Perspectives for the use of biochar in horticulture

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Referaat
Biochar geproduceerd met verschillende productieparameters is doorgemeten in een substraatlaboratorium en een deel van de producten is gebruikt in potgrondmengsels in kortere en langere teeltproeven. Het uitgangsmateriaal blijkt van invloed op voeding en zoutniveau, de zuurgraad en het ionenuitwisselend vermogen, eigenschappen die de normale teelt kunnen verstoren. Er wordt aangegeven welke uitgangsmaterialen geschikt zijn wat betreft nalevering van voeding en zoutgehalte. Er wordt een methode beschreven en gevalideerd voor het compenseren van de zuurgraad. De productieparameters blijken van invloed op fytotoxiciteit, watergedrag, en afbreekbaarheid van de biochar, eigenschappen die direct van invloed zijn op teeltresultaten. Tenslotte wordt geteeld op mengsels met biochar en volgen aanbevelingen voor verdere compensatie van ongewenste eigenschappen. In deze proeven was het mogelijk zonder productieverlies te telen op mengsels met 20%-v/v biochar. De metingen maken aannemelijk dat dit percentage opgevoerd kan worden tot minstens 25%-v/v bij gebruik van geschikte metingen en menging met potgrondmaterialen met complementaire eigenschappen. Voldoende poreuze biochars blijken in de experimenten in staat microleven te huisvesten maar om deze potentieel veelbelovende eigenschap goed te benutten zal de biochar na productie ingrijpender moeten worden voorbehandeld.

Abstract
Biochars were produced using different production parameters and consequently measured in a rooting media laboratory and used as a constituent in potting soil mixes for 8 and 12 week growth experiments. The biochar feedstock influences the biochar's eventual nutrient and salt content, the pH, the buffer capacity and the cation exchange capacity all of which can potentially hamper normal growth. It is indicated which materials may result in effective biochars regarding nutrient and salt content. A method to measure and compensate unwanted pHs is described and validated. The production parameters are shown to influence growth defining properties such as phytotoxicity, water behaviour and degradability. Finally growth experiments with mixes of biochar and other rooting media constituents were performed and used for further advice on compensating unwanted properties. In the experiments up to 20%-v/v biochar was used without adverse effects on production. De lab results indicate the maximum amounts could at least be 25%-v/v when mixing with rooting media constituents with sufficiently complementary properties. Porous biochars are shown to be able to host microbial life but to fully utilize this promising trait more rigorous pretreatment of the biochar particles are suggested.

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Inhoud

Samenvatting

Doel van de studie was biochars te beoordelen met criteria voor tuinbouwsubstraten. In laboratoriumproeven werden eigenschappen van zuivere biochar gemeten. In plantenproeven werden de eigenschappen van biochar in mengsels verder bestudeerd.

Uitgangsmateriaal Biochars van: Gewasresten kastomaat, gewasresten paprika, hout chips van beukenhout en vermalen bossnoeihout.

pH Er zijn geen biochars zonder voorgaande pH behandeling bruikbaar voor glastuinbouw. Door de hitte behandeling bevatten biochars hoge gehalten geoxideerde kationen. Na_2O, K_2O, CaO en MgO. Door die geoxideerde kationen zijn ze erg basisch (pH 9-12). De hoge pH kan worden geneutraliseerd door een gelijkwaardige hoeveelheid onbekalkt (=zuur) veen. Een methode om de juiste dosering zuur veen te bepalen is ontwikkeld. De buffercapaciteit van biochar is hoog (258 mmol zuur / kg d.s.) waardoor één kubieke meter biochar geneutraliseerd moet worden met 3 kubieke meter zuur veen. Van biochar uit bossnoeihout kan dus maar 25% (v/v) in een venige potgrond gebruikt worden.

EC Biochars gemaakt van tomaten gewasresten of paprika gewasresten zijn ongeschikt voor gebruik in de glastuinbouw door hun te hoge gehalte aan voedingszouten en het te hoge natriumgehalte. Biochars gemaakt van houtproducten bevatten weinig zouten en zijn wel geschikt.

C:N, OUR, N-fixation De C:N verhouding van biochar uit houtproducten is hoog (50-100) wat aangeeft dat de biochar weinig voedingszouten zal leveren en waarschijnlijk erg stabiel is. De Oxygen Uptake Rates (OURs van <3) bevestigen dat de biochars niet gemakkelijk door micro-organismen worden omgezet dus stabiel zijn. We zagen wel dat een hoeveelheid nitraat van betekenis werd vastgelegd (nitraatfixatie). Dit geeft aan dat toch biochar door bacteriën wordt omgezet zodat een aanvullende nitraatgift nodig is om tijdelijke tekorten te voorkomen. In de glastuinbouw zijn materialen met een lage hoeveelheid eigen voeding gewild omdat alle voeding in de irrigatiegiften wordt meegegeven. Daarbij zijn stabiele materialen een voordeel maar is een vooraf te bepalen extra gift nitraat acceptabel *et al.* praktijk voor andere potgrond materialen.

Toxiciteit De biochar van paprika gewasresten gaf geen groeiremming in kiemtesten. De biochar van tomaten gewasresten met houtchips en 100% houtchips, lieten wel groeiremmingen zien in kiemtesten. Dat duidt op water oplosbare giftige stoffen die de kieming en vroege groei van planten remmen. Het biochar productieproces moet zo ontworpen zijn dat het condenseren van vluchtige toxische koolwaterstoffen in de biochar niet meer mogelijk is. Eerder werk door ECN liet al zien dat dit technisch haalbaar is.

Watervasthoudend vermogen Het watervasthoudend vermogen van biochar (30-60) is soms laag door de grove korrelstructuur (4-8 mm) die natuurlijk wel fijner gemaakt kan worden. Opvallend is dat die droge aard vrijwel volledig verloren gaat in mengsels tot 25%-v/v biochar in 75%-v/v veen, die zich blijven gedragen als 100%-v/v veen. Op zich zouden veel veensoorten baat hebben bij een wat droger materiaal. Het watervasthoudend vermogen van biochars is gunstig vergeleken met veel andere, drogere, materialen die door veen gemengd worden om het droger te maken.

Meeldauw experiment De mate van aantasting door meeldauw was niet verschillend in chrysanten op veen en op biochar. De productie op veen met biochar was gelijk aan de productie op veen zonder biochar toevoeging. Fusarium experiment. Er werd geen effect van Fusarium toediening op planten gevonden en daarom ook geen effect van biochar toediening op de aantasting van planten door Fusarium. De productie op potgrond met biochar was gelijk aan de productie op potgrond zonder biochar. Daarnaast kon biochar worden geladen met opgeloste organische stoffen (DOC). DOC is mogelijk van betekenis voor het gebruiken van biochar als drager voor micro-organismen die ziekten kunnen onderdrukken. Het experiment toont ook aan dat het toevoegen van biochar (15%-v/v) aan venige potgrond geen invloed heeft op de pH, EC of voedingshoeveelheden (mits de pH is gecompenseerd met de juiste hoeveelheid zuur veen). Dit betekent dat telers de biochar in hun mengsels kunnen gebruiken zonder hun bemestingsstrategie aan te passen. Het experiment laat zien dat dit kan zonder verlies aan opbrengst of kwaliteit.

Algemeen Biochar is potentieel geschikt als wortelmedium tot 25%-v/v. De metingen laten zien dat veel biochars plantengroei remmen, onvoorspelbaar wisselen van kwaliteiten, veel te hoge pH en EC hebben en de opbrengst verlagen. Alle genoemde nadelen zijn te ondervangen of te voorkomen.
Het is mogelijk dat biochar de drager kan zijn van nuttige micro-organismen maar de biochar zal moeten worden voorbehandeld met in volgorde:

a. Een pH neutralisatie.
b. Toevoegen van een gemakkelijk verteerbare koolstofbron.
c. De benodigde micro organismen.

1 Introduction

1.1 Motivation and background

The Ministry of Economic Affairs in Holland asked the national Top Technology Institutes to propose several cross institutional development projects to boost developments in several national priority areas (Top Sectoren). In the new TO2 project, partners ECN (Energy Centre Netherlands), TNO (Applied Physics), LEI (Agricultural Economics Institute) and Wageningen UR's Greenhouse Horticulture Unit decided to define a project focussed on reducing the energy use in the greenhouse horticultural industry. In greenhouse horticulture much emphasis was already on the minimisation of energy use when using conventional energy sources. Therefore in Work Package 1 (WP1) efforts were concentrated on energy from sustainable biogene sources and included the reuse of any rest products (priority area Horticulture and Propagation, T&U, as well as priority area Energy and High Tech Systems, HTSM). In WP2 efforts were aimed at the use of thin photovoltaic film (WP2, priority areas Energy, HTSM and T&U) and in WP3 efforts concentrated on the development of high tech sensor systems for greenhouse energy control (WP3, priority areas T&U and HTSM) .

The aim of the overall project was to find a more sustainable energy production system with emphasis on electricity generation, a higher energy efficiency and valorisation of biogene rest products of intensive horticultural production systems.

1.1.1 Biogene energy sources and the transition towards a biobased economy

The use of non-renewable feedstocks for energy supply and production of synthetic products has caused an exponential increase in CO_2 levels in the atmosphere since the start of the industrial revolution. Therefore, on a national and European level, agreements have been made to reduce CO_2 emissions to the atmosphere and to stimulate the transition towards a biobased economy. In a biobased economy, non-renewable carbon- and energy sources are substituted by renewable sources such as biomass. Biomass can replace fossil fuel by using pyrolysis; a process in which biomass is heated without oxygen supply. The process delivers combustible gas (energy), oil tar (to produce synthetics) and a charcoal-like rest product; biochar. Biochar can potentially be used to replace peat in soil potting media. Peat is a non-renewable organic material which is harvested in large quantities for the production of potting soil leading to degradation of nature areas and to a decrease in the amount of carbon that is sequestrated in peat areas. Thus, the use of renewable carbon sources for production of energy and production of potting soil is another step forward in the transition towards a biobased economy.

