

Organic fertilisers and nitrogen availability

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Abstract

Liquid organic fertilisers allow growers to abandon the use of conventional de novo (mined or synthesised) fertilisers without the need for major technological adaptations in the greenhouse. It was decided to run a cultivation experiment to find practical information for producers and growers when using liquid organic fertilisers in cultivation. The objective was to compare crop production between plants grown with liquid organic fertilisers and plants grown with conventional de novo fertilisers. Cucumbers were propagated in coir and peat and planted on slabs of respectively coir and peat. The plants on the two rooting media received either liquid organic or chemical fertilisers, resulting in four treatments in total. Plants fed with liquid chemical fertilisers were shown to initially produce 10-20% more fresh weight per unit time than plants fed with liquid organic fertilisers. When organic fertiliser was used during cultivation, ammonium accumulated to levels of 10-20 mmol L⁻¹, while nitrate levels dropped to below 1 mmol L⁻¹. To explain the results, the conversion of nitrogen was studied. Conversion of organic nitrogen into nitrate requires enzymatic hydrolysis to ammonium, followed by bacterial oxidation to nitrate. This microbiological process depends on two physiological groups of naturally occurring aerobic soil bacteria; ammonia oxidising bacteria (AOB) and nitrite oxidizing bacteria (NOB). In conclusion nitrate concentration rather than the concentrations of organic nitrogen or ammonium, were growth rate determining. The low conversion rate of ammonium into nitrate by bacteria present in substrate limited the leaf area growth of cucumbers. Suggestions to overcome the problem are made.

Keywords: bio fertiliser, ammonium, nitrate, amino acids, bio reactor

INTRODUCTION

The label “organic” qualifies products grown without the use of most chemical crop protectants and conventional de novo (mined or synthesised) fertilisers. Presently the use of the label “organic” for products grown on hydroponic systems is forbidden in Europe (EGTOP, 2013) but allowed in countries like the USA and Australia (Raviv, 2010). The development of high grade liquid biological fertilisers anticipates a future acceptance of most hydroponic growing for “organic” production in Europe. Biological fertiliser is used to describe fertilisers derived from plant or animal sources and accepted for the production of “organic” plant products.

At present biological fertilisers are not applicable in automated dosing and distribution systems. Most biological fertilisers are either solid or, when liquid, contain large amounts of organic matter. Liquid biological fertilisers with organic matter are prone to block irrigation lines by obstruction, precipitation or the formation of bacterial blockage. Irrigation lines may become anaerobic in between irrigation cycles, and dark coloured organic fertiliser solutions cannot be disinfected with the common UV equipment (Şahin et al., 2005; Meador et al., 2012). There seems to be a market for clear biological fertiliser solutions fit for the present automated irrigation via drip lines (Raviv, 2010). Several attempts have been made in the past (Agele et al., 2011). Horticoop offers a system with two concentrated solutions, including a mix of organic nitrogen fertilisers. These fertilisers

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should be dosed straight after dilution to prevent anaerobic decomposition of organic nitrogen forms.

In a broader context the use of organic fertilisers produced from every available organic waste-stream or even dedicated organic production is a solution for severe problems related to conventional de novo fertilisers. The main argument against conventional de novo fertilisers is the unsustainability of diffuse emission of elements into the environment. The levels released are far above levels released by nature and they accumulate (Sutton and Ayyappan, 2013; Bindraban et al., 2015). Release over the last 50 years is causing environmental and drinking water problems; continuing this system for another 200-300 years would be disrupting the global ecosystem. Other arguments are the likely though disputed depletion of sources such as phosphate (Withers et al., 2014) and the high energy input in the production of nitrates.

To test practical use of liquid biological fertilisers in greenhouse horticulture, a comparison in a cultivation experiment was made between biological and common conventional de novo fertilisers. The goal was to find the yield level with organic fertilisers compared to conventional de novo fertilisers and to prepare a user protocol for the use of biological fertilisers.

MATERIALS AND METHODS

Two experiments were conducted in a 12.0×12.8 m glasshouse compartment of the Venlo multispans facility at Bleiswijk research station of Wageningen UR, The Netherlands (Latitude 52°1'N, Longitude 4°3'E). Cucumber seeds (*Cucumis sativa* L., cultivar 07PL48, Rijk Zwaan, Fijnaart, The Netherlands) were sown on 3 July (first experiment) and 16 Augustus (second experiment). Planting took place on 27 July and 5 September.

