation point of most common indoor plants (Burchett *et al.* 2011). However, there has been almost no testing of both indoor plant light compensation points or the actual photosynthetic performance of indoor plants *in situ*. These data were thus collected for the test plant species in the current project.

Leaf-chamber based light compensation points (LCPs) (the light intensity at which CO_2 flux equals zero) are used to estimate the light intensity at which the photosynthetic CO_2 removal by the plant shoots would be exactly balanced by their respiratory CO_2 emissions.

Leaves from the plants in the potting mix treatment group were tested using a leaf chamber infra-red gas analyser (IRGA: LI-COR 6400 portable photosynthesis system; LI-COR Inc.) with an enclosed chamber leaf area of 6 cm^2. Light of variable intensity was provided in the leaf chamber by a built in red/blue light emitting diode. Chamber relative humidity was continuously monitored, and ranged from 40 to 60%. Tests were carried out between 9.00 am and 5.00 pm, under conditions when natural photosynthesis could be expected to be in progress in C3 plants such as the test species. Young, fully opened mature leaves were sampled. Four leaves per plant, for 4 plants were tested. CO_2 at 400 ppm was used as the test concentration as this is at the lower end of average ambient indoor air CO_2 concentrations (Hess-Kosa 2002). The intensity of photosynthetically active radiation (PAR) provided to the leaf was gradually increased, step-wise, at intervals of 0, 2, 4, 6, 8, 10, 20, 50 μmol PAR m^{-2} s^{-1}. Each intensity level was maintained for 3–5 minutes to allow the photosynthetic response to stabilise and a meaningful reading to be obtained before increasing to the next intensity. The final CO_2 concentration present in the chamber is the resultant of any photosynthesis and/or leaf respiration. Readings are therefore recorded as CO_2 flux, as μmol CO_2 per m^{-2} leaf area s^{-1}. The LCP was estimated by interpolation from the light response curve produced.

2.7.2 Measurement of whole-potted-plant CO_2 fluxes

Whilst photosynthetic rate has generally been used in the past to test the CO_2 removal capacity of plants (Barton *et al.* 2010), this variable does not reflect the true performance of any plant system, as all plants have both non-green tissues plus microorganisms associated with their growth substrates which will have their own carbon use / release profile (Somova and Pechurkin 2001). Thus to estimate the true influence of the test plants on indoor air quality their carbon flux performance was measured on a whole plant plus substrate basis.

Glass test chambers, 26 x 20 x 30.5 cm (interior volume 15 L) were used for the trials, equipped with a portable Infrared Gas Analyser (IRGA) CO_2 monitor (TSI IAQ-CALC, TSI Inc., MN, USA) set to record CO_2 concentrations at 1-minute intervals. Fans (50 mm diameter 12 V DC) were fitted to maintain air circulation. Two light sources were used to attain various test intensities: ambient laboratory lighting was simulated by Wotan 'daylight' incandescent tubes with modified shading for the chamber to achieve 10 μmol photosynthetically active radiation (PAR) m^{-2} s^{-1}, and a 500 W sodium arc discharge lamp for a test intensity of 350 μmol PAR m^{-2} s^{-1}. All light levels were measured using a LI-COR light meter recording PAR wavelengths only, to account for the different wavelength profiles between light sources.

Four randomly selected samples, each in independent chambers were tested for each treatment. Plants for this analysis were sampled from the treatments using a formal random number process. Each potted plant was placed in the chamber set up with test illumination, which was then sealed and CO_2 concentration readings continued over a 40 min period at the tested light intensity. This period was selected because in preliminary testing it was found that after this interval, chamber CO_2 levels became low enough to affect the draw down rate (ie. the rate of removal became nonlinear and thus not representative of the draw down rate that would occur in an open system), and at the same time chamber humidity rose to levels that could affect stomatal function and thus photosynthesis. All whole potted–plant chamber trials were performed at a starting CO_2 concentration of ≈ 1000 ppmv, which is the World Health Organisation guideline for maximum allowable indoor CO_2 concentrations (WHO 2000), and the standard specified by American Society of Heating, Refrigeration and Air Conditioning Engineers for sealed, air-conditioned buildings (ASHRAE 2011). Previous work (Brennan 2011) has shown that the proportional rate of CO_2 draw down by two other indoor plant species is independent of the starting CO_2 concentration between 350 and 2000 ppmv; thus similar draw down rates could be expected for ambient conditions as those detected here.

The two test light intensities were selected on the following basis:

- 350 μmol PAR m^{-2} s^{-1} was the maximum intensity found within 0.5 m of any indoor light source within the two building investigated in previous UTS office studies (Wood *et al.* 2006; Burchett *et al.* 2010; Burchett *et al.* 2011), and thus it represented a practical maximum indoor lighting intensity; and
- 10 μmol PAR m^{-2} s^{-1} which was the most common, 'well-lit' office light level found in the UTS office studies, and used in follow-up laboratory studies (Brennan 2011; Burchett *et al.* 2011)

  Data were adjusted for differences in the initial CO_2 concentrations by expressing changes in chamber air as percentages of initial CO_2 concentration.

2.8 VOC Removal

To allow comparison of the VOC biodegradation rates of hydroculture and potting mix based plant treatments a chamber experiment was performed. The potted-plants were watered to saturation and allowed to drain for 1 h before being placed one in each test-chamber (as used in 2.7.2), with lids sealed and lights on at 50 PAR μmol m^{-2} sec^{-1}. A dose of 25 ppm (80 mg m^{-3}) benzene (Analar grade; BDH Chemicals Australia Pty Ltd, Port Fairy, Vic.) was injected into each chamber, this being five times the allowable occupational 8-h averaged maximum exposure limit in Australia (Environment Australia 2003). Samples of 1.00 mL of chamber air were withdrawn in a gas-tight syringe at regular time interval as described below. The four experimental plant treatments were tested in duplicate, and benzene concentrations analyzed using a gas chromatograph (GC; Shimadzu GC-17A, see Table 2.2 for specifications). Subsequent samples were taken at 12 hour intervals over the next 7 days. On day 7 the benzene readings approached undetectable levels for all experimental replicates.

Table 2.2 Gas chromatography parameters for benzene vapour estimation

Instrument	**Shimadzu GC-17A**
Column	J&W Scientific DB5 Megabore
Stationary phase	(5%-Phenyl)-methylpolysiloxane (nonpolar)
Mobile phase	Nitrogen
Flow rate	4 $mL.min^{-1}$
Injector port temperature	250ºC
Oven temperature program	Isothermal 70^0C
Detector	FID
Benzene retention time	~1.67 min

2.8.1 Benzene chamber control

To account for any reduction in benzene due to chamber leakage, absorption or adsorption (hereafter referred to as 'leakage'), leak tests were carried out on each of the six 15 L experimental chambers before and after use. This involved sealing the chambers and injecting 25 ppm of benzene into each chamber with a 5 μL volumetric (gas chromatography) syringe. The chambers were left for 30 min to allow the benzene vapour to equilibrate before a 1.00 mL sample of chambers headspace gas was withdrawn using a 1 mL gas tight syringe and immediately analysed using gas chromatography. Subsequent readings were taken daily over the next 7 days.

The results of the leak tests are shown in Figure 2.6. To correct for leakage, the data for individual chambers was modelled using least squares linear regression, and a correction applied to all experimental data calculated from the model at an equivalent time as the experimental reading. Average daily leakage across all experimental chambers ranged from 2% to 5%.

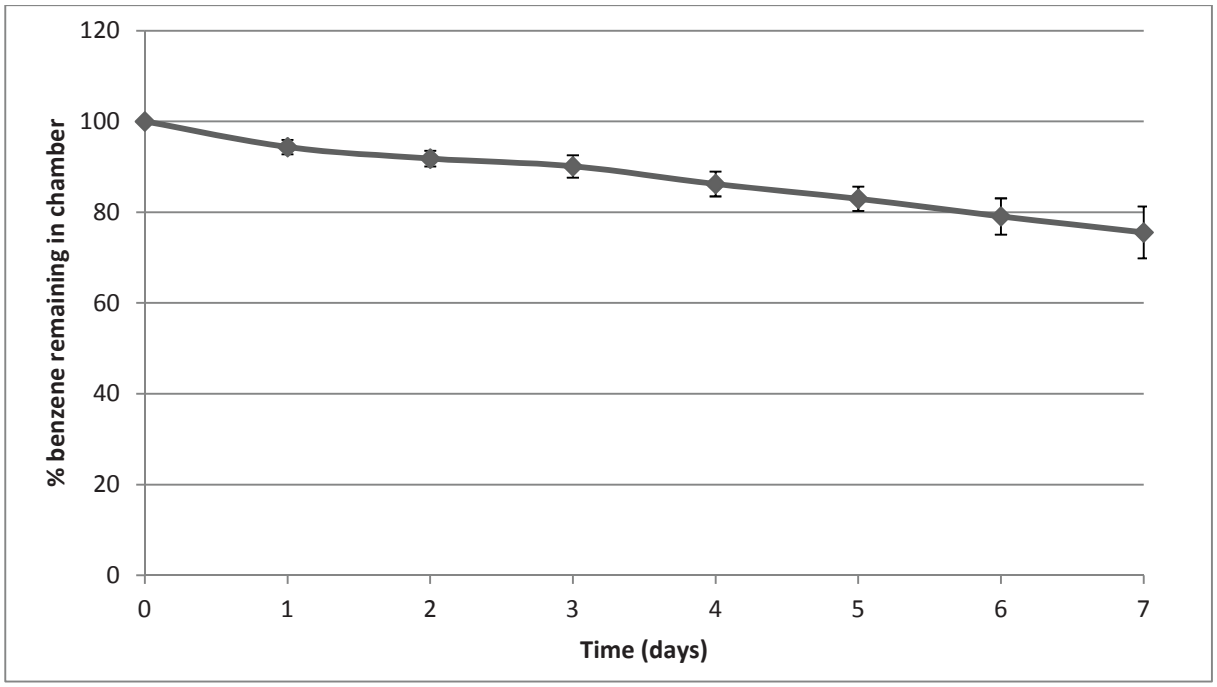

Figure 2.6 Benzene leakage rates, averaged across all 15 L chambers (Means ± SE; n=5).