1.1.2 Biochar as an ingredient in potting soil

The use of biochar as a soil amendment or as ingredient for the production of novel potting soil has received increasing attention during the last decade because of the observed positive effects of biochar addition on soil quality and plant health. Mixtures of biochar and other common substrates, such as peat, have been successfully used for cultivation of plants. In some studies, biochar has been shown to have positive effects on plant health due to an increase in disease suppressiveness of the substrate (Elmer and Pignatello, 2011; Trifonova *et al.* 2009). However, biochar addition can also have adverse effects on plant growth due a high salt content, high pH and presence of phytotoxic compounds (Jaiswal *et al.* 2014). Whether biochar can be used to substitute part of the peat in potting soil depends on the properties of the biochar and the ratio in which biochar is mixed with other substrates. When used in horticulture, biochar should have a low salt content because of the adverse effects of salt on plant growth. The pH of biochar is too high to be used in horticulture without pH treatment. The pH can be lowered by mixing biochar with acid substrates such as peat. Other important criteria are the water holding capacity, the stability of the biochar, the nutrient content and the absence of phytotoxic compounds. The properties of biochar depend on the nature of the source material and the process conditions during pyrolysis. Thus, to use biochar as an ingredient of potting soil, the biochar production process should be optimized in order to produce biochar with favourable properties and the optimal mixing ratios between biochar and other ingredients should be formulated and tested.

1.2 Research objectives

In this subproject, the following questions will be answered:

i. What are the properties of biochar produced from different types of feedstocks?
ii. Can biochar be used an ingredient for potting soil and under which conditions (i.e. mixing ratio with peat).
iii. What is the effect of biochar addition on plant growth?
iv. What is the effect of biochar addition on plant health and disease suppressiveness.

1.3 Approach

ECN delivered biochar produced from different feed stocks and from different process conditions. These biochar samples were analysed in the laboratory of Wageningen UR, Glastuinbouw and assessed for their suitability as a peat substitute in potting soil. Based on the biochar properties, optimal mixing ratios between biochar and peat were formulated and the performance of these mixed substrates was used in two pot experiments to assess the effect of biochar addition on plant growth and plant health. Special attention was given to the ability of biochar to enhance disease suppressiveness because previous studies have shown that biochar can reduce symptoms of pathogenic organisms such as Fusarium oxysporum (Elmer and Pignatello, 2011). The positive effect of biochar on disease suppression can be explained by colonization of biochar with plant-growth promoting bacteria and fungi which can supress pathogenic bacteria due to competition for resources. The capability of biochar to enhance disease suppressiveness of substrates would give biochar an added value compared to conventional potting media. Overall, the objective of this study is to test whether biochar can be used as an ingredient for potting soil with similar or better properties compared to conventional potting soil.

1.4 Organisation

The project was organised by Chris Blok (Wageningen UR, Glastuinbouw) and performed by Inge Regelink (Wageningen UR, Alterra), Jantineke Zijlstra and Barbara Eveleens (Wageningen UR, Glastuinbouw). The characterization of biochars and the practical work for the greenhouse experiment was performed by Popko Bolhuis (Wageningen UR, Alterra), Barbara Eveleens and Eduard Hummelink (Wageningen UR, Alterra). At ECN, Lydia Fryda and Rian Visser were involved.

2 Material and Methods

2.1 Characterization of various biochars

2.1.1 Biochars

All biochars were produced by ECN (Petten) in a MILENA pilot gasifier, at 670 °C gasification temperature, except for sample 5 which was produced at 750 °C in order to compare with the char produced at 670 °C (Table 2.1). All biochars were continuously collected from the bed in order to ensure the biochar was produced under stable and continuous conditions.

Table 2.1
Overview of the biochars produced by ECN and pyrolysis conditions.

Number	Code	Biomass	Additives	Temp.	Remarks
1	Beech/ Tomato	80% beech wood + 20% tomato leaves	5% kaolin	670	
2	Wood/ Tomato	80% wood (Purmerend) + 20% tomato leaves	5% kaolin	670	
3	Wood chips -1	Wood cuttings from stadsbosbeheer Purmerend (Batch voorjaar 2015)		670	
4	Paprika waste (650)	Residues from paprika (Spain)		670	
5	Paprika waste (750)	Residues from paprika (Spain)		750	
6	Wood chips -2	Wood cuttings from stadsbosbeheer Purmerend (delivered in July, 2015)		670	Used in greenhouse experiment
7	Wood chips -3	Same feedstock as biochar 5, but processing conditions are optimised to remove dust (delivered in August, 2015)		670	
8	Beech	Beech wood chips			Used in climate chamber experiment

2.1.2 Elemental composition and element supply

Dry weight and bulk density were determined after drying at 110 °C. The organic matter content was determined by loss on ignition at 500 °C. Water-extractable nutrients were determined in an extract with a solid-solution ratio of 1:1.5 (v/v). The exchangeable cations were determined by extraction with concentrated $BaCl_2$. Water-extractable nutrients and exchangeable cations were analysed by Groen Agro Consult, a commercial laboratory for analysis of environmental samples. The specific surface area (SSA), elemental contents, levels of organic compounds were determined by Eurofins (Germany) using their standard analytical procedure for biochar characterisation.

2.1.3 Phytotoxicity test

Biochars may contain certain phytotoxic compounds. Therefore, phytotoxicity was tested with a phytotoxic test (bioassay) using water extracts (1:2 v/v) from the biochars. Water-extracts were filtered. Thereafter, the pH values of the extracts were adjusted to pH 5.5 using concentrated acid, the EC values were adjusted to 2 mS/cm by dilution with demi water, and nutrients (NPK) were added. As such, differences in pH, salt or nutrient availability cannot influence test results. A control treatment with demi water and nutrients was included. The bioassays were carried out using three different species: *Sorghum saccharatum* (sorghum), *Lepidium sativum* (garden cress) and *Sinapis alba* (mustard).
For each treatment, 4 plates with 30 seedlings were prepared leading to 120 seedlings per treatment. After incubation for 3 days at 25 °C, in darkness, photos of the plates were taken and the number of germinated seedlings was counted and root- and shoot length of seedlings were measured. Statistical analyses on the data were performed in GenStat.

2.1.4 Oxygen uptake rate

The oxygen uptake rate (OUR) method determines the maximum oxygen uptake rate measured under conditions ideal for microbial degradation. Substrates were mixed with a nutrient solution (NPK) to ensure that microbial activity was not limited by nutrients or moisture. The pH of the substrate-nutrient mixture was adjusted to pH 5.5 and a nitrification inhibitor was added.
The substrates were put in a closed vessel and placed on a horizontal shaker (150 rpm) for five days at a temperature of 30°C. The pressure in the vessels was continuously measured over the course of the incubation period. CO_2 was scrubbed from the gas phase. Therefore, the decrease in pressure can be completely attributed to consumption of O_2. The O_2 concentration was plotted as a function of time and the maximum O_2 consumption rate (dO_2/dt) was derived from this graph. The results are expressed as oxygen consumption per unit of time and mass of dry organic matter (mmol/kg/h).

2.1.5 Acid-neutralising capacity

The acid-neutralizing capacity of the biochar was determined by titration using a commercial titration unit (Titrino). A step-wise titration procedure was used in which concentrated acid (HCl) was dosed for five minutes followed by an equilibration period of 45 minutes without acid dosing. During dosing, acid was dosed until a pH of five was reached. During equilibration, the pH slowly increases again due to buffering of the substrate. This procedure was repeated ten times. The titration took therefore nine hours and was performed in duplicate. The total amount of acid dosed during the titration was set equal to the acid-neutralising capacity of the substrate. The same procedure was used to determine the base neutralizing capacity of peat however, using concentrated KOH.

2.1.6 Water holding capacity

The water content was determined over a range of suction strengths using a suction plate device. First, the samples were nearly saturated (-3 cm). Thereafter, the water potential was decreased to -31.5 and -50 cm and the water content was measured when equilibrium was established. The drying steps were followed by a rewetting step in which the sample was again brought to near-saturation in order to determine the effect of the drying on water uptake.

2.2 Pot experiment

A pot experiment in a climate chamber was performed to assess whether the addition of biochar to substrate affects plant growth and plant health. More specific, the experiment tested the effect of biochar addition on the disease suppressiveness of the Gerbera plants against Mildew infection. Mildew is a bio trophic fungus that feeds on living plants. The biochar was produced from beech wood.

Treatments

a. Standard substrate (peat).
b. Standard substrate with 20 % (v/v) biochar.
c. Standard substrate + fungicide (chemically induced disease suppression).
d. Standard substrate + SAR elicitor (biologically induced disease suppression).

Each treatment consisted of 20 plants. In treatment C, a chemical fungicide is applied. Treatment D includes a biological treatment (SAR elicitor) that induces disease suppression against Mildew through stimulation of natural processes in the plant (i.e. activation of the salicylic acid route). The experiment was performed in a climate chamber (20 °C, relative humidity: 85%, 16 hours light, 8 hours darkness). After three weeks, plants were infected with Mildew (Figure 2.1).

Substrates

Treatment A, C and D were cultivated on a standard peat substrate produced by Jiffy Substrates.
The substrate with biochar (treatment B) was produced by mixing 3 litres of biochar with 2 litres of acid peat in order to neutralise the high pH of the biochar. This peat/biochar was mixture was then mixed with a standard substrate. The volume of biochar in the final substrate was 20% (v/v).

Assessments and harvest

After five weeks, a plant health assessment was performed and the leaf chlorophyll content was measured (SPAD measurement) (Figure 2.1). After six weeks, plants were harvested and fresh weights were analysed.