The Horticoop fertiliser concept consists of two concentrated solutions and an enzyme (Figure 1; Table 1). The first solution, F1, contains 8%-w/w CaO and 0.9%-w/w MgO derived from algal mass after enzymatic removal of most organic carbon. The second solution, F2, with a density of 1.20 kl L⁻¹ and pH 5.5, contains 9%-w/w N and P, K, Mg and sulphate. The nitrogen is the concentrate of protein rich by-products processed with enzymes, mainly unspecified proteins, amino acids and urea. Micro elements are of a conventional de novo origin as this is allowed in both, organic and Eco growing (The Commission of the European Communities, 2006). The enzyme solution consisted of endoprotease to convert the protein-N into amino acids and ammonium in a process which also lowers the pH. Dosing was started halfway the cultivation phase when pH levels became too high.



Figure 1. Nutrient dosing of F1, F2 and Endoprotease. Dosatrons in the back.



Table 1. Drain sample analyses for four treatments and three dates.

Parameter	F1	F2	Mix 1:1	Chemical ¹	20-07 CCsBf	20-07 CCsCf	20-07 CPsBf	20-07 CPsCf	27-07 CCsBf	27-07 CCsCf	27-07 CPsBf	27-07 CPsCf	06-08 CCsBf	06-08 CCsCf	06-08 CPsBf	06-08 CPsCf
N-total		17.3	17.3													
pH		5.5	6.0	5.5	7.4	6.8	7.3	6.2	5.1	6.4	5.1	5.8	8.3	5.8	8.5	5.8
EC (dS m ⁻¹)	0.9	1.6	2.5	2.5	2.4	3.6	2.9	1.6	2.3	0.9	2.5	2.7	2.6	3.0	2.0	3.2
NH ₄ (mmol L ⁻¹)				1.6	3.9	0.3	13.0	4.4	0.5	0.0	0.7	0.8	4.6	0.8	4.5	2.8
K (mmol L ⁻¹)		8.0	8.0	10.0	9.4	0.0	8.0	3.2	8.5	5.5	10.7	9.2	12.7	10.7	6.8	10.4
Na (mmol L ⁻¹)					1.8	6.2	1.3	0.5	1.4	1.8	1.7	0.2	2.6	0.3	1.5	0.6
Ca (mmol L ⁻¹)	4.0		4.0	5.0	3.9	1.5	3.5	1.7	7.8	0.0	8.3	5.7	2.3	6.0	3.2	4.1
Mg (mmol L ⁻¹)	0.6	1.4	2.0	1.7	1.7	1.2	2.0	1.9	2.6	0.0	3.1	1.8	1.3	2.0	1.7	4.0
Si (mmol L ⁻¹)					0.9	0.1	0.0	0.0	0.0	0.3	0.0	0.0	1.4	0.0	0.1	0.0
NO ₃ (mmol L ⁻¹)				20.0	0.1	11.4	0.0	7.0	0.9	3.2	0.4	19.2	0.4	21.8	0.2	21.5
Cl (mmol L ⁻¹)					1.8	13.1	1.5	0.5	1.2	3.2	1.5	0.1	2.1	0.3	1.3	0.3
SO ₄ (mmol L ⁻¹)		1.4	1.4	1.7	1.4	0.7	1.9	2.0	2.1	0.0	2.6	1.7	1.1	1.8	2.4	2.6
HCO ₃ (mmol L ⁻¹)	9.2		9.2		10.7	2.3	11.2	0.6	0.5	0.1	0.5	0.2	21.2	0.1	17.2	0.2
P (mmol L ⁻¹)		1.3	1.3	1.6	0.8	0.6	1.2	1.7	1.5	0.1	2.0	1.5	0.5	1.7	0.4	2.1
Fe (μmol L ⁻¹)		15.0	15.0	18.8	2.0	2.3	11.9	13.0	16.0	3.9	20.0	12.0	5.4	19.0	3.8	8.2
Mn (μmol L ⁻¹)		10.0	10.0	12.5	9.2	3.0	8.7	5.6	20.0	0.0	25.0	7.4	7.8	12.0	8.6	7.0
Zn (μmol L ⁻¹)		5.0	5.0	6.3	4.9	13.9	4.1	10.3	9.0	0.0	10.8	2.9	3.4	5.9	2.4	11.0
B (μmol L ⁻¹)		25.0	25.0	31.3	13.0	43.0	14.0	34.0	65.0	15.0	76.0	22.0	21.0	35.0	70.0	30.0
Cu (μmol L ⁻¹)		0.8	0.8	0.9	0.5	0.1	0.2	0.3	0.8	0.0	0.7	0.3	0.0	0.8	0.0	0.9
Mo (μmol L ⁻¹)		0.5	0.5	0.6	0.0	0.0	0.0	0.0	0.6	0.0	0.7	0.3	0.0	0.6	0.7	0.0
Sum cation (mel L ⁻¹)	9.14	10.76	19.90	25.00												
Sum anion (mel L ⁻¹)	9.20	4.01	13.21	25.00												

CPsCf = Cultivation on Peat with Chemical fertiliser; CPsBf = Cultivation on Peat with Biological