2.9 VOC effects on bacterial community level physiological profiles (CLPPs)

Biolog EcoPlates were used as an indicator of bacterial community differences between the hydroculture and potting mix media, before and after exposure to benzene, to provide an indication of whether community metabolic changes on adaptation to benzene were similar in both media. This was not considered to be an exhaustive test of the bacterial communities, but it is an economical and commonly-used alternative to molecular procedures such as metagenomic or metabolomic studies.

Biolog EcoPlates are commercially manufactured microtitre plates loaded with a mixed mineral medium, a tetrazolium dye and 31 different carbon sources in triplicate, plus triplicate blank wells. CLPPs are characterised by the reduction of the dye linked to carbon source utilization. Thus the plates provide a profile of a bacterial community based on their differential use of these substrates.

For plants in potting mix; before and after exposure to benzene, (see section 2.8 above), 5 g dry weight equivalent samples of roots were excised from the upper part of the pot. Any closely adherent potting mix was left on the root material, in an attempt to sample root plus rhizosphere bacteria only. The material was extracted with 45 mL autoclaved de-ionised water in a 100 mL flask and shaken at 200 rpm for 30 minutes using a Griffin Flask Shaker (Griffin

& George Ltd, London, U.K.).. Tenfold serial dilutions was prepared and the 10^{-3} dilution was used to inoculate the Biolog Ecoplates. By means of an 8-channel pipette, one plate per sample was inoculated with 125 µL per well. The plates were incubated at 23^{o}C in the dark for 7 days and colour development was read immediately after inoculation and at 24 h intervals for the following 7 days with the use of an electronic plate reader (FLUOstar Optima microplate spectrophotometer, (BMG Labtech Pty Ltd)), at a wavelength of 590 nm. The same procedure was used for plants in hydroculture, except that 5 g of roots and the adherent perlite/vermiculite substrate was assessed in place of potting mix. Mean optical density (OD) for each of the 32 well types was calculated as the average of the three within-sample replicates. The ODs for the 31 carbon source wells were then corrected for any colour development in the blank water well due to particle-associated scattering or utilisation of any carryover carbon sources from the sample. Blank-corrected wells with a negative absorbance were treated as having an absorbance value of zero.

2.10 Hazardous Mould risk analysis.

An investigation into whether the hydroculture potted-plants could be a likely significant source of hazardous airborne mould spores was performed after completion of the experimental period. Previous work (Brennan 2011) has shown that the common pathogenic moulds are culturable on artificial media, grow well in competition with other species and are easily identified; thus a course screening assay only was performed. Samples from the growth medium (1 g) and the solutions remaining in pot reservoirs (1 mL) were taken aseptically from 3 randomly selected hydroculture pots. Sample aliquots were serially diluted in autoclaved deionised water and the 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} dilutions were used to inoculate, using the spread plate method, Rose Bengal Chloramphenicol Agar plates (RBC; Oxoid) containing (per litre) mycological peptone 5 g, glucose 10 g, di-potassium phosphate 1 g, magnesium sulphate 0.5 g, rose-bengal 0.05 g and agar 15.5 g. Inoculated plates were incubated in the dark at 23^{o} C for 7 days. When colony development was inadequate after this period, plates were allowed to incubate for another 7 to 14 days. The number of mould colony forming units (CFU) was counted and colonies were identified to genus level based on gross morphology using differential interference contrast light microscopy according to keys found in Mycology Online (2012) and 'Descriptions of Medical Fungi' (Ellis *et al.* 2007).

For comparison, 3 replicate 1 g potting mix samples were collected from the surface of randomly selected pots to a depth of 15 mm, this being the depth considered most likely to be

responsible for any release of spores into the air. Potting-mix samples were taken aseptically and serially diluted in autoclaved deionised water to 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}, then plated out and assayed for quantification and qualification of fungal spores, by the methods described above.

2.11 Data analysis

All data is expressed as means ± SE.

All comparisons of means were performed using GLM ANOVA . Pairwise comparisons were made using Tukey's HSD *post hoc* test. Prior to all analyses, data was checked for normality and homogeneity of variance. No data required transformation.

At day 47 plant height and leaf number was measured and compared between treatments, this being the duration at which plants were considered hardened and were inoculated.

At day 133, fresh and dry weights for shoots (stems, leaves) and roots and leaf area were determined for all treatments.

For CO_2 removal data collected from each sample, data were adjusted for differences in the initial CO_2 concentrations by expressing changes in the chamber air in terms of percentages of initial CO_2 concentration recorded over time**.**

CO_2 removal was compared amongst treatments, by fitting an ordinary least squares linear regression line to data from all samples and comparing the mean gradients.

Percentage of benzene removed per treatment was compared at time periods 24, 48 and 72 hours after injection of benzene.

Comparison of the multivariate CLPP data set before and after benzene exposure was made using principal components analysis (PCA) using Minitab Ver. 14 (Minitab Inc. 2003). PCA is a multivariate pattern analysis technique for reducing multidimensional datasets to fewer, and thus more easily interpretable, dimensions (Gomez *et al.* 2006). As is the case with all pattern analyses, it is not a significance test and therefore does not provide a p-value. In this instance, there were 31 dimensions (one for each carbon source). The first principal component is the dimension that reflects the greatest variance between samples (ie. the EcoPlates) in this multidimensional space. The second principal component reflects the second greatest variance and so on (Moldovan 2006).

To statistically compare CLPPs, an analysis of similarities (ANOSIM: PRIMER 6.1.6, PRIMER-E Ltd 2006) was used. This analysis is a rank matrix equivalent to MANOVA, that is based on a randomization process that makes the analysis more robust to the complex assumptions inherent with multivariate hypothesis testing procedures (Heath 1995). A 2-factor ANOSIM was performed with the fixed factors *treatment* and *data*. As ANOSIM in PRIMER cannot perform pairwise comparisons, after global significance was tested the two factor design was reduced to one factor with a dummy variable, and pairwise comparisons made using a sequential Bonferroni correction procedure.

3. RESULTS AND DISCUSSION

3.1 Transplant success of tissue cultured plantlets to potting mix and hydroculture treatments.

Generally healthy growth of plantlets was observed in both potting mix and hydroculture treatments (Figures 3.1 & 3.2). However, greater transplant shock was experienced by plants in potting mix, as indicated by the initial low growth rate recorded between days 1 and 12 and high cumulative mortality over the 47 days — 13.8% for potting mix compared to 2.5% for hydroculture plants. Known causes of transplantation shock include infection with soil pathogens, environmental stresses and injury to the plant's roots preventing it from developing a fully-established root system, as well as the complete change in hydration and nutrient supply mechanisms (Sundstrom 1989; Chandra *et al.* 2010). However, the potting mix plants that did establish themselves grew at the same rate as the hydroculture plants, there being no significant differences in leaf number or plant height after 47 days ($p= 0.282$ and 0.440 respectively).

The better transplantation survival success of the hydroculture plants over those in potting mix could be due to the reduced microbial load in this medium and hence the reduced chance of microbial infection, although this was not tested here. Transplant shock is mainly associated with the age at which seedlings are challenged to acclimatise to new environmental conditions (Close *et al.* 2004), so a possible contributing factor to success is that the hydroponic conditions better resemble those of the plant tissue culture medium from which the plantlets originated. A related possibility is that the tissue cultured plants had been insufficiently hardened. Hardening refers to the transitional period in which the plants are nurtured from laboratory to land transfer and is commonly measured as mortality percentages (Smith *et al.* 1990). It is considered that most losses during hardening occur due to the inability of plantlets to maintain adequate hydration (Sundstrom 1989; Kaymak 2011). Conditions that supply seedlings with moisture or reduce evaporative loss from leaves after planting reduce seedling death (Close *et al.* 2004). The improved transplant success of tissue culture explants into hydroculture over traditional potting mix has not been previously reported. The current results may therefore indicate that tissue culture explant losses and reduction in growth rate caused by transplant shock may be reduced by hardening plants from *in vitro* to *ex vitro* with hydroculture rather than standard potting mix.

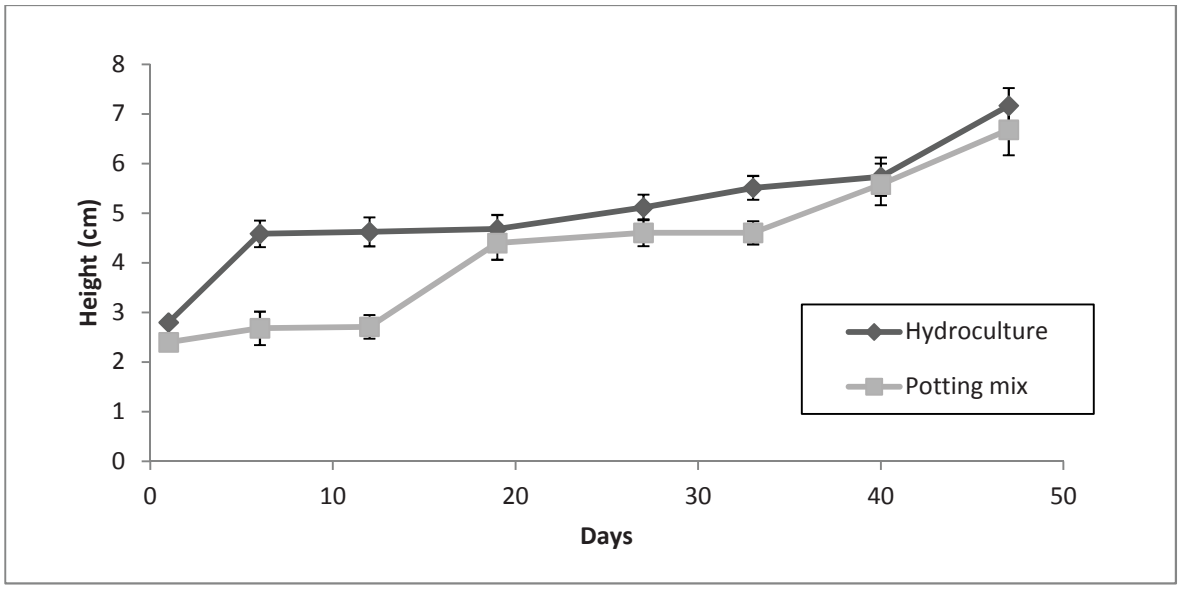

Figure 3.1 Changes in plant height for hydroculture & potting mix plants over 47 days (Means ± SEM; Final n = 31 potting mix, 79 hydroculture).