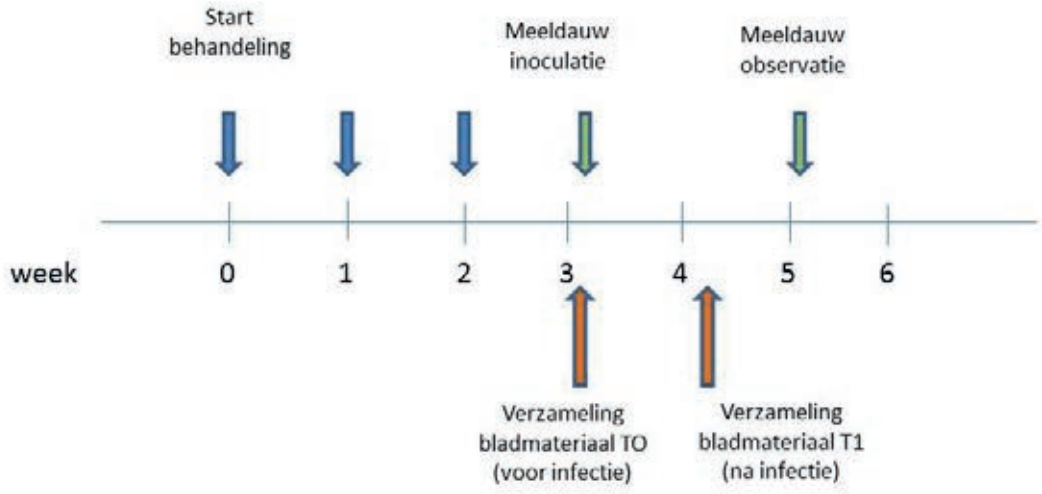

Figure 2.1 *Time schedule of the gerbera experiment. The objective of the experiment was to test the effect of biochar addition on the disease suppressiveness of plants against mildew infection.*

2.3 Greenhouse experiment

A greenhouse experiment was performed to assess whether the addition of biochar to peat substrate affects plant growth and plant health. More specific, it was tested whether (i) biochar addition enhances the ability of chrysanthemums to supress diseases caused by Fusarium oxysporum and whether (ii) addition of rhizobacteria to substrate enhances the colonization of biochar with bacteria.

Treatments

The plant-growth experiment included four treatments:

a. Peat.
b. Peat + plant-growth promoting rhizobacteria.
c. Peat + biochar.
d. Peat + plant-growth promoting rhizobacteria.

All treatments were infected with the plant-pathogen Fusarium oxysporum. Treatment B and D were treated with a commercial product (Compete Plus) containing plant-growth promoting organisms that are known to increase disease suppressiveness of the soil.

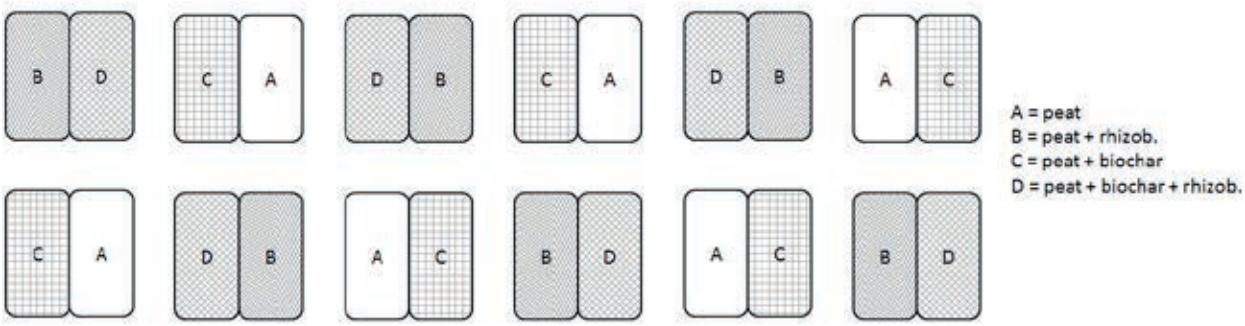

Figure 2.2 *Setup of the plant growth experiment. Each set of two blocks represents one table. Each block consists of 30 plants. Treatments with and without rhizobacteria were kept on separate tables and received water from separated storage tanks to prevent spreading of rhizobacteria.*

For each treatment, there were six blocks with 30 plants each, resulting in 180 plants per treatment and 720 plants in total. The blocks were distributed over the tables as shown Figure 2.2. For each block, 14 plants were guard plants and 16 plants were surrounded by other plants. Treatments with and without rhizobacteria were kept on separate tables and received water from separated storage tanks to prevent spreading of rhizobacteria to treatments without added rhizobacteria. For the first two weeks, plants were watered manually on top of the containers to ensure mixing of the rhizobacteria throughout the containers. From day 14 onwards, plants were watered from below by temporarily flooding the table (every one or two days, depending on weather conditions).

Substrates

Biochars were produced by ECN from 100% wood. Biochars were delivered in two batches, which were mixed in a 1:1 ratio for the plant-growth experiment. Acid peat (milled Baltic white peat) was ordered from Jiffy Substrates.

Two substrates were prepared; one with peat and one with peat and biochar. The pH of the peat substrate was adjusted to pH 5.0 by addition of 4 kg/m^3 dolomite (calcium magnesium carbonate). The peat /biochar substrate was prepared by mixing peat and biochar in an 85:15 v/v ratio. The pH was adjusted to pH 5.0 by addition of 2 kg/m^3 of calcium carbonate. The mixing ratios needed to prepare a neutral substrate (pH 5.0) were established in a preliminary mixing experiment. NPK fertilizer was added to both substrates (1 kg/m^3). The substrates were prepared two weeks before the start of the experiment. The pH and nutrient content were analysed shortly before the start of the experiment.

Plants

The pot-experiment was performed with chrysanthemums which were reproduced by stem cuttings. Ten days after planting the unrooted cuttings on a standard peat substrate, the plants had formed a sufficient rooting system and were transplanted into the final pots. Plants were grown in containers of 0.7 L.

Rhizobacteria

Rhizobacteria were added every two weeks in the form of Compete Plus, which is a commercially available product. Compete Plus contains a mixture of plant growth promoting rhizobacteria, fungi, microbial nutrients and humic acids. Compete Plus is a product of Plant Health Care B.V. (Oisterwijk) and is developed to enhance disease suppressiveness of soils and substrates. Rhizobacteria were added four times. Addition of rhizobacteria was started one day before planting the chrysanthemums into the final pots and was repeated once every two weeks (Figure 2.3). Compete Plus is a product in powder form and a fresh solution was prepared for each addition. For the first addition, each pot received 100 mL of solution prepared with 1 g/L Compete Plus. For the latter additions, each pot received 50 mL of solution prepared with 0.4 g/l Compete Plus. The rhizobacteria were poured on top of the pots in order to ensure that the rhizobacteria were well spread throughout the pots.

Plant-pathogen

All treatments were infected with the plant-pathogen Fusarium oxysporum, isolated from chrysanthemums. Fusarium was added to the chrysanthemums on day 14 of the experiment in a concentration of 10^4 spores per pot. Because there were only minor indications of infection, fusarium was added again in a concentration of 10^6 spores per pot on day 49.

Assessment and harvest

After 70 days, plants were harvested and dry weight, fresh weight and nutrient content were determined (Figure 2.3.)

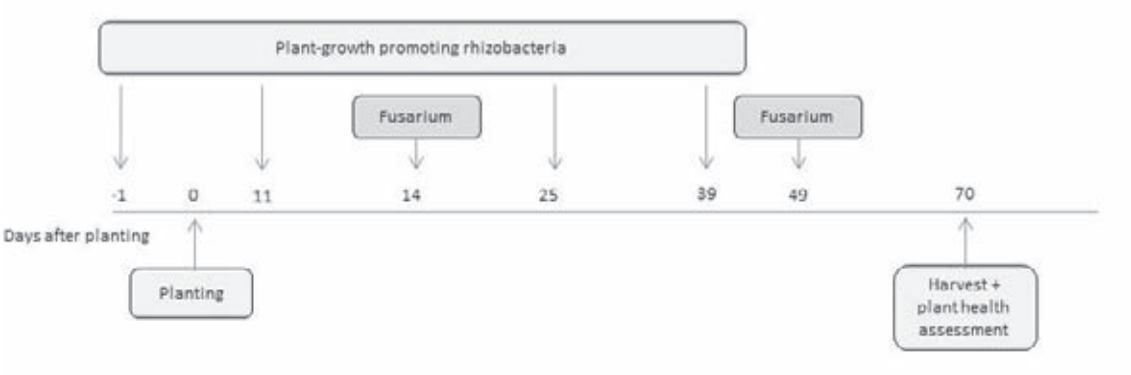

Figure 2.3 *Timeline of the greenhouse experiment with chrysanthemums in which the effect of biochar on plant growth and disease suppression was tested.*

3 Results

3.1 Characterisation of biochars

3.1.1 EC, pH and elemental composition

Table 3.1 shows the pH, nutrient and salt concentrations of the biochars, when extracted with water in a 1:1.5 volume ratio (substrate/water). Water-extractable nutrient concentrations give a good indication of the level of nutrients that are directly available for plant uptake.

Biochar materials produced from feedstocks containing tomato leaves or paprika waste are characterized by strongly alkaline pH values, high EC values and high concentrations of potassium (K), chloride (Cl), sodium (Na) and sulphate (SO_4). The high pH value (11-12) and high salt content (6.8–13 mS/cm) are problematic for application in horticulture since a pH of about 5.5 and an EC value below 1 mS/cm is required in the final substrate. The more nutrient cations a feedstock material holds, the higher the pH of the resulting biochar will be as these oxidise into alkaline oxides (Na_2O, K_2O, CaO and MgO). Two of the tested biochar materials were produced from feedstock that contained 80% wood and only 20% tomato leaves. It is immediately apparent that addition of small amounts of nutrient-rich waste materials to the feedstock can have a strong negative effect on biochar quality.

Biochar produced from wood (beech or residual wood) has a very low EC value (<0.6 mS/cm), low Na and SO_4 concentration and somewhat lower pH value (9.4 – 11). The EC value of the biochar meets the required EC value for substrates. However, the pH value is still much higher than the desired pH value (5.0–6.0) meaning that the biochar should be mixed with other acidic substrates to produce a mixture with the desired pH. Another apparent difference between biochar produced from wood and paprika waste is the specific surface area, which is 2 to 3 times higher for biochar produced from wood compared to paprika waste. The production of biochar from residual wood chips was repeated twice by ECN in order to produce sufficient material for the greenhouse experiment. The pH, EC and water-extractable nutrient content of these three batches are very similar meaning that ECN is able to produce biochar with a constant quality of the properties discussed. It appears that only biochar that has been produced from wood (without addition of nutrient-rich biomass) has a suitable EC value for application in horticulture.

The concentrations of water-extractable nutrients give an indication of the easily available nutrients. Water-extractable nutrients (Table 3.1) except potassium are very low in all biochar samples, irrespective of the nature of the source material. Thus, biochars do no deliver most plant nutrients in the short term except for potassium levels which are too high when using vegetable residue and may induce shortages of other elements by over supply. On top of that potentially harmful elements not necessary for plant production such as Na, Cl, SO_4 and HCO_3 are also present in vegetable residue in quantities high enough to reduce growth at dosages of over 10%-v/v.

The concentrations of exchangeable cations (Table 3.2) give an indication of the amount of nutrients that can become available in the mid-term due to exchange with other cations. The cation exchange complex is low in Na and rich in K, Ca and Mg, which are all plant nutrients. The total nutrient content gives an indication of the amount of nutrients that become available for plant uptake in the longer term (Table 3.2). Phosphorus (P) is likely present as calcium-phosphate minerals which slowly dissolve over time. The P content of biochar is however rather low; i.e. biochar should not be regarded as a P fertilizer. With respect to nitrogen (N), the story is more complicated because it is the C/N ratio rather than the total N content that determines the effect of biochar on N availability. Because of the very high C/N ratio of biochar (65-110), mineralisation of biochar will at first lead to N fixation rather than N mineralisation. This can be prevented by addition of extra N in the form of calcium nitrate. Materials with a low nutrient content are suitable for application in horticulture because nutrients are delivered through the water.

To summarize, biochars produced from source materials that contain nutrient-rich biomass (i.e. tomato leaves, paprika waste) have too high salt contents and too high pH values to be used in substantial amounts in horticulture. Biochars produced from beech wood or residual wood chips are of much better quality because of their low salt content but neutralisation of the pH is still required.

Table 3.1

Basic characteristics and nutrient supply biochars produced from different source materials and under varying temperature[1] in a 1:1,5 extract.

	Paprika (650 C)	Paprika (750 C)	Beech/ Tomato	Wood/ Tomato	Beech wood2	Wood – batch 1	Wood - batch 23	Wood - batch 33
pH (-)	12	12	11	12	11	9.4	9.9	10
EC (mS/cm)	9.6	11	6.8	13	0.68	0.53	0.61	0.71
NH_4 (mmol/l)	< 0.1	< 0.1	< 0.1	0.1	< 0.1	< 0.1	< 0.1	< 0.1
K (mmol/l)	61	84.3	49.1	94.3	3.2	3.6	3.6	4.5
Na (mmol/l)	5.1	7.4	0.5	1	0.1	0.2	0.2	0.3
Ca (mmol/l)	0.3	0.5	0.3	3	0.3	0.2	0.3	0.2
Mg (mmol/l)	0.2	0.1	0.1	< 0.1	< 0.1	0.2	0.2	0.1
Si (mmol/l)	0.1	0.2	0.3	0.3	< 0.1	< 0.1	< 0.1	< 0.1
NO_3 (mmol/l)	0.1	0.1	< 0.1	0.2	< 0.1	< 0.1	0.1	< 0.1
Cl (mmol/l)	47.8	65.2	23.6	48.5	0.1	0.2	0.4	0.6
SO_4 (mmol/l)	4.1	4.3	10.8	21.4	0.3	0.4	0.2	0.2
HCO_3 (mmol/l)	18.9	19.5	3.3	7.2	3.6	3.2	3.7	4.3
P (mmol/l)	<0.05	<0.05	<0.05	<0.05	< 0.05	0.1	<0.05	<0.05
Fe (µmol/l)	< 0.4	< 0.4	0.4	0.9	< 0.4	0.5	0.6	0.5
Mn (µmol/l)	< 0.1	< 0.1	0.2	< 0.1	< 0.1	2.2	0.7	0.2
Zn (µmol/l)	< 0.1	< 0.1	0.1	0.1	< 0.1	0.2	0.1	0.1
B (µmol/l)	28	35	13	9	10	8	7	6
Cu (µmol/l)	0.2	0.1	0.1	5	< 0.1	<0.1	<0.1	<0.1
Mo (µmol/l)	<0.1	<0.1	0.5	0.9	< 0.1	<0.1	0.10	<0.1
Dry weight (%)	95.5	95.5	96.7	97.1		97.8		
WHC (%)	284	234	171	210		217		
Bulk density (kg/m^3)	104	129	131	113		102		
SSA (m^2/g d.s.)	39	29	59	81		119		
As (% w/w d.s.)	35	34	19	28		13		
total C (% d.s)	59	59	77	68		82		
total H (% d.s)	1.2	1.3	1.3	1.4		1.5		
Total N (% d.s)	0.8	0.9	0.7	1.0		0.8		
C/N ratio (mol/mol)	86	76	128	79		119		

[1] Nutrient concentrations and pH were determined in a 1:1.5 (v/v) water-extract. Dry-weight, water-holding capacity (WHC), bulk density, specific surface area (SSA), ash content and total H, C and N contents were determined by Eurofins (data supplied by ECN). Composition of feedstock is given in Table 2.1.

[2] Biochar used in pot experiment with gerbera.

[3] Biochar used in greenhouse experiment with chrysanthemums.

Table 3.2

Total element content and exchangeable ($BaCL_2$) cations.

	Beech/Tomato	Wood		Beech/Tomato	Wood
DS (%)	100	100	$BaCl_2$ – K (mmol/l)	-	3.2
K (mmol /kg d.s)	285	254	$BaCl_2$ – Na (mmol/l)	-	0.2
Na (mmol /kg d.s)	< 10	11.9	$BaCl_2$ – Ca (mmol/l)	-	1.5
Ca (mmol /kg d.s)	341	515	$BaCl_2$ – Mg (mmol/l)	-	1.3
Mg (mmol /kg d.s)	126	119			
N-tot (mmol /kg d.s)	301	469			
P-tot (mmol /kg d.s)	21	69			
Fe (mmol /kg d.s)	4.4	14.6			
Mn (mmol /kg d.s)	10	5.7			
Zn (mmol /kg d.s)	0.09	1.4			
B (mmol /kg d.s)	2.5	3.2			
Mo (µmol /kg d.s)	< 10	13.4			
Cu (µmol /kg d.s)	107	154			

3.1.2 Phytotoxicity

The results of the phytotoxicity test (Figure 3.1) provide insight into the presence of toxic compounds that affect germination and early growth of seedlings. The tested biochar materials were produced from three different feedstocks; paprika waste, wood chips and tomato leaves and wood chips only.
The biochar produced from paprika waste performed rather well in the phytotoxicity test as there was no significant difference in root and shoot length compared with the control treatment. In contrast, the biochar produced from wood and tomato leaves showed significant adverse effects on root and shoot development indicating that this biochar contains water-soluble phytotoxic compounds. The biochar produced from wood chips is of better quality however, still a significant reduction in root length of Sorghum was found. Thus, also this biochar is not completely free from toxic compounds. The presence of phytotoxic compounds is a point of concern and further research is needed in order to understand how the presence of phytotoxic compounds in biochar can be prevented.

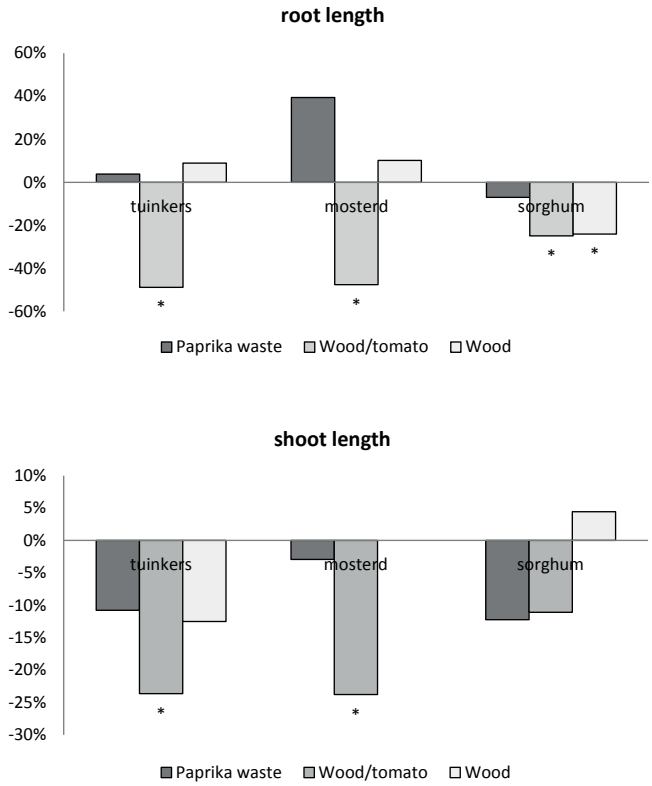

Figure 3.1 *Results of the phytotoxicity test for three biochars produced from paprika waste, wood and tomato leaves, and wood chips only. The bars show the average difference in root (upper figure) and shoot length (lower figure) between treatments and the control. The asterisks denote a statistically significant reduction in root or shoot length. Biochar produced from wood/tomato shows significant phytotoxic effects.*

3.1.3 Stability

The oxygen uptake rate (OUR) is a measurement for the maximum oxygen uptake rate under ideal conditions (i.e. ideal pH, EC and sufficient amount of nutrients present). The OUR of biochar is very low and similar to the OUR of peat (Table 3.3) meaning that the organic material in the biochar is very stable. A low OUR is beneficial for application in horticulture because it means that degradation of the material, and consequently shrinkage of the substrate, will not occur. A consequence of the low OUR is that biochar is a poor source of carbon for micro-organisms. Thus, if one aims to stimulate microbial activity in potting soil, an additional carbon source like fresh wood fibre or coir may be needed.

Table 3.3
Maximum oxygen uptake rate (OUR) of biochar and peat.

	Oxygen uptake rate (mmol/kg/h)
Biochar Woodchips	2.5
Peat	1.9
Compost	5 - 10

3.1.4 Acid-neutralising capacity

The pH of biochar is too high to be used directly as a substrate in horticulture. The pH can be neutralized by mixing biochar with acidic peat. To calculate the mixing ratio between biochar and acid peat, the acid-neutralizing capacity of the biochar and the base-neutralizing capacity of the peat were determined. For this test, biochar made from wood chips was used, which has a lower pH value (pH 9.9) compared to biochars produced from feedstocks containing greenhouse residues (pH 11-12).