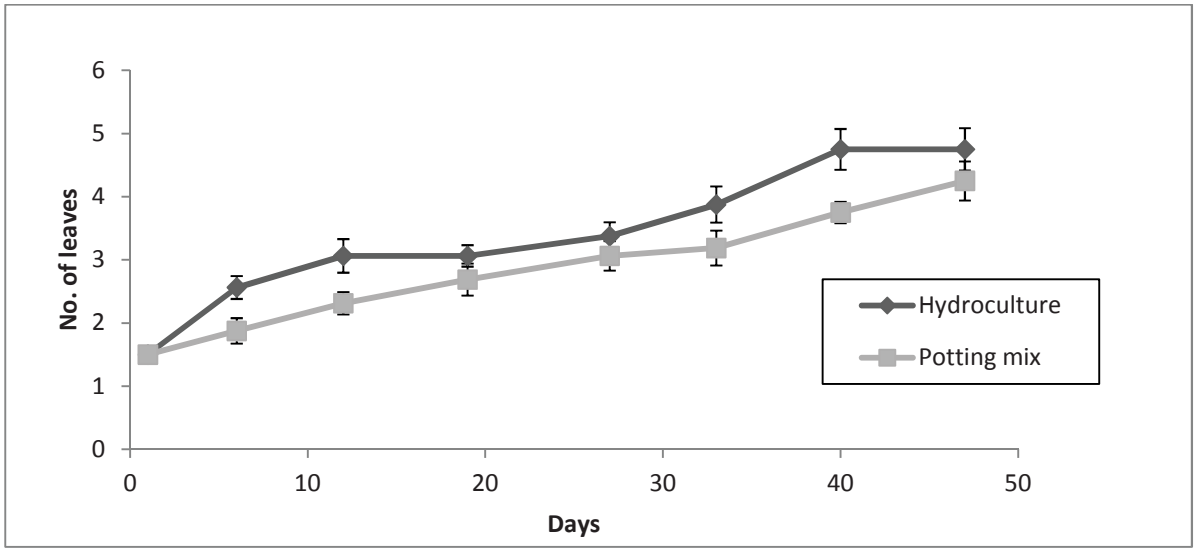

Figure 3.2 Leaf number of plants in hydroculture & potting mix plants over 47 days growth (means ± SEM; Final n = 31 potting mix, 79 hydroculture).

Table 3.1 Mortality rates of hydroculture and potting mix plants over 47 days

Treatment	Initial number	Final number	Mortality %
Hydroculture	81	79	2.5
Potting mix	36	31	14

3.2 Inoculation treatments and effects of bioaugmentation.

No augmentation of survival or growth was found with either inoculation treatment. Both methods reduced survival compared with the potting mix treatment (Table 3.2). Inoculation method 2, the addition of a root preparation containing AMF, had the greater impact in terms of mortality, the results also showing that the mortality rate resulting from this method was increased whether the material was applied alive or autoclaved.

Table 3.2 Plant mortality rates across all treatments. At Day 133 (86 days after inoculation).

Treatment/Survival	Initial number	Final number	Mortality %
Hydroculture	16	15	6.25
Potting mix	30	30	0.00
Hydro inoculation method 1	36	35	2.70
Hydro inoculation method 2	36	32	11.10
Hydro inoculation method 2 control	16	15	6.25

Among survivors, however, in terms of growth parameters measured at harvest (day 133) no differences were observed in leaf area ($p = 0.369$), shoot fresh weight ($p = 0.739$), shoot dry weight ($p = 0.912$), root fresh weight ($p = 0.972$) and root dry weight ($p = 0.995$) across all treatments, (Table 3.3). Also, among leaf numbers and shoot heights (Figures 3.3, 3.4), the only significant differences were between hydroculture inoculation method 2 and inoculation 2 control (p values, 0.0079 and 0.0002 respectively).

Table 3.3 Leaf areas (m^2), shoot fresh weight (FWT; g) and dry weight (DWT; g); root FWT and DWT for five treatments: potting mix; hydroculture control; hydroculture with inoculation method 1; hydroculture with inoculation method 2; and hydroculture inoculation method 2 control. (Means ± SE; n=4), and GLM ANOVA p-values amongst treatments for each data variable.

Treatment	Leaf area (m^2)	Shoot FWT (g)	Shoot DWT (g)	Root FWT (g)	Root DWT (g)
Potting mix	0.103±0.02	71.5±7.1	11.9±1.2	61.0±11	11.7±1.8
Hydroculture	0.115±0.003	74.6±4.0	11.9±0.3	68.5±8.4	12.0±0.7
Hydro inoc 1	0.10±0.16	70.3±3.5	10.7±0.5	65.5±5.3	11.5±0.6
Hydro inoc 2	0.10±0.05	66.2±5.8	10.4±0.8	65.6±5.4	11.5±0.8
Hydro inoc 2 (Control)	0.10±0.05	70.3±1.2	11.6±0.9	66.4±4.7	11.7±0.6
ANOVA p-value	p=0.369	p=0.739	p=0.912	p=0.972	p=0.995

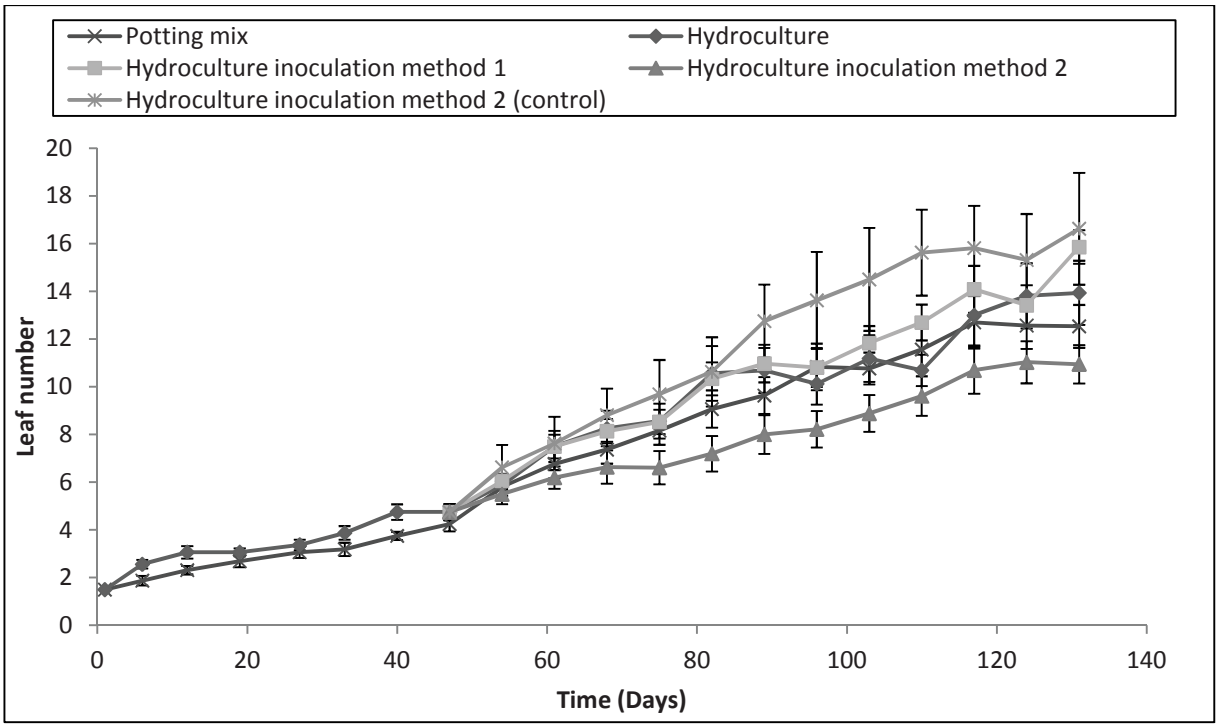

Figure 3.3 Changes in leaf number for all treatments over 133 days (Means ± SE; n = 15 hydroculture control; 35 inoculation method 1; 32 inoculation method 2; 15 inoculation method 2 control; 15 standard potting mix).

Table 3.4 GLM ANOVA comparing leaf number between five treatments at Day 133. (n= 15 hydroculture control, 35 hydroculture with inoculation method 1, 32 hydroculture with inoculation method 2; 15 hydroculture inoculation method 2 control; 15 standard potting mix).

Source of Variation	Degrees of Freedom	Mean squares	F-ratio	P-value
Between treatments	4	53.81	3.65	0.010
Error	62	14.73		
Total	66			