The results of the acid titration of biochar (Figure 3.2) show that biochar has a high acid-neutralizing capacity. In total, 258 mmol acid per kg dry matter are needed to acidify the pH of biochar to pH 5.0. The titration curve shows that multiple acid dosages are needed to reduce the pH to the desired pH due to the rather slow buffering of the biochar. Similar slow buffering processes occur when biochar is mixed with acid peat and the final pH of the mixture can only be established after an equilibration period of at least 7 days.

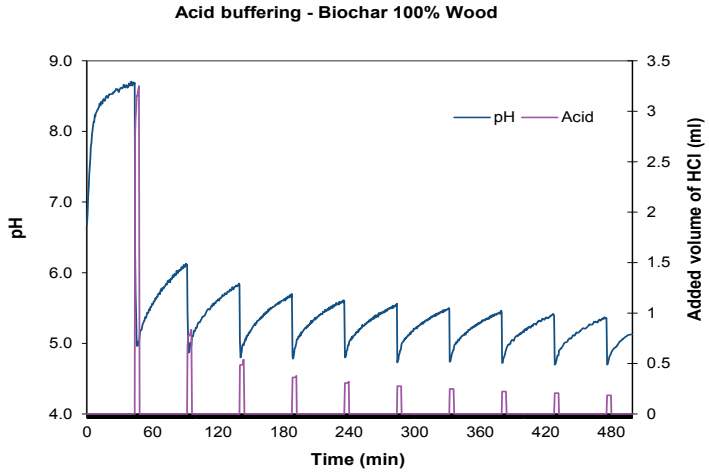

Figure 3.2 *Acid neutralising capacity of biochar from wood chips as determined by titration. The blue line is the pH; the pink line is the acid dosage. The acid-neutralizing capacity of biochar is very high (258 mmol/kg) and the pH-buffering capacity is particularly high at pH 5 to 6.*

The amount of peat needed to neutralise the biochar can be calculated from the acid neutralising capacity of the biochar and the base-neutralising capacity of acid peat (Table 3.4). The acid neutralising capacity of biochar (258 mmol/kg) is 2.9 times higher compared to the base neutralising capacity of peat (90 mmol/kg) meaning that a mixture of 25 kg of biochar and 75 kg of acid peat has a neutral pH. In terms of mixing, it is more relevant to express the mixing ratios based on a volume basis using the bulk density of both materials. The bulk density of biochar (102 kg/m^3) and peat (91 kg/m^3) is in this particular case (!) similar and the volumetric mixing ratio amounts to 1:3; i.e. a mixture of 25 L biochar and 75 L acid peat has a neutral pH.

A general phenomenon that occurs when mixing substrates is interstitial filling, meaning that the total volume of the mixed substrate is smaller than the sum of the volumes before mixing. To which extent interstitial filling occurs is apparent when comparing the bulk density and porosity of the separate and mixed materials (Table 3.5). The data show that bulk density and porosity of the mixed substrate and the peat substrate are equal. It can therefore be concluded that interstitial filling has a negligible effect; i.e. mixing 25 L biochar and 75 L acid peat gives 100 L of substrate. It is stressed that bulk density differences and interstitial filling depend on the properties of all materials to be mixed and therefore have to be evaluated for each new mixture. Disregarding the influence of either could lead to very serious mistakes in pH and in very low air contents in the mixtures.

Table 3.4

Acid buffering capacity of biochar and base buffering capacity of peat, determined by titration[1].

Substrate	Acid buffering capacity		Base buffering capacity	
	mmol/kg d.m.	mol/m^3	mmol/kg d.m.	mol/m^3
Biochar (wood chips)	258	26		
Peat[2]			90	9
Mixing ratio (v/v)	25% biochar + 75% acid peat = neutral			

[1] Titration with a commercial titration unit using concentrated HCl or KOH. See Figure 3.5.

[2] Acid milled white peat, no fertilizer or calcium-carbonate added.

Table 3.5

Bulk density and volumetric contribution of solid particles and pores in peat, biochar and peat-biochar mixture[1].

Substrate	Bulk density	Solid fraction	Porosity
	(kg/m^3)	(% v/v)	(% v/v)
Biochar (wood chips)	100 ± 2.6	6.2 ± 0.17	93.8 ± 0.17
Peat[2]	91 ± 0.87	5.8 ± 0.06	94.2 ± 0.06
Peat/biochar mixture (85:15)	91 ± 1.7	5.8 ± 0.11	94.2 ± 0.11

[1] Data collected for determination of pF curves. Peat.

[2] Acid milled white peat, no fertilizer or calcium-carbonate added.

3.1.5 Water holding capacity

The water holding capacity was measured at different suction strengths to represent the wetting and drying cycles that occur in substrates during plant growth. In horticulture, the water holding capacity is commonly expressed on a volume basis because this allows the volume of plant-available water based on the volume of the pot to be calculated and because it allows the volume of air to be calculated. Even under saturated conditions (i.e. after watering), substrates need to contain a sufficient volume of air (>15%) to prevent fungal growth and rotting due to anaerobic conditions.

Peat has a high water holding capacity varying between 35 and 74% (v/v) (Figure 3.3). The water holding capacity of the biochar (produced from wood chips) is much poorer and varies between 25 and 56% (v/v) depending on the suction strength (Figure 3.4). Biochar consists of relatively coarse particles and the spaces in between these particles are too large to retain moisture. Therefore, biochar itself has a poor water holding capacity. However, once the biochar is mixed with peat, the water holding capacity is again very similar to the water holding capacity of the peat alone (Figure 3.5). This can be explained by the fact that the large spaces in-between the biochar particles are now filled with peat. Under nearly saturated conditions, the peat and peat-biochar mixture contain at minimum 20% air meaning that the substrates contain a sufficient amount of oxygen. For both biochar and peat, less than 10% of the material volume is occupied by solid particles showing the porous nature of both materials. To conclude, a peat/biochar mixture containing 15% biochar has a similar water holding capacity as peat and is thus suitable for application as a substrate in horticulture.

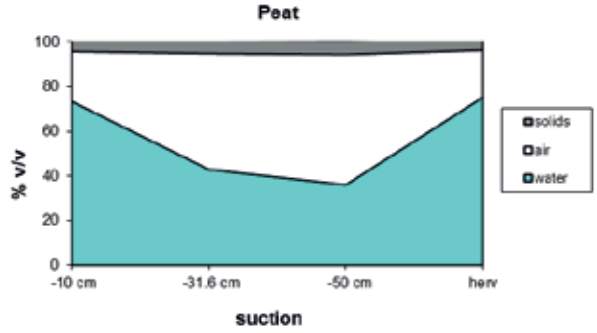

Figure 3.3 *Water content of peat substrate as a function of suction strength. The peat material is able to retain a large volume of water over a range of suction strengths. Under saturated conditions (-10 cm), the peat material contains still a sufficient volume of air meaning that no anaerobic conditions will occur.*

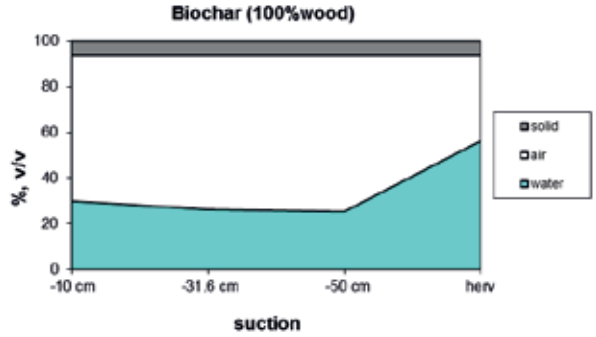

Figure 3.4 *Water content of biochar from wood chips as a function of suction strength. The water holding capacity of biochar is very low compared to peat and too low to ensure sufficient water supply to plants. The higher water-content after re-saturation compared to the initial situation can be partly explained by re-arrangement of the biochar particles leading to an increase in the presence of small pores.*

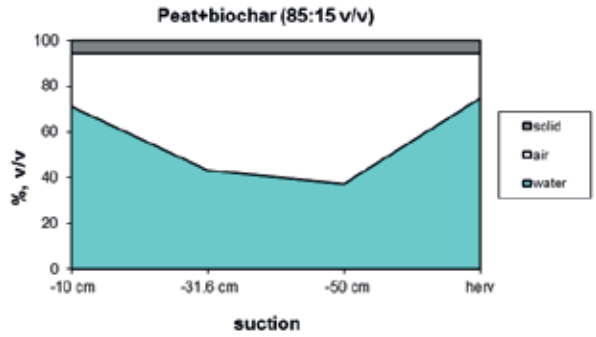

Figure 3.5 *Water content of a peat/biochar mixture (85:15 % v/v) as a function of suction strength. The water holding capacity of the peat/biochar mixture is very similar to the water holding capacity of peat because the large pores in between the biochar particles are now filled with peat. Based on the water holding capacity, the peat-biochar mixture is suitable for application in horticulture.*

3.2 Pot experiment

A pot experiment was performed with gerbera plants cultivated on substrates with and without biochar in a climate controlled room. Plants were infected with mildew to assess the effect of biochar addition on the ability of plants to supress mildew infection.

3.2.1 Substrate quality

A standard peat substrate and a substrate with 20% biochar from beech wood were used. The pH was higher in the substrate with biochar (Table 3.6), meaning that the amount of acidic peat used to neutralise biochar was not yet sufficient. At the end of the pot experiment, the nitrate concentration was much lower in the substrate with biochar compared to the standard substrate. This indicates that some nitrogen fixation occurs due to microbial degradation of biochar. Degradation of biochar can lead to nitrogen fixation because the C:N ratio of biochar (>65, Table 3.1) is much higher than the C:N ratio needed for bacterial growth. Shortage of nitrogen can be easily prevented by addition of extra nitrogen in the form of calcium-nitrate. However, in this case, the extent to which nitrogen fixation occurred was only minor because no effects of nitrogen shortage were observed in plant yield and leaf chlorophyll content (Figure 3.3 and 3.8).

Table 3.6
Nutrient concentrations, pH and EC of the standard substrate (peat) and substrate with 20% biochar[1].

Substrate	Peat	Peat+biochar
pH (-)	5.3	6.4
EC (mS/cm)	0.65	0.54
NH_4 (mmol/l)	<0.1	<0.1
K (mmol/l)	2.3	2.4
Na (mmol/l)	0.3	0.4
Ca (mmol/l)	0.7	0.4
Mg (mmol/l)	0.5	0.3
Si (mmol/l)	<0.1	<0.1
NO_3 (mmol/l)	2.7	0.6
Cl (mmol/l)	0.1	0.1
SO_4 (mmol/l)	0.8	1.1
HCO_3 (mmol/l)	< 0.1	< 0.1
P (mmol/l)	0.65	0.65
Fe (µmol/l)	4.7	2.8
Mn (µmol/l)	0.4	0.2
Zn (µmol/l)	0.8	0.8
B (µmol/l)	6	8
Cu (µmol/l)	0.2	0.4
Mo (µmol/l)	0.1	0.2

[1] Measured in a 1:1.5 (v/v) soil/water extract. Biochar was produced from beech wood.

3.2.2 Plant growth and disease suppression

Fresh weight of gerbera plants was similar for plants cultivated on peat and on peat with biochar (Figure 3.6). Thus, biochar addition did not lead to any phytotoxic or other growth-reducing effects.

Fresh weight of the plants receiving SAR elicitor, a product to stimulate biologically induced disease suppression, was lower compared to other treatments. This product is known to cause a reduction in yield.

Nineteen days after inoculation with mildew, plants were assessed for symptoms of mildew infection (Figure 3.7-8). Gerbera's cultivated on peat and peat/biochar show symptoms of mildew infection and the area of leaf spots was similar for both treatments. Thus, biochar addition does not affect the level of incidence of mildew infection. Gerberas receiving fungicide did not show any symptoms of mildew infection. Generally, the incidence of infection with mildew was rather low since less than 5% of the plant surface was affected. The low incidence of infection might be due to the fungicide treatment that the gerbera plants received at the grower, prior to the start of the experiment.

Overall, it can be concluded that cultivation of gerbera on substrates containing 20% of biochar was successful and gave similar yields as compared to standard substrates. Biochar addition did not reduce the incidence of mildew infection.

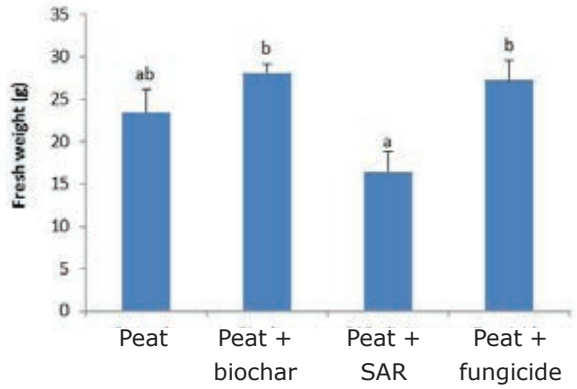

Figure 3.6 *Average fresh weight of gerbera after six weeks. No significant effects of treatment were found (P<0.05).*

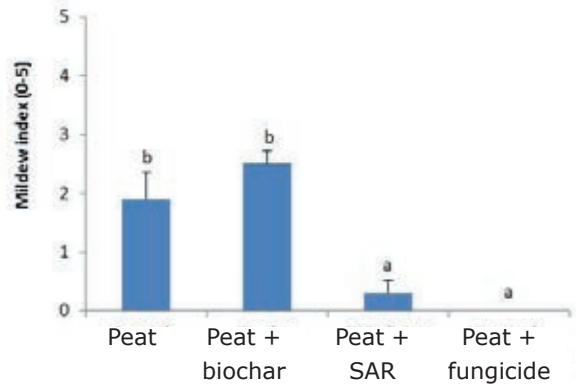

Figure 3.7 *Symptoms of mildew infection on gerbera plants, 19 days after inoculation. Symptoms were quantified on a scale between 0 and 5. Letters denote significant differences between treatments (P<0.05).*

Figure 3.8 *Pictures of gerbera plants cultivated on standard peat substrate and on peat substrate with 20% biochar. Pictures were taken after 6 weeks. No differences were observed.*

3.3 Greenhouse experiment

A greenhouse experiment with 720 chrysanthemums was performed in order to test whether biochar addition affects plant growth, plant health and disease suppression. Chrysanthemums were cultivated on a standard substrate (peat) and substrate with 15% biochar and plants were infected with Fusarium oxysporum to assess the effect of biochar on Fusarium infection.

Additionally, two treatments received commercially available product containing plant-growth promoting organisms (Compete Plus, Plant Health Care BV, The Netherlands) in order to stimulate colonization of biochar and thereby enhancing the disease suppressiveness of the substrate. At the start of a pot experiment, substrates are almost free of microbes and addition of extra plant-growth promoting organisms might therefore lead to better and faster colonization of the biochar. An increase in the abundance of plant-growth promoting bacteria should lead to a decrease in the incidence of pathogens due to competition for resources and antagonism.

3.3.1 Substrate quality

Over the course of the experiment, the pH was similar in the peat and peat/biochar mixture meaning that the amount of calcium-carbonate needed to neutralize the pH of both substrates were correctly calculated from the acid neutralising capacity of the biochar (paragraph 3.1.4). The EC value and nutrient concentrations were also equal for all treatments (Table 3.7). Nitrate concentrations were high and similar in the peat and peat/biochar mixture meaning that no effects of nitrogen fixation were observed. Overall, this means that addition of biochar, with the measures taken here, does not require any adjustments in the fertilization scheme.

Table 3.7 *Nutrient concentrations, pH and EC of the peat and peat+biochar substrates sampled at the start and end of the greenhouse experiment.*[1]

	---------- Start ----------		----------End----------			
Substrate	Peat	Peat+biochar	Peat	Peat+ Compete Plus	Biochar	Biochar + Compete Plus
pH (-)	4.9	4.8	6.1	6.2	6	5.9
EC (mS/cm)	1	1.1	1.5	1.6	1.6	1.5
NH_4 (mmol/l)	2.4	2.5	0.2	0.2	< 0.1	0.1
K (mmol/l)	2.5	3.9	4.7	4.9	5.9	5.6
Na (mmol/l)	0.3	0.3	0.7	0.8	0.9	0.8
Ca (mmol/l)	0.8	0.4	2.8	2.9	2.8	2.6
Mg (mmol/l)	1.4	0.7	2.7	2.9	2.2	2.2
Si (mmol/l)	0.2	0.1	< 0.1	< 0.1	< 0.1	< 0.1
NO_3 (mmol/l)	4.2	3.9	6.8	7.4	6.8	6.4
Cl (mmol/l)	0.2	0.4	< 0.1	< 0.1	0.1	0.1
SO_4 (mmol/l)	1.1	1.3	3.2	3.4	3.2	3.4
HCO_3 (mmol/l)	< 0.1	< 0.1	0.4	0.5	0.4	0.4
P (mmol/l)	1.4	1.5	1.6	1.6	1.6	1.6
Fe (µmol/l)	23.8	15.7	28.2	23.2	28.6	31.6
Mn (µmol/l)	3.6	2.8	1.8	1.2	1.3	1.2
Zn (µmol/l)	0.8	0.5	9.2	6.4	6.9	5.4
B (µmol/l)	14	17	4	< 4	8	8
Cu (µmol/l)	0.3	0.5	0.8	0.7	0.8	0.8
Mo (µmol/l)	0.1	< 0.1	0.8	1.2	0.8	0.9

[1] Measured in a 1:1.5 (v/v) soil/water extract. Biochar was produced from residual wood from Staatsbosbeheer.

At the start of the experiment, dissolved organic carbon (DOC) concentration was measured in the two substrates (Table 3.8). The high concentration of DOC in peat can be explained by the presence of organic acids that are naturally present in peat. Addition of the biochar leads to a strong decrease in the DOC concentration, indicating adsorption of organic acids on biochar through hydrophobic or electrostatic interactions. The adsorption of organic acids on biochar is relevant for developing biochar which can harbour bacterial life to be used in disease suppression. Biochar is itself a poor substrate for microbes (poorly degradable = not a proper carbon source). If biochar can be loaded with biodegradable organic compounds, biochar may become a much more suitable habitat for organisms.

Table 3.8

Concentration of water-extractable dissolved organic carbon in substrates.

	Dissolved organic carbon(mg/kg)
Peat	1392
Peat +15% biochar	723

3.3.2 Plant health and plant growth

No effect of treatment on the plant fresh- and dry weight was observed (Figure 3.9-11) and no differences in nutrient content were observed except for somewhat higher potassium content in plants grown on biochar (Table 3.9). This is consistent with the observation that pH, EC and nutrient availability are very similar in treatments with and without biochar. Thus, for the tested biochar, biochar addition had no consequences for nutrient uptake or plant growth.

No effect of fusarium incidence was found meaning that the effect of biochar addition on disease suppressiveness could not be tested.

Figure 3.9 *Chrysanthemums grown on peat and peat/biochar substrate, with and without addition of plant-growth promoting rhizobacteria.*

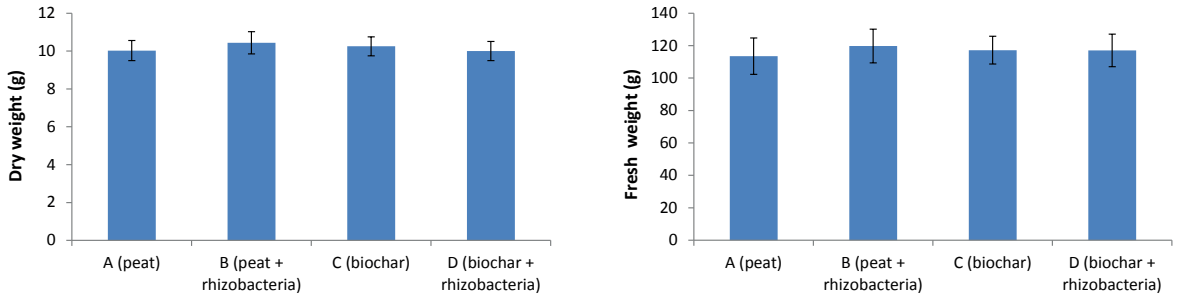

Figure 3.10-11 *Dry- and fresh weight of chrysanthemums cultivated on peat and peat with 15% biochar. Plant-growth promoting rhizobacteria were added to two of the treatments. No differences between treatments were found.*

Table 3.9

Nutrient content of chrysanthemums grown on peat or peat+biochar substrate, with and without addition of plant-growth promoting rhizobacteria.