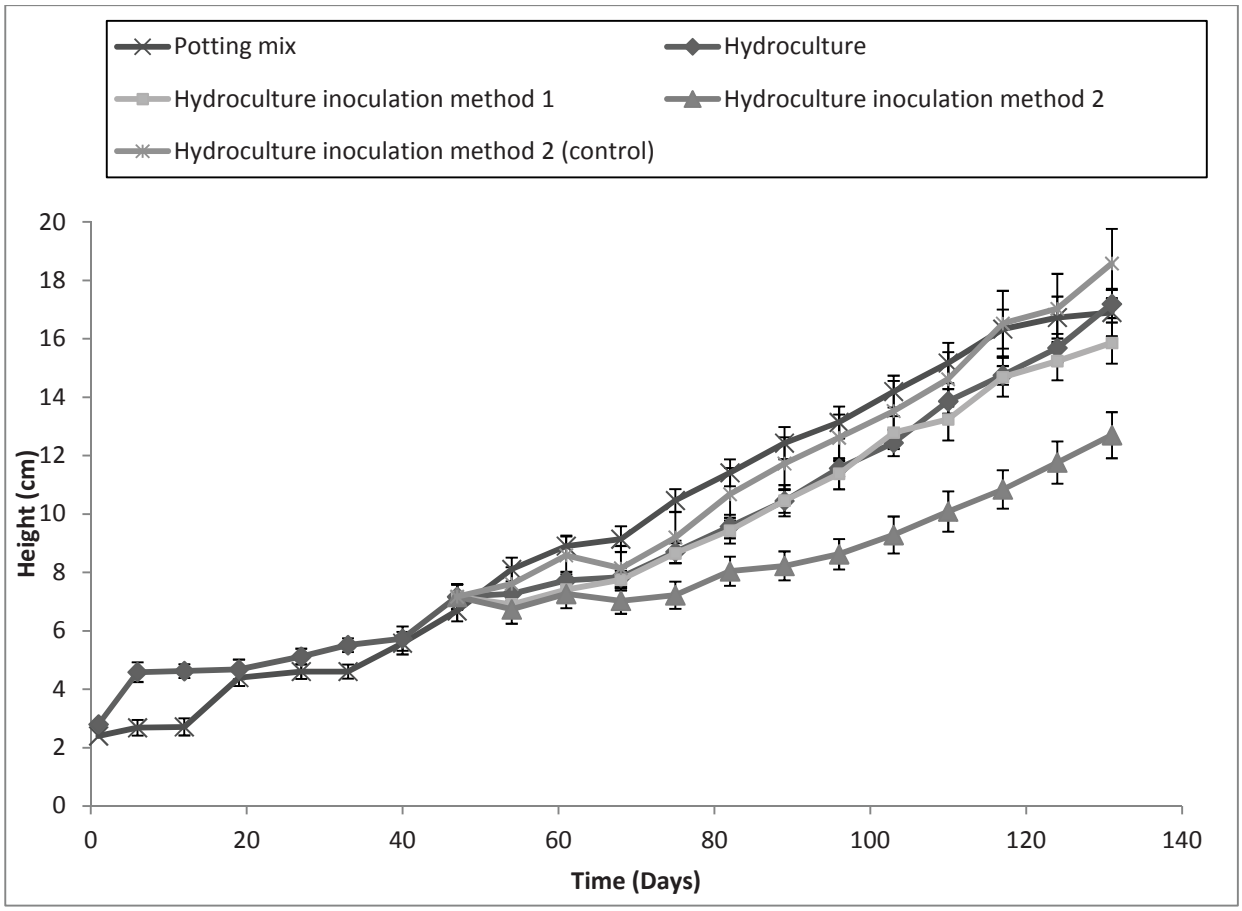

Figure 3.4 Changes in plant height for all treatments over 133 days (Means ± SE; n = 15 hydroculture control; 35 inoculation method 1; 32 inoculation method 2; 15 inoculation method 2 control; 15 standard potting mix).

Table 3.5 GLM ANOVA comparing plant height of five treatments at Day 133. (n= 15 hydroculture control, 35 hydroculture with inoculation method 1, 32 hydroculture with inoculation method 2; 15 hydroculture inoculation method 2 control; 15 standard potting mix)

Source of Variation	Degrees of Freedom	Mean squares	F-ratio	P-value
Between treatments	4	67.187	7.72	0.000
Error	58	8.702		
Total	62			

The observation that overall growth, was similar across all four experimental treatments, is in contrast to the findings of a number of other studies (Hopke 2009; Vestberg and Cassells 2009), which showed that introducing rhizospheric plant growth promoting rhizobacteria (PGPR) and/or AMF significantly increased plant biomass. As outlined in Chapter 1, it has been hypothesised that when AMF colonise roots, they reduce susceptibility or improve the vigour of host plants to withstand challenges from root pathogens (Lekberg and Koide 2005), and they also improve the bioavailability of nutrients, and resistance to drought (Gaur and Adholeya 1999)**.** AMF inoculation has also been used during *ex vitro* acclimitisation to reduce duration of hardening, and mortality of micropropagated plants (Gaur and Adholeya 1999). In this experiment, colonisation rates of recipient plants were found to be low, only achieving 8.3% **(Table 3.6),** with no observed positive effects on growth were brought about.

It is unknown whether the low colonisation rates and negative effects on growth were due to the inoculation method used or the AMF inoculum itself. The use of the most appropriate AMF inoculum is complicated by the fact that the most effective AMF species may differ between donor and recipient plants and may have variable effects on nutrient uptake, increased pathogen resistance or improved water relations (Gosling *et al.* 2006). Failure to find the most appropriate AMF/host/inoculation method may explain why the inoculum used failed to have any beneficial effect. In this study, a broad spectrum inoculum was used from a donor plant that was as taxonomically close as possible within the plant material resources available to this project. However, since the inoculation methods used here were somewhat crude, and no selective pressures against co-occurring pathogens were put on the treatment other than the subsequent exposure to benzene, it is possible that plant soil pathogens were introduced into the treatments. As such plant pathogens are mostly fungal (Alexopoulos *et al.* 1996), it is also possible that Inoculation Method 1 would be less likely to introduce them, and this outcome was reflected in the lower mortality rate from this treatment, also the observed growth rates which were closer to those of the hydroculture control plants. Soggy black roots were observed in some of the fatalities, thus making fungal disease a plausible cause of death (Okumura *et al.* 1999).

Table 3.6 Arbuscular mycorrhizal fungal (AMF) colonization as percentage of root length colonized in *Syngonium podophyllum* in inoculated hydroculture (method 2),; hydroculture inoculated with sterile roots (method 2, control); and potting mix treatments. (Means ± SE; n=3).

	% Colonisation			
	Inoculum	Hydroculture w/AMF	Hydroculture AMF Control	Potting mix
Arbuscular	9	0	0	0
Vesicular	22	0	0	0
Hyphal	37	8.33±4.06	0.33±0.33	1.66±1.20

Figure 3.5 Condition of plants in all treatments at final harvest (Day 133).

3.3 Light compensation point for *Syngonium podophyllum* grown in lab conditions.

The light response curve from leaf-chamber measurements for *S. podophyllum* grown for 133 days under laboratory lighting conditions is presented in Figure 3.6. These findings confirmed this species was capable of net photosynthetic uptake of CO_2 at very low light levels. The light compensation point (LCP); the intensity at which CO_2 flux equals zero, was at the extremely low level of 1.35 µmol PAR m^{-2} s^{-1} demonstrating that the plant is fully capable of removing CO_2 at light levels that have previously been described as too low for photosynthesis (Llewellyn and Dixon 2011). The intensity at which the CO_2 removal rate

reached saturation (asymptotic levels) was at approximately 10 µmol PAR m^{-2} s^{-1}, again confirming this species' adaptation to heavy shade conditions.

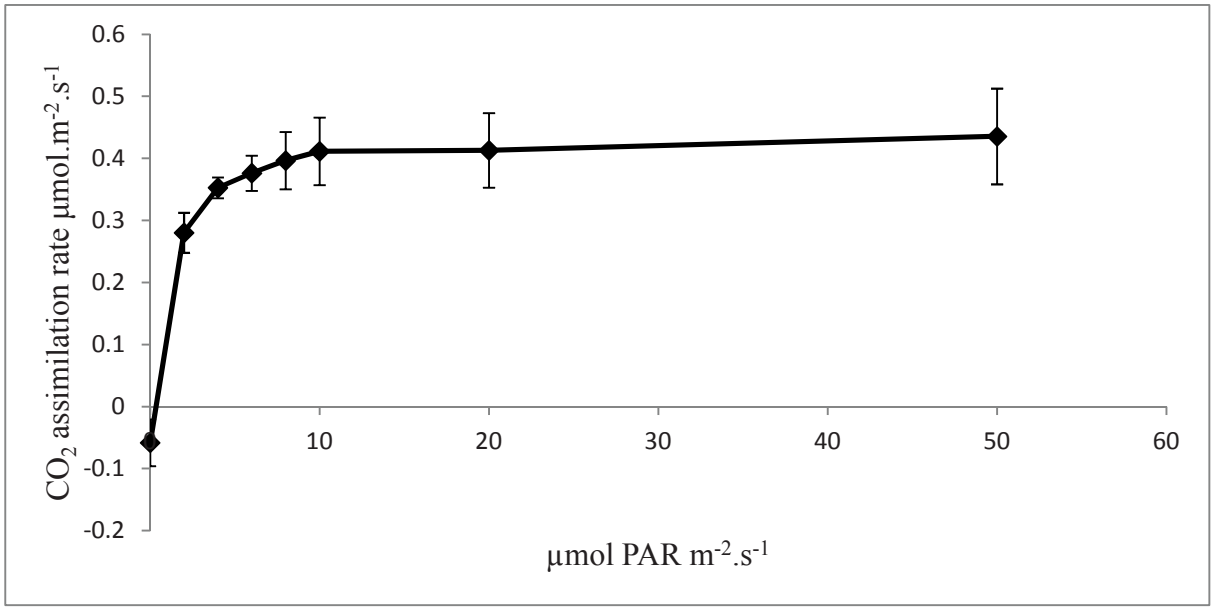

Figure 3.6 Light response curve of *Syngonium podophyllum* grown in laboratory conditions for 133 days (Means ± SE; n=4).

3.4 Whole potted plant CO_2 fluxes.

3.4.1 Under normal indoor light intensity

The results of the whole potted-plant microcosm (PPM) tests conducted at 10 µmol PAR m^{-2} s^{-1} are presented in **Figure 3.7.** At this light intensity all three hydroculture treatments showed net CO_2 reductions. Only those plants growing in potting mix did not do so, and on the contrary, tended to show a slight trend towards an increasing CO_2 level (linera regression, p=0.000). This was confirmed with the ANOVA with *post hoc* Tukey's tests of the mean regression gradients for this data, revealing a significant difference in CO_2 draw down rates between unaugmented hydroculture and potting mix (p = 0.0022). The greatest reduction in CO_2 concentration was recorded in the control hydroculture treatment (i.e. no bioaugmentation), with a net CO_2 reduction of 27% ± 6.5 from an initial concentration of 1000 ppm over the 40 minute period. This was followed by the Inoculation method 1 treatment, which showed a 10% ± 3.5 CO_2 removal from 1000 ppm over the 40 minutes.