	Peat	Peat+ rhizobacteria	Biochar	Biochar + rhizobacteria
droge stof (%)	6.9	7.5	6.8	6.4
K (mmol/kg)	1832	1906	2009	2730
Na (mmol/kg)	< 10	< 10	< 10	< 10
Ca(mmol/kg)	571	486	484	512
Mg (mmol/kg)	398	364	277	315
N-tot (mmol/kg)	4134	3944	3752	3910
P-tot (mmol/kg)	719	641	699	791
Fe (mmol/kg)	2.3	1.9	2	2.4
Mn (mmol/kg)	6.5	5.5	6.1	6.6
Zn (mmol/kg)	0.43	0.41	0.46	0.44
B (mmol/kg)	7.5	6.7	7.2	8.6
Mo (µmol/kg)	55.3	38.8	40.4	47.4
Cu (µmol/kg)	223	194	183	194

3.3.3 Bacterial biomass and SEM analysis

Abundance of bacteria and fungi in substrates was checked using semi quantitative PCR technique (qPCR) with primers sets, and 338F and 518R and 5.8s and ITSrev for bacteria and fungi respectively. Additional qPCR was performed to establish the numbers of Firmicutes (bacterial phylum to which Bacillus species are categorised).

At the end of the greenhouse experiment 3 mixed substrate samples of each treatment where collected by mixing substrate from 5 pots and manually removing plant roots. After manual homogenisation of the sample by sieving them through 2mm sieve, 3 subsamples were collected for DNA isolation. DNA was isolated using the commercially available PowerSoil DNA Isolation kit (MoBio Laboratories, USA) according to manufacturer's protocol. qPCR was performed using SYBR Green chemistry with reagents supplied by Promega Inc. (USA).

Results

Numbers of bacteria (on basis of copy numbers of 16S gene per gram of substrate) did not differ significantly between treatments. Numbers of bacteria were highest in peat+biochar substrate with addition of Compete Plus (Figure 3.12). However, the increase in numbers was still not statistically significant.

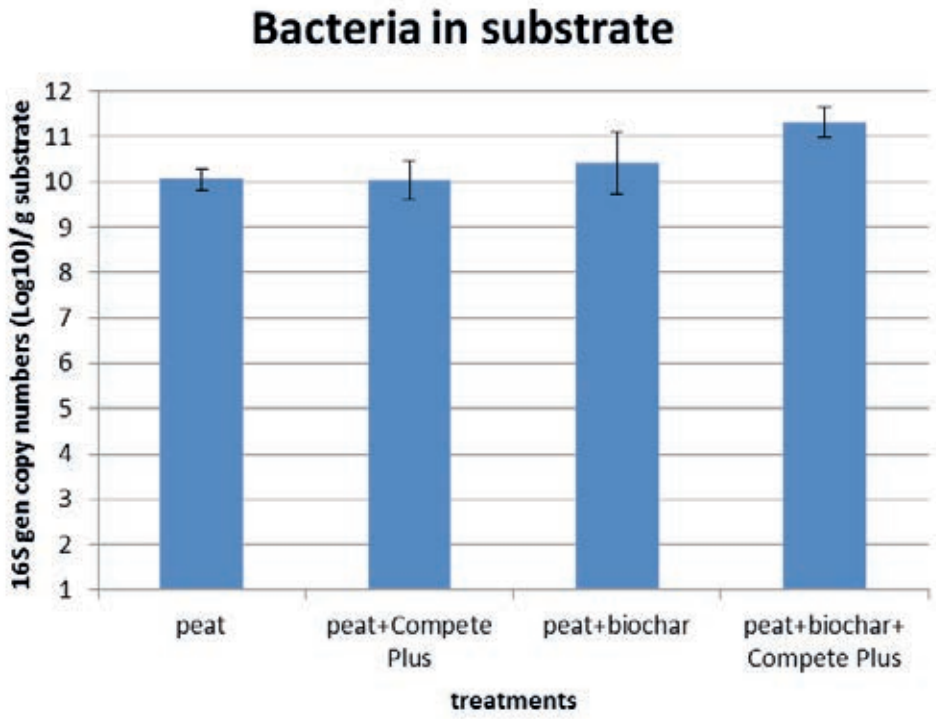

Figure 3.12 *Total Bacteria numbers (on basis of log10 of copy numbers of 16S gene per g dry substrate).*

Hypothetically, addition of Compete Plus should have resulted in higher number of Firmicutes in the studied substrate as Bacillus species are members of this bacterial phylum. In the treatment with peat+biochar addition of Compete Plus resulted in the statistically significant increase of numbers of Firmicutes.
However, we still measured very low abundances of this group of bacteria in all treatments (Figure 3.13). It seems that this group of bacteria is not abundant in the studied substrate. It might be the result of substrate physical and chemical characteristics (e.g. pH; organic matter quality). With the information available it is difficult to pinpoint the exact reason why the numbers of this phylum are so low. In other studies on microbial diversity of peat authors also have measured low abundances of this group. It was however never below 1% of relative abundance of all bacteria (now <<0.1%). Phylum Acidobacteria is often found to dominate peat as they are better suited for lower pH and type of organic matter available in this substrate.

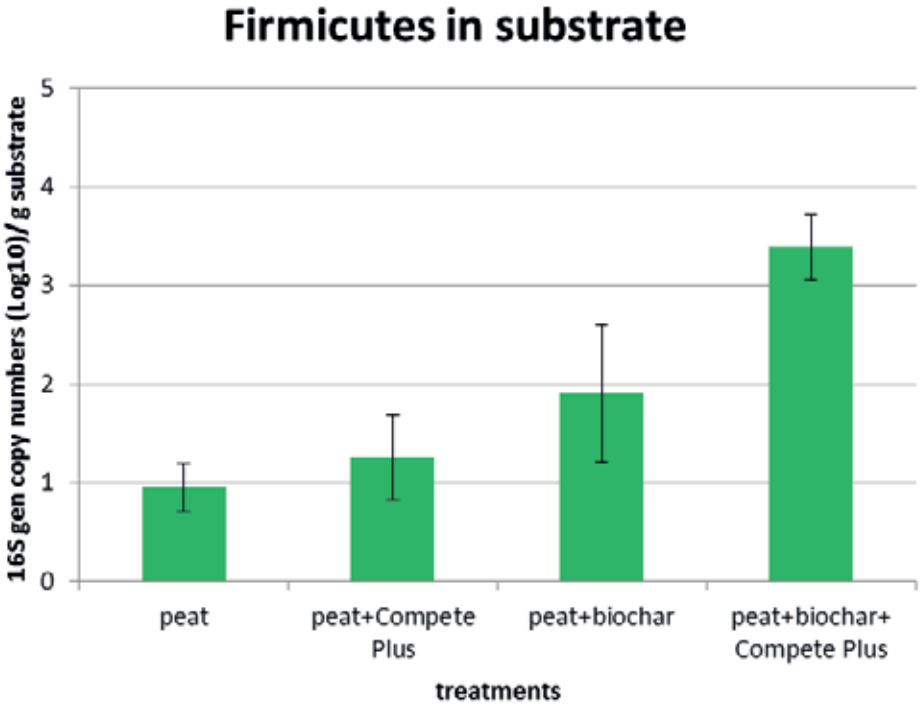

Figure 3.13 *Firmicutes numbers (on basis of log10 of copy numbers of 16S gene per g dry substrate).*

Additionally fungal abundance was checked in different treatments (Figure 3.14). There was no statistically significant difference in fungal numbers (on basis of 18S rDNA gene) in four treatments tested.

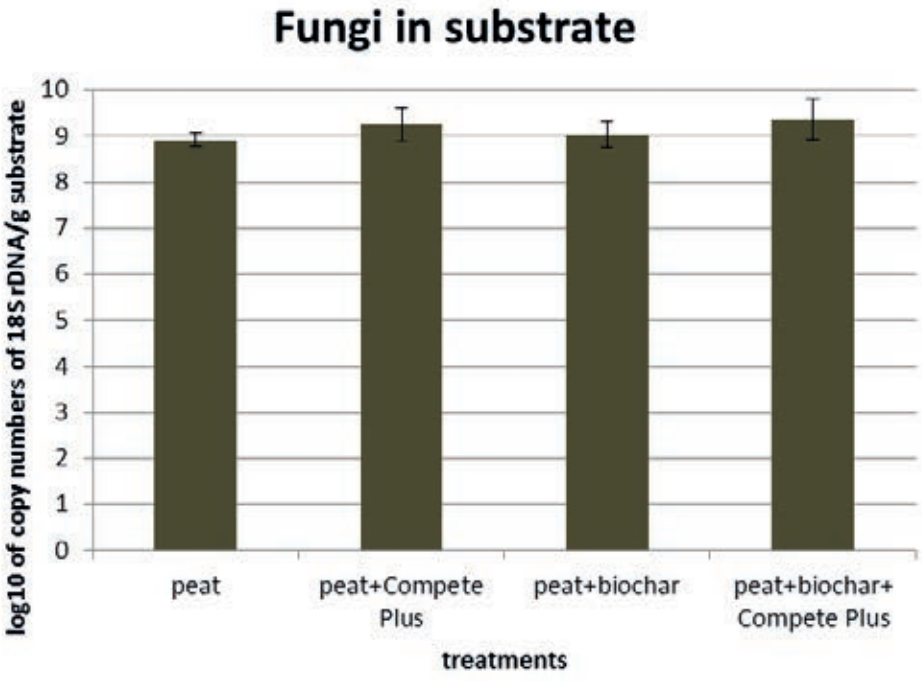

Figure 3.14 *Fungal numbers (on basis of log10 of copy numbers of 18S gene per g dry substrate).*