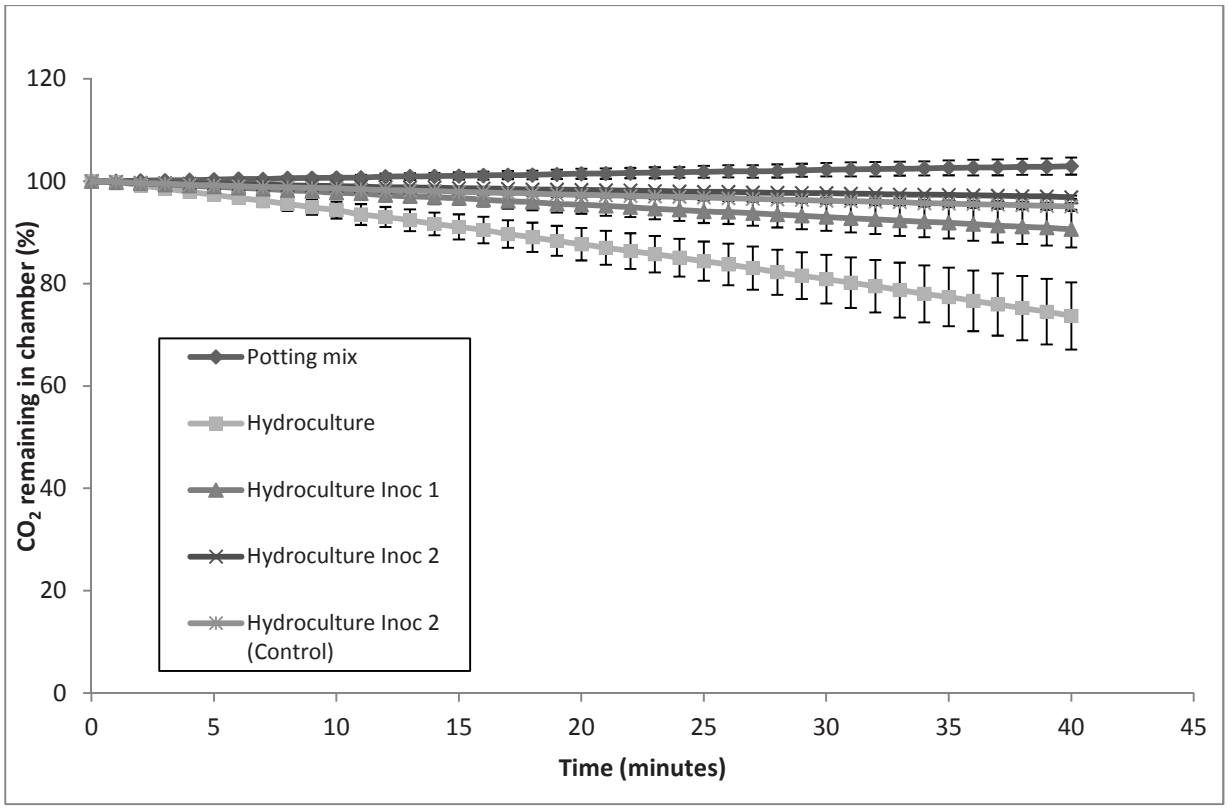

Figure 3.7 CO_2 changes under a 10 µmol PAR m^{-2} s^{-1} for *S. podophyllum* grown in potting mix, hydroculture, and inoculated hydroculture (Means ± SE; n=4).

Table 3.7 GLM ANOVA comparing CO_2 removal among the five treatments, after a 40 minute test period at a light intensity of 10 µmol PAR m^{-2} s^{-1} (n = 4).

Source of Variation	Degrees of Freedom	Mean squares	F-ratio	P-value
Between treatments	3	0.307	11.78	0.003
Error	8	0.026		
Total	11			

Tukey's *post hoc* test for the ANOVA in Table 3.7 revealed differences in the rate of CO_2 removal between: hydroculture control and hydroculture inoculation method 1 ($p = 0.041$), control and hydroculture inoculation method 2 ($p = 0.0088$), and the hydroculture control and potting mix ($p = 0.0022$).

The inability of the inoculated hydroculture plants to remove CO_2 as efficiently as the control could have been because of an increased microbial load; however, as the benzene degrading capacity of these plants would indicate (see section 3.4), it seems more likely to have been

due to reduced physiological capacity of the plants as a result of the inoculation treatment. In future experiments this could easily be tested by the inclusion of a manipulation control treatment group: none was used in the current experiments as it was not relevant to the hypotheses (ie. I was interested in testing the gross effects of the inoculation methods, and thus the treatments had to include the manipulation as well and the inoculation itself). In any case, it appears that the inoculation methods reduced the PPM's ability to reduce CO_2. Hydroculture with no inoculation is thus the recommended choice.

It was anticipated that at this very low light intensity, this species would either be at or near the photosynthetic compensation point, however net reduction of CO_2 by all but 1 treatment indicates *Syngonium podophyllumn* would be capable of reducing CO_2 in common indoor lighting conditions, and is thus an effective plant species for reducing indoor CO_2 concentrations.

3.3.2 Under maximum indoor light intensity

Figure 3.8 presents the results for all treatments, for CO_2 fluxes when plants were tested under a 350 μmol PAR m^{-2} s^{-1} light intensity. It can be seen that, with the increased light intensity, all treatments showed the ability to remove substantial net amounts of CO_2, as has previously been observed in other indoor plant species (Brennan 2011; Burchett *et al.* 2011). Again, hydroculture with no bioaugmentation showed the greatest net CO_2 reduction, on this occasion producing a reduction of 60% ± 1 of 1000 ppm over 40 minutes. Under this intensity, however, the lowest performing treatment was not that of potting mix, but that of hydroculture with inoculation method 1, which removed 29.5% ± 5 CO_2 of 1000 ppm over the 40 minutes. The potting mix treatment reduced 37% ± 3 of CO_2 over 40 minutes. Tukey's test for the ANOVA (Table 3.8) revealed significant differences between hydroculture control and both inoculation Method 1 ($p = 0.003$) and the potting mix ($p = 0.016$).

A light intensity of 350 μmol PAR m^{-2} s^{-1} light is rarely encountered indoors, unless sunlight is directly shining on the plants: and indoor plants are typically placed out of the reach of such high intensity light. However this intensity is sometimes used for spotlights or highlights in foyers or hallways, although, again, plants are unlikely to be placed close to the lamps. However, the intensity was chosen as representing the maximum ever possibly to be met by indoor plants, and therefore useful to test the robustness or scope of plant responses. Taken together, the results reported here indicate that, with moderately increased light regimes, and

aided by reduced bacterial loads in the growing media through the use of hydroculture, indoor plants could be a realistic tool in substantially reducing indoor CO_2 levels.

As only one species was tested here, future work is required to test the ability of other indoor species in hydroculture growth scenarios that reduce the microbial load. Furthermore, the development and selection of plants in hydroculture should be made with species that have already been shown in potting mix to have a greater potential to reduce CO_2 under different acclimatization regimes and different light intensities, as demonstrated in (Burchett *et al.* 2011). Burchett et al (2011) further demonstrated that different species can reduce CO_2 at different rates depending on the light intensity to which they have been acclimatized. Plants in the current experimentation were maintained at 50 µmol PAR m^{-2} s^{-1} since, on the basis of previous studies (Brennan, 2011; Burchett et al., 2011), this light intensity was considered likely to maintain the fastest growth rates, and is similar to the light intensity found in commercial glasshouses (F. Torpy and P. Irga – unpublished data).

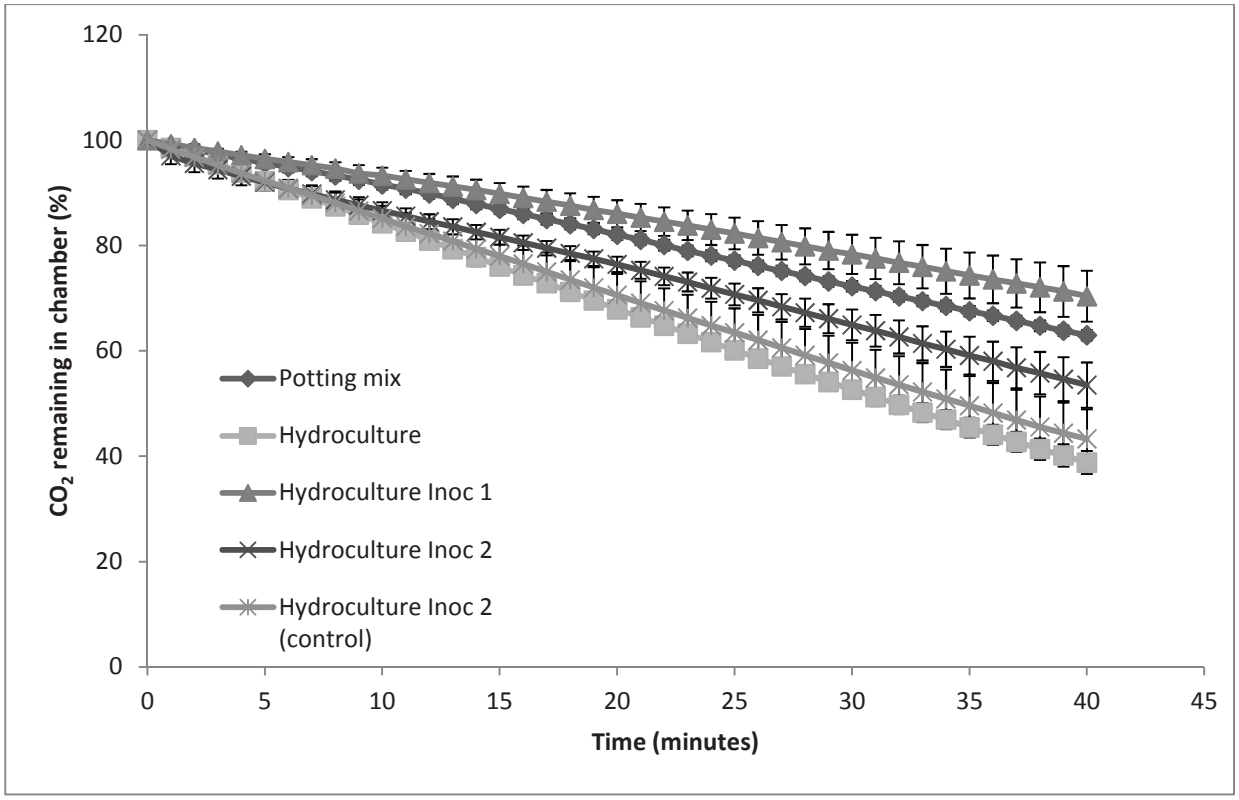

Figure 3.8 Displays all treatments (Potting mix, hydroculture, and inoculated hydroculture plants) CO_2 changes under 350 µmol PAR m^{-2} s^{-1} light regime (Means ± SE; n=4).

Table 3.8 GLM ANOVA comparing the mean rates of CO_2 removal among the five treatments, after a 40 minute test period at a light intensity of 350 μmol PAR m^{-2} s^{-1} (n = 4).

Source of Variation	Degrees of Freedom	Mean squares	F-ratio	P-value
Between treatments	3	0.36	10.36	0.004
Error	8	0.0347		
Total	11			

3.4 Benzene removal

3.4.1 Comparison of rates among treatments

Benzene removal rates for each treatment are shown in Figure 3.9, and a comparative summary of amount removed after 24, 48, and 72 h after leak corrections were made, and also the time taken to achieve 2/3 benzene removal is presented in Table 3.9. Removal was achieved in all treatments, and it can be seen that there were strong similarities of response across the three hydroculture treatments. The potting mix treatment showed the highest removal rates, showing double the efficiency after 1 day than the other treatments. The potting mix treatment showed removal rates similar in pattern to exponential decay of substrate utilisation following the Monod model (i.e. approximating first order kinetics) (Littlejohns and Daugulis 2008). The hydroculture treatments, in contrast, showed a more gradual removal rate with a steady rate of decline throughout. There were no significant differences in overall removal rates among inoculation treatments and the hydroculture control, as confirmed by the GLM ANOVA (Table 3.9), while the potting mix treatments significantly reduced more benzene than the hydroculture treatments at 24, 48 and 72 hours (Table 3.9).

Table 3.9 Percent removal of benzene from 15 L chambers at 24, 48 and 72 hours and time taken to obtain 2/3 removal for all treatments, with significance tests at the times indicated (GLM ANOVA) (Means ± SE; n=4). (Times taken to remove 65% of benzene were interpolated from Figure 3.9).

There were no significant differences between treatments with the same letter at alpha = 0.05

	% Removal of 25 ppm benzene			
Treatment	24 h	48 h	72 h	65% removal (h)
Potting mix	42.8 ± 13**a**	70.8 ± 1.2**a**	81.9 ± 4.0**a**	41.0
Hydro. control	21.0 ± 2.1**b**	45.4 ± 4.7**b**	59.6 ± 4.5**b**	80.5
Hydro. Inoc. 1	17.2 ± 3.2**c**	39.9 ± 5.0**b**	64.6 ± 2.1**b**	74
Hydro. Inoc. 2	25.4 ± 4.2**b**	44.7 ± 3.9**b**	64.0 ± 1.9**b**	74

Why is potting mix more efficient than the other treatments?

Despite attempts to bioaugment the plants in hydroculture, the potting mix treatment was nevertheless much more efficient at removing benzene. If the hydroculture without inoculation and potting mix treatments are compared, a variety of reasons can be proffered as to why the potting mix is more efficient. Firstly, it can be assumed the potting mix has a more diverse bacterial community structure, or at least is able to sustain a more diverse community, because the medium provides a diverse environment that is more hospitable for microorganisms. In addition, it is possible that any root exudates would not be plentiful enough to sustain such abundant bacterial communities in hydroculture. This possibility is explored further in the CLPP analysis (section 3.5), which examined the possible effects on the rhizospheric bacterial communities of the two growth media resulting from benzene exposure. Nonetheless, the hydroculture treatments were able to remove benzene at rates high enough to be able to propose their routine use for reducing indoor VOC levels.

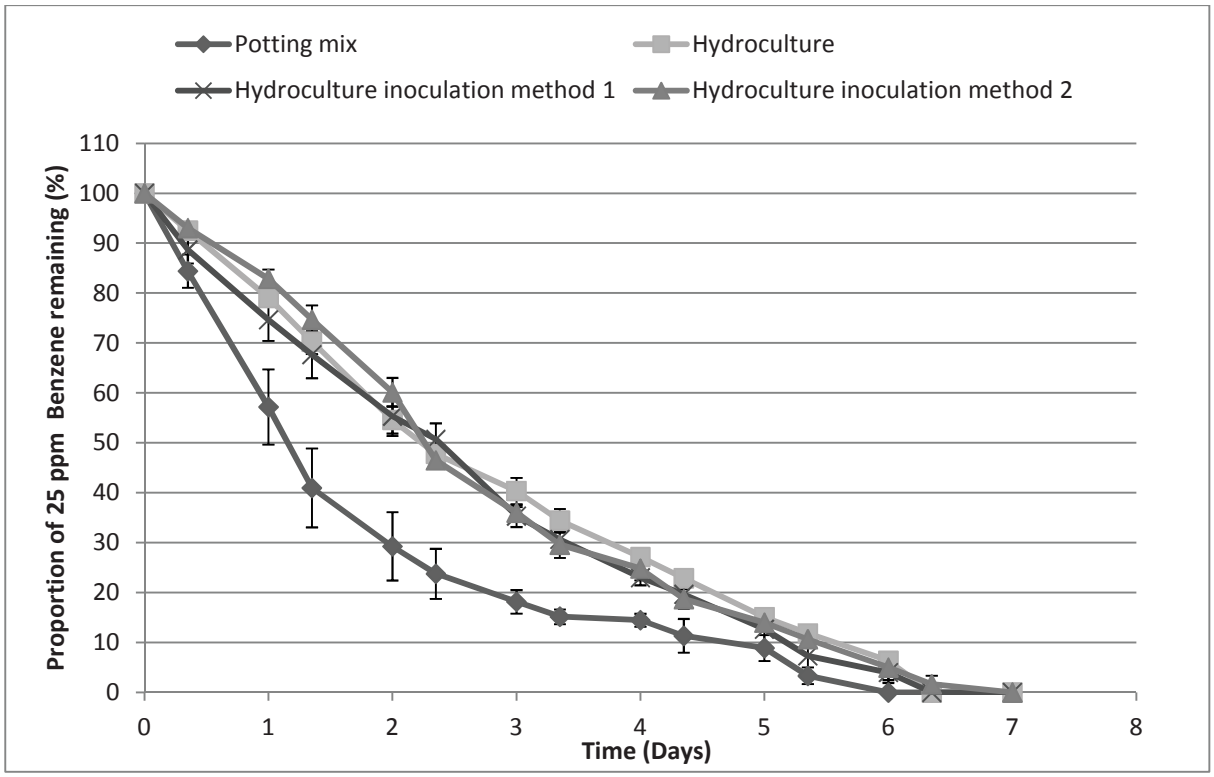

Figure 3.9 Removal rates of a 25 ppm dose of benzene, for the four treatments, corrected for chamber leakage (Means ± SE; n=4).

Failure of improved VOC removal with bioaugmentation

The fact that the overall removal rates were about equal amongst the two inoculation treatments and the hydroculture control provides another indication that the bioaugmentation methods applied here had to a large degree failed. However, bioaugmentation efforts have been found to fail rather frequently, due to lesser efficiency, competitiveness and adaptability, relative to the indigenous members of natural microbial communities (Megharaj *et al.* 2011). If this is true for the inoculation methods utilised in this study, it would explain why the control was equally as efficient in benzene removal. As shown in Figures 2.4 and 2.5, it was demonstrated that the inoculums had the capacity to remove benzene, thus the fate of the bacterial community in the inoculas most likely did not survive until the test period. It was therefore assumed that the bacterial population of the inoculated treatments subsequently became identical or similar to that of the control, in terms of benzene degrading taxa, and hence they were not assessed for their CLPP.

It was likely that the main reason for failure of enhanced bioremediation was the implementation of the bioaugmentation strategy. Other studies have found that co-culture with a known microbial consortium while the plant is still in tissue culture had the possibility

of a better symbiosis established (Pandey *et al.* 2000; Chandra *et al.* 2010); as evidenced in the biohardening and biopriming of some species grown in tissue culture (Harish *et al.* 2008). This would involve the use of microbial inoculants, primarily bacterial and mycorrhizal, as propagule priming agents both as *in vitro* co-cultures and on transplanting. This was not possible in the current project, as plants were inoculated after it was confirmed that they had completed the hardening stage (47 days after removal from tissue culture had occurred), possibly leading to their demise as they were introduced into an already competitive environment. The plants were not inoculated at an earlier stage as commercially available tissue culture plants are too well-developed, and thus needed to be removed from tissue culture as soon as possible. What is yet to be determined is if the bacteria involved in VOC degradation are the same ones that are involved in growth promotion. They are rhizospheric, but to what extent they benefit the plant, if at all, is unknown.

The inoculation methods; both included crude microbial communities only. This was justified because it had been previously demonstrated that a broad consortium is required to aerobically degrade benzene (Wood *et al.* 2002). Furthermore, its implementation needs to be simple and cheap if is to have any practical value.

Syngonium podophyllum has been shown in other studies to reduce VOCs, Wolverton et al., (1989) recording reductions of formaldehyde. In addition, Yoo et al., (2006) demonstrated *S. podophyllum* ability to remove benzene only by the aerial plant parts, with a maximum rate removal of only 0.103 $\mu g.m^{-3}.h^{-1}cm^{-2}$. This means no comparisons can be made with the current experiments. Other studies have shown that the aerial parts of the plant removal rates are trivial compared to that of the rhizospheric bacterial communities present in soil (Schmitz *et al.* 2000; Xu *et al.* 2011). However, when compared with another species of the same family, *Spathiphyllum wallisii*, *S. podophyllumn* has very similar patterns of benzene removal, and at similar rates ie approximately 4-5 days to remove 25 ppm without induction (Wood *et al.* 2002). It would be expected that the rate of benzene removal for all treatments can be increased with re-exposure to benzene, as demonstrated with a range of species in other species (Orwell *et al.* 2004; Orwell *et al.* 2006; Tarran *et al.* 2007).

3.5. Community Level Physiological Profiles of the rhizosphere of plants in potting mix and hydroculture before and after exposure to benzene

Principal Components Analysis (PCA) comparisons of pre- and post-benzene exposure of hydroculture (unaugmented control) and potting mix pot data (Figure 3.10) indicate differences in CLPPs between treatments both before and after exposure; i.e a multivariate difference among the four sets of data. Three distinct groupings can be observed on the biplot for this analysis (Figure 3.10): from potting mix plants pre- and post- benzene exposure, and hydroculture pre-exposure. A less distinct grouping was formed for post-exposure hydroculture pots, but three of the five data points are intermixed with the clusters of pre-exposure hydroculture and post-exposure potting mix replicates.