3.4 SEM images

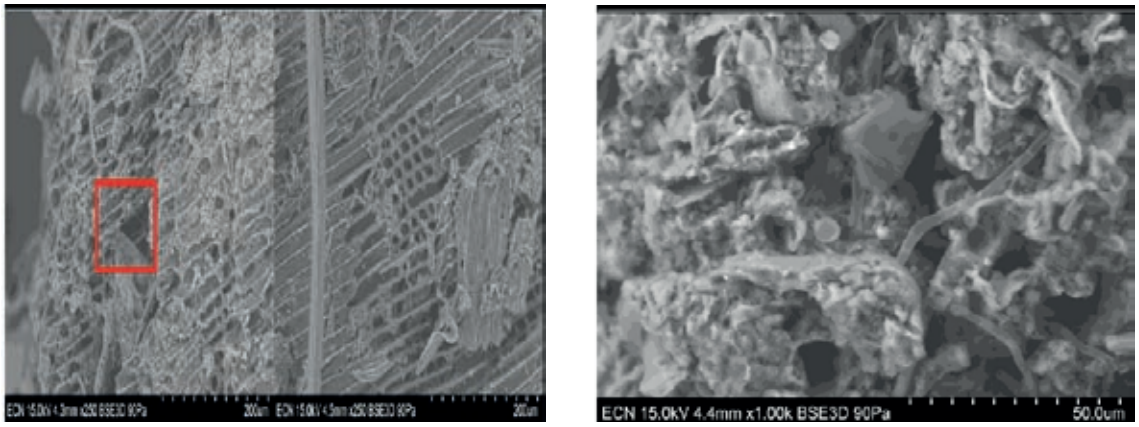

Figure 3.15-16 *SEM images by courtesy of ECN of biochar particles, one showing xylem vessels (left) and one showing fungal hyphae (right).*

SEM images provided by ECN showed both peat and biochar particles (Figures 3.15 and 3.16). In the pictures on biochar particles clear fungal hyphae were visible on the biochar surface giving evidence of colonization of the material.

4 Discussion: Perspectives for biochar as peat alternative in potting soil

Various biochars, produced from different feedstock materials, were analysed on a range of relevant properties to assess their suitability for application in horticulture. Biochar produced from source materials containing nutrient-rich biomass (i.e. tomato leaves, paprika waste) appears unsuitable for use as potting soil because of its high salt content and high pH. Even biochar produced from feedstock with only 20% tomato leaves and 80% wood chips appear to be too saline to be used as an ingredient for potting soil.

In contrast, biochars produced from beech wood or mixed residual wood have a very low EC value. Moreover, these biochars have a slightly lower pH and a higher specific surface area compared to biochars produced from tomato or paprika waste. Biochar should be mixed with acidic and "wet" substrates such as peat in order to neutralize the pH and to improve the water holding capacity. The maximum peat/biochar mixing ratio is limited by the acid-neutralising capacity of the biochar (and the base-neutralising capacity of the acidic peat). Amounts up to 25% (v/v) for the tested biochar; i.e. a mixture of 25 L biochar and 75 L acidic peat has a neutral pH. Overall, the laboratory analyses reveal that biochars from wood have suitable properties for use in horticulture when used in a mixture with peat. Peat/biochar mixtures, containing 15 – 20% biochar, have very similar properties (EC, pH, water holding capacity, nutrient availability) as standard peat substrates and therefore show good perspectives for application in horticulture. However, the presence of some phytotoxic compounds in biochar from wood chips remains a point of concern that needs further attention (even though the problem was reported as technically solvable in ECN reports on production).

In two pot experiments, plants were grown on mixtures of peat and biochar (beech wood and residual wood). In both experiments, nutrient availability and plant growth were similar in the biochar treatment compared to the control treatment without biochar. There were no signs of phytotoxic or growth reducing effects of biochar. Thus, the pot experiments confirm that peat/biochar substrates, with substantial biochar content (15 – 20%), can be used in horticulture without yield reduction and without adjustment of the fertilization scheme.

Results of the pot experiment with gerbera show that the incidence of mildew infection is similar for gerberas cultivated on substrate with 20% biochar and the control treatment without biochar. Thus, biochar addition does neither decrease nor increase incidence of mildew infection in gerberas. In the experiments with chrysanthemums, no fusarium incidence was found meaning that the effect of biochar addition on disease suppressiveness could not be tested. More research is needed in order to elucidate under which conditions biochar additions can enhance disease suppressiveness of substrates.

Bacteria, Firmicutes and Fungi

Testing for bacteria, Firmicutes and Fungi revealed levels of $10^{\wedge 10}$ for bacteria and fungi and $10^{\wedge 3}$ for Firmicutes expressed as gene copy numbers per gram wet substrate. This corresponds with a rather rich microbial community for bacteria and fungi but is low for Firmicutes. Even though the statistics were indecisive this does not mean microbial harbouring on biochar particles did not occur. It is possible that the non statistical but higher level of bacterial activity on biochar enriched samples is "real" but hidden in variation due to the limited number of replicates taken from the treatments and the considerable variation in biochar level due to sample taking. Furthermore it was argued that the pH of the biochar particles probably was 5-7 on the surface but possibly 7-9 in the deeper parts of biochar particles, preventing most bacteria to colonize the deeper parts. Another reason for not colonizing the deeper parts is of course the low level of degradation (OUR <3), showing biochar particles to be a poor environment for microbials needing carbon for growth. Possibly, colonization of biochar can be enhanced by loading the biochar with biodegradable organic acids, which would make biochar a more attractive environment for microbes. The evidence in Table 3.8 is that 15% biochar can halve the amount of free organic carbon in a peat sample. It is assumed the biochar can absorb the organic acids. If so, in similar ways it is possible to pre-treat biochar to a) be externally and internally brought to pH levels favourable for bacterial growth and b) be treated with small organic molecules which can attach to the surface and which can feed a population of microbials establishing themselves on the biochar particles.

Relation between SSA, CEC, DBD

NB ECN has evidence that lower temperatures and careful application of steam as a reactant can produce a biochar with an intact structure as shown in the SEM images. In these images the cell structures like xylem vessels are still readily recognisable. Various other reported biochars with other forms can lose this structure and then become much more dense than the biochars reported here. Obviously such biochars will not be reactive in the substrate or soil. In this way properties like specific surface area (SSA), cation exchange capacity (CEC) and internal porosity of the biochar as shown by the dry bulk density of the material at one particle size are all linked together. NB the restriction 'at one particle size' is given to make sure density differences due to interstitial filling are not included as they are unrelated to the production process.

Role of particle sixe distribution PSD

The properties important for use as growth substrate such as the water and air content at different suction forces, are related to the particle size and the distribution of various particle sizes. The very uniform particle size of the current product (3-4 mm) allows for high air content in between the particles (air content within the particles is not taken into account). The density is therefore low (120 kg/m^3). If a large portion of 3 mm particles would be mixed into the sample, the density would increase with 20-40%. By controlling the PSD it is possible to create a whole range of biochar properties from just one or two production materials and some crushing and sieving equipment.

5 Conclusions

The suitability of biochar for application in horticulture was studied using lab experiments, pot and greenhouse experiments.

Biochars from feedstocks containing nutrient-rich materials, such as paprika waste or tomato waste, have salt contents of >> 1 dS/m and are therefore not suitable for application in horticulture. Directly proportional to the salt content is the alkalinity caused by the oxides of cations such as sodium, potassium, calcium and magnesium. Again the high content of these cations make the material unsuitable for application in horticulture as the resulting pH is >10 and the maximum amount which can be used in mixtures is < 10%-v/v. Biochar produced from wood (beech wood or residual wood) has a very low salt content. Moreover, this biochar has a much lower alkaline pH as alkalinity is related to the salt content as explained. Additionally biochar from wood has a higher surface area compared to biochar produced from feedstocks with sweet pepper or tomato waste. A higher internal surface area is regarded as an advantage for binding cations, organic acids molecules and harbouring microbial life although direct proof of this line of thought must still be found.

Nutrient contents of biochars are low, except for potassium. Because of the high C:N ratio of biochar, N fixation will occur upon microbial degradation of biochar. This means the nutrient addition to rooting media with biochar need to be adapted by lowering potassium supply and increasing the nitrate supply in a predictable way.

The very low oxygen-uptake rate of biochar is positive since it indicates that biochar is poorly degradable, reducing the need for additional nitrate and reducing the risks of shrinkage of the substrate due to microbial degradation of biochar.

Biochars from wood chips perform better in the bio-essay for phytotoxic compounds compared to biochar from paprika waste. Nevertheless, the bio-essay indicates the presence of some phytotoxic compounds. Based on previous work it should be possible to prevent the condensation of volatile carbohydrates back into the biochar by redesigning the production path.

The high pH of biochar needs to be neutralised and one way to do so is by mixing biochar with acidic substrates such as peat. The acid-neutralising capacity of biochar is the limiting factor controlling the maximum amount of peat that can be substituted for biochar. For biochar produced from wood chips, 25 m^3 of biochar should be mixed with 75 m^3 of peat in order to obtain a mixture with a neutral pH (pH 5.0).

The poor water holding capacity of biochar is diminished when biochar is mixed with peat. A peat/biochar mixture has a similar water holding capacity as peat alone. If mixed in appropriate ratios, biochar/peat mixtures have very similar properties (EC, pH, water holding capacity, nutrient availability, stability) as compared to standard peat substrates.

Yields and plant nutrient contents were similar for gerberas and pot chrysanthemums grown on substrates with 15-20% biochar as compared to the control treatment without biochar.

Addition of biochar to substrates did neither decrease nor increase incidence of mildew infection in gerberas. More research is needed to find specific conditions under which biochar can enhance disease suppression.

Overall, biochar produced from wood can replace at least 15% (v/v) of peat in potting soil without causing any effect on pH, EC, nutrient availability, water holding capacity, stability, plant growth or plant health.

Overall the hypothesis that biochar can act as a carrier for desirable bacterial soil life and can be used to introduce disease suppression in a soil plant system has not been proven. There are indications this reasoning is valid and can be fruitful when pre-treating biochars: a) soaking biochar with a proper pH b) consequently adding easily digestible organic substrate like organic acids c) adding a proper mix of bacteria and allowing it to establish itself on the biochar. All measures to be taken prior to mixing biochar with other rooting media constituents.

6 Literature

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To explore the potential of nature to improve the quality of life

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