There was little apparent difference between pre-exposure hydroculture and post-exposure potting mix CLPPs, which indicates that the hydroculture medium already possessed the bacterial community that can degrade benzene. This result makes it unsurprising that neither bioaugmentation method produced any differences in rates of benzene exposure by the hydroculture treatments in the test-chamber trials (section 3.4). The pre- and post-exposure CLPP results of the potting mix treatment formed two distinct clusters, varying mainly along the first principal component.

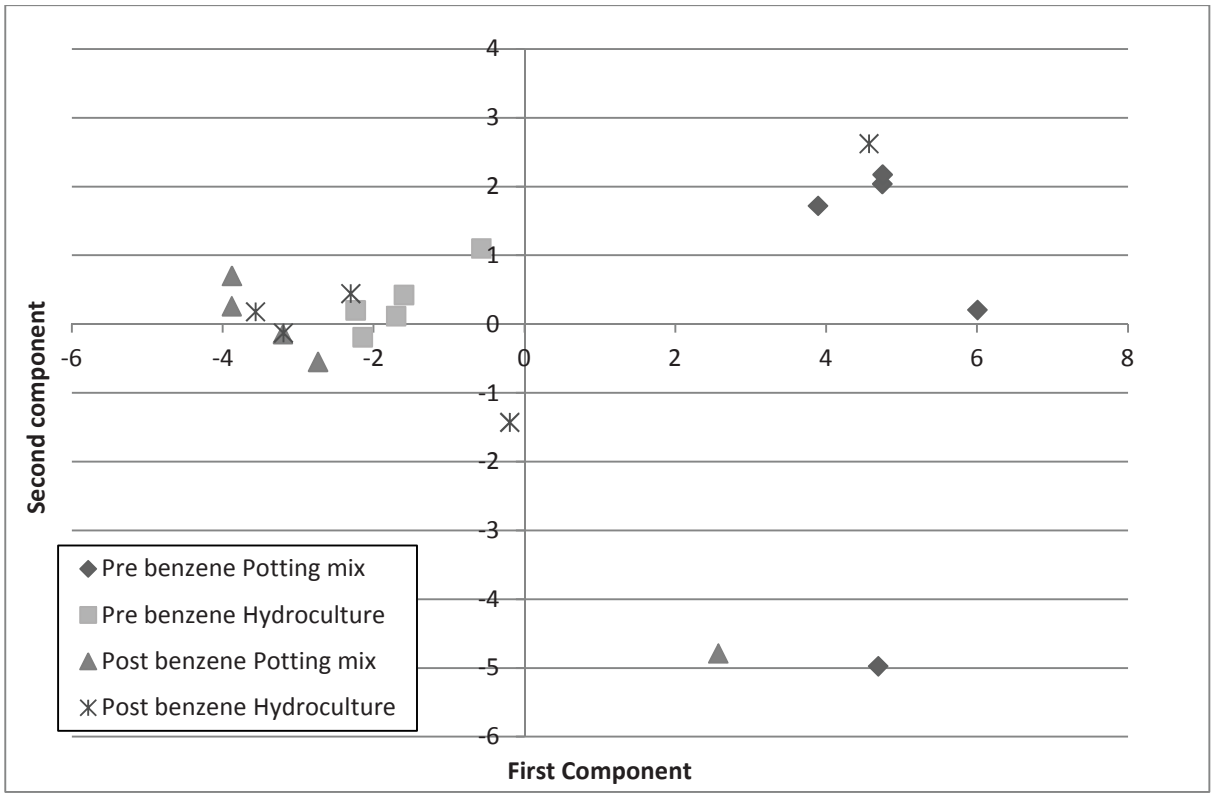

Figure 3.10 Ordination biplot produced from PCA of CLPP data of pre- and post-benzene exposure of hydroculture and potting mix treatments. Scores of each CLPP data for the first and second PCs are plotted (n=5).

ANOSIM of the CLPP data revealed a Global R statistic of 0.348, and a global p-value of 0.03. Pairwise comparisons showed significant differences in CLPP between hydroculture and potting mix without any exposure to benzene ($p = 0.04$) and potting mix treatments before and after exposure to benzene ($p = 0.04$).

Thus the pattern of differences in CLPPs observed in the potting mix bacterial community pre- and post-benzene exposure, demonstrated a significant shift in carbon source usage between these two bacterial communities. Whether these differences in CLPPs occurred as a result of changes in the proportions of bacterial taxa, or a change in the metabolism of individual bacterial types, or a combination of both, cannot be determined from the data obtained.

The ANOSIM also confirmed that while the CLPP pre-benzene exposure of the potting mix treatment differed from that of the pre-exposure hydroculture treatment, post-benzene exposure there were no significant differences between these two communities. Thus it would appear that after exposure to benzene, the potting mix community became more like that

which was already present in the hydroculture medium. In a previous study (Burchett et al. 2001), it was found that benzene exposure of the potting mix of *Spathiphyllum 'Petite'* almost halved the culturable bacterial density, although four, unidentified, aerobic bacterial taxa became more abundant post-exposure. It seems likely that similar changes in bacterial composition were responsible for the change in the potting mix CLPP found in this investigation.

The predominant degraders of organopollutants in the oxic zone of soils are chemo-organotrophic bacteria able to use a large range of natural and xenobiotic compounds as carbon sources and electron donors for the generation of energy (Fritsche and Hofrichter 2005). These bacteria generally fall within two phyla; the *Proteobacteria* and *Actinobacteria*, particularly by Gram-negative bacterial genera *Pseudomonas, Acinetobacter* and *Burkholderia* and the Gram-positive genus *Rhodococcus* (Cavalca *et al.* 2004; Kim and Jeon 2009; Martínková *et al.* 2009). Identifying bacterial species was beyond the scope of the current project, however, it is likely that aerobic, mesophilic taxa such as Pseudomonads would form an active component of the benzene degrading community, as has been found previously (Wood *et al.* 2002). As mentioned previously (Section 1.9), these earlier studies found that pure cultures from the potting mix of indoor plants failed to achieve significant benzene degradation rates, indicating that for the observed benzene biodegradation; the process was a consortial activity as has been found to be the case in other studies (Littlejohns and Daugulis 2008).

That there was no significant change in the hydroculture bacterial community before and after benzene exposure, and that effective benzene removal was detected for the hydroculture control treatment is strong evidence that whilst the bacterial community present in hydroculture is reduced in diversity compared to potting mix, the species present encompass at least some of involved with VOC removal, thus indicating that hydroculture plants should still be an effective means of reducing indoor VOC concentrations

3.6 Qualitative screen for pathogenic fungal spores from indoor *S. podophyllum* grown in hydroculture and potting mix.

No *Aureobasidium pullulans* was identified in the assay; which was the most dominant pathogenic species found by Engelhart et al., (2009). *A. pullulans* has a world-wide distribution and can be found in many indoor habitats (Gostinčar *et al.* 2011). However, it is regarded as being a health concern only when found in high concentrations indoors when it can cause a variety of localised respiratory infections (Hawkes *et al.* 2005; Engelhart *et al.* 2009). It very rarely causes systemic infections, although isolated cases have been reported (Kaczmarski *et al.* 1986).

Assessment of the hydroponic reservoir liquid nutrient revealed no fungal spores from the dilutions tested. The coarse procedure used does not rule out the presence of pathogenic fungi but shows there fewer than $1x10^3$ CFU/mL were present. As most common pathogenic moulds are capable of growth on RBC (Hocking 2003), this suggests that the nutrient solution is unlikely to be a source of dangerous moulds.

The results for fungal presence, composition and relative abundance, from the potting-mix and hydroculture growth medium samples are presented in Table 3.10. The hydroculture samples showed a little more diversity than was found from the potting mix samples, with a total of 7 genera (10 morphological species) identified, compared to 5 genera (9 morphological species) in the potting mix. No species of *Aspergillus* was found, and the most abundant genera isolated from both potting mix and hydroculture included *Cladosporium* and *Penicillium*.

Table 3.10 Number of morphological species found per fungal genera, frequency of occurrence and mean counts expressed in CFU/mL found in potting-mix and hydroculture medium (n=3).*Values are of samples found only once and hence not true means. Total CFU/mL is the average of total colonies found, estimated from the spread plate method and is not the total of the mean CFU/mL of the genera found.

Hydroculture			**Potting mix**		
Genera	Frequency (%)	Mean count (CFU/mL)	Genera	Frequency (%)	Mean count (CFU/mL)
Cladosporium spp.	25.85	$6x10^4$	*Cladosporium* spp.	66.11	$3.93x10^6$
Penicillium spp.	20.51	$4.5x10^4$	*Penicillium* spp.	17.09	$1.02x10^6$
Rhizopus sp.	18.73	$4x10^4$ *	*Scopulariopsis* spp.	10.08	$6x10^5$
Fusarium sp.	18.73	$4x10^4$ *	*Rhizopus* sp.	3.36	$2x10^5$ *
Verticillium sp.	12.81	$3.6x10^4$ *	*Botrytis* sp.	3.36	$2x10^5$ *
Acremonium spp.	11.62	$2x10^4$			
Total	100.00	$1.76x10^5$	Total	100.00	$1.55x10^6$

Other than *Rhizopus,* all of the species found in the potting mix sample are commonly found indoors (Adan and Samson 2011). *Rhizopus* spp. tend to be found in decaying vegetation and soils (Alexopoulos *et al.* 1996). In nature, most fungi are saprophytes on a diverse range of organic substrates and also commensal on plants and animals (Hillis *et al.* 2012). Through the use of hydroculture, the presence of decaying material is substantially reduced (provided proper maintenance of plant material, with dead or dying plant parts removed, as is standard in the interior plantscape industry).

Buildings with central HVAC systems and low air exchange, such as those present at UTS, may have 90 % lower airbourne fungal loads than outdoor levels (Burchett *et al.* 2010), or even less (Levetin 2004). In buildings considered acceptable for human occupation; the diversity of indoor fungi should parallel those outdoors (Hess-Kosa 2002). However, in buildings considered to have unacceptable fungal contamination, high concentrations of one or two spore types may dominate the air samples (Horner *et al.* 2008). Often these species are not present in the outdoor air or present in low concentrations (Levetin 2004). *A. pullalans* is a species often found in such situations (Hawkes *et al.* 2005). Despite the presence of mould spores in the potting mix and hydroculture samples, the presence of indoor plants has been shown to have no detectable effect on indoor airbourne spore concentrations, provided adequate building and HVAC design (Reponen *et al.* 1992; Brennan 2011).

It should be noted that this assay was a preliminary screen only; primarily designed to indicate the most abundant moulds. A full assessment of the mycoflora of the two media was beyond the scope of this investigation, thus further investigation is thus required.

4. SIGNIFICANCE OF FINDINGS

4.1 Comparisons of the performance of plants in hydroculture and potting mix

4.1.1 Survival and growth

Initially a greater transplant shock was experienced by plants in potting mix than those in hydroculture, as evidenced by the initial low growth rate between days 1 to 12, and twice the mortality rate over the first 47 days (Figure 3.1, Table 3.1). Transplantation success depends on a number of factors, however in this study it appears that the higher rate of transplant success in hydroculture was a result of the conditions in hydroculture being more similar to those in the original tissue culture than those in the potting mix, including a reduced microbial load in the hydroculture and hence a reduced chance of microbial infection. The potting mix plants that did establish themselves, however, grew at the same rate as those in hydroculture, there being no significant differences in leaf number or plant height after 47 days.

Implication for horticulture: For this species, growth of tissue culture plantlets in potting mix or hydroculture were similar. The reduced mortality in hydroculture indicates that this growing medium may be preferable if transplant losses are a significant cost to the nursery industry.

4.1.2 Responses to hydroculture bioaugmentation treatments

Over the 86 days post-inoculation of bioaugmented hydroculture plants, no further mortalities occurred in the potting mix treatment, whilst 6.25% mortality was recorded in the hydroculture control (Table 3.2). Over the same period, hydroculture with inoculation method 1 (bacterial preparation from donor-plant potting mix) showed only a 2.7% mortality, but with inoculation method 2 (with AMF colonised roots) a higher mortality rate was observed (11.1%), whether the preparation was applied live or sterilized (method 2, control) (6.25%).

Both inoculation methods had no recorded positive effects on growth compared to that of the hydroculture control and potting mix.

Implication for horticulture: The efforts trialled here for bioaugmentation to improve growth in this species were unsuccessful. However, the methods used were somewhat crude, and

further studies with more elaborate methods, referred to above, might produce more positive outcomes.

4.1.3 CO_2 removal

The leaf - based light compensation point (LCP) (intensity at which CO_2 flux equals zero) of the test plant, *S. podophyllumn,* was 1.35 μmol PAR m^{-2} s^{-1}, demonstrating that this species is fully capable of removing CO_2 at extremely low light levels (Figure 3.6). When whole-potted-plant CO_2 removal rates were tested at an indoor light intensity of 10 μmol PAR m^{-2} s^{-1} (Figure 3.7), all three hydroculture treatments showed net CO_2 reduction, however, bioaugmentated plants in hydroculture removed net CO_2 at a reduced rate than the hydroculture control. Plants growing in potting mix did not reduce net CO_2 at this intensity; on the contrary, in this case the potted-plant-microcosm actually emitted about 2-5% CO_2 over the 40 min test period. When tested at the maximum indoor light intensity recorded in any UTS studies (350 μmol PAR m^{-2} s^{-1}), whole-potted-plants of all treatments showed large increases in CO_2 reduction rates (Figure 3.8).

The results indicate that this species (with a light compensation point well below the test light intensities of 10 and 350 μmol PAR m^{-2} s^{-1}, as well as the culture conditions of 50 μmol PAR m^{-2} s^{-1}), has the capacity to respond positively to intervals of relatively high light intensities, such as will occur naturally as sun spots or flashes on the forest floor habitat, at various time during the day (Vierling and Wessman 2000).

Implications for horticulture: The results here confirm that, with a moderate increase in light intensity, the species could be developed and used to remove significant amounts of indoor CO_2. The results also suggest that hydroculture as a growth medium makes for greater efficacy of CO_2 removal than potting mix, presumably because of the extra load in microbial respiration carried in the latter system.

4.1.4 VOC removal

In the case of benzene removal, the potting mix treatment showed significantly higher reductions than any of the three hydroculture treatments (Figure 3.9, Table 3.9). The result is consistent with the previous finding of this laboratory (Wood *et al.* 2002; Orwell *et al.* 2004) that it is the microorganisms of the potting mix that are the main agents of VOC degradation:

the reduced microbial community in the hydroculture treatments slowing the removal of the VOC. The result is also consistent with the finding reported above, that net CO_2 removal rates were lower in the potting mix treatment that in hydroculture, although the leaf areas of the treatments were about equal (Table 3.3). No significant differences in VOC removal were observed among the hydroculture treatments, which confirmed that efforts to increase removal rates through bioaugmentation were not successful. Nonetheless, all the hydroculture treatments removed benzene at rates that are very robust, even though not at the same levels as the potting mix.

Implications for horticulture: The results clearly indicate that potted plants in hydroculture, or possibly with hydroponic irrigation in arrangements such as plant walls / vertical gardens could, like those in potting mix, be recommended for indoor air VOC and CO_2 reduction.

4.1.5 Effects of benzene exposure on substrate bacterial communities

Previous studies have shown that benzene exposure resulted in changes in the potting mix bacterial community level physiological profile (CLPP) of *Spathiphyllum* 'Petite' (Moldovan 2006). In the current study with *S. podophyllum*, it was also found that, in both hydroculture and potting mix, the VOC exposure caused changes in the CLPPs. Principal Components Analysis (Figure 3.10) showed that three distinct clusters formed; for potting mix pre- and post-benzene exposure, and hydroculture (unaugmented control) pre- exposure. The ANOSIM test showed that the CLPP pre-benzene exposure of the potting mix treatment differed from that of the pre-exposure hydroculture treatment, but that post-benzene exposure there were no significant differences between these two communities. Thus it would appear that after exposure to benzene, the potting mix community became more like that which was already present in the hydroculture medium. The fact that there was little difference between clusters for pre-exposure hydroculture and post-exposure potting mix, indicates that the hydroculture medium already possessed the bacterial community that degrades benzene. This would help explain why the bioaugmentation inocula failed to produce any increases in VOC removal over uninoculated controls (Figure 3.9).

Implication for horticulture: This is the first investigation into the effect of benzene on the CLPPs of rhizospheric bacteria of hydroculture plants. Whilst the bacterial community present in hydroculture is reduced in diversity compared to potting mix, the species present

encompass at least some of involved with VOC removal, thus indicating that hydroculture plants should still be an effective means of reducing indoor VOC concentrations.

4.1.6 Hydroculture and hazardous fungi

A case study by Engelhart *et al.,* (2009) implicated hydroponics as a source of origin for pathogenic fungi, specifically *Aureobasidium pullulans.* A screen of the mycobiota present in the medium of both hydroculture and potting mix, revealed sufficient community diversity, with no one fungal genera dominating, as is usually the scenario for health risks to occur. However, further, more detailed investigation is required on this matter.

Implications for horticulture: Hydroculture nutrient solution and supporting media did not harbour any pathogenic fungi and are thus unlikely to pose a major health risk. However indoor plants grown in both hydroculture and potting mix should be researched further as aetiological agents for infectious diseases.

4.2 Further investigations

This is one of very few studies of the air cleansing properties of indoor plants in hydroculture, and only one species was able to be investigated here. This species should now be tested for removal rates with other VOCs. Future research is also needed on the growth characteristics and air quality capacities in hydroculture of other commonly used indoor species.

The transfer of plant materials to hydroculture media is becoming more widely used in indoor horticulture, because of the increasing technology and usage of plant walls and similar arrangements (Liu and Baskaran 2005; Grewal and Grewal 2012), indicating that research on hydroculture plants' ability to improve air quality could be of great value.

Investigations into alternate and more refined methods of bioaugmentation in hydroculture for indoor species are also needed, since increased ability for VOC removal would be highly advantageous. The current work did not exhaust all possibilities; in particular the use of a benzene degrading bacterial community extract from the potting mix of the same plant species was not available for the current project. Similarly, methods to biostimulate benzene degrading bacteria already existing in the rhizosphere of hydroculture should be explored.

CO_2 removal capacities of hydroculture plants of this species need to be tested at a range of indoor light intensities ranging up from 10 μmol PAR m^{-2} s^{-1}, and appropriate lighting fittings

developed as part of the technology of plant wall installation. There is a continuing need for extended research studies into the photosynthetic responses and capacities of indoor plant species in both potting mix and hydroculture.

The studies here were all conducted on very young plants. It would be valuable to investigate the full capacity of mature potted-plants, in larger volume pots which are standard for office plants, to discover whether the promise of air quality improvement capacities at the immature stages in hydroculture are realised, or exceeded, in plants in regular use 'in the field'.

4.3 Concluding remarks

This is one of the first investigations made of the possibility that hydroculture plants can remove benzene. Furthermore, this is the first investigation into the effect of benzene on the CLPPs of rhizospheric bacteria of hydroculture plants. The findings presented here demonstrate the VOC removing potential of hydroculture plants. Whilst the rate of removal was somewhat slower than traditional potting mix plants, the simultaneous capacity of the system for effective CO_2 removal, plus the potential of the system to be improved with further research, is evidence that hydroculture is a more effective system for functional indoor plants than the potting mix systems that are used now.

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APPENDIX

Table 1. Water holding capacity of Perlite: Vermiculite ratios.

	% Water holding capacity
Volume rations Perlite: Vermiculite	
100%:0%	35.6
75%:25%	29.8
50%:50%	28.2
25%:75%	28.1
0%:100%	25.7

Table 2. Cyco Grow Platinum Series A and B formulation (S J Enterprises Pty Ltd)

Adjusted to 1mL/L (Nutrient concentrations used in the experiment)

Nutrient	**% weight/volume**
Calcium Nitrate	0.004
Potassium Nitrate	0.002
Potassium Phosphate	0.006
Magnesium Nitrate	0.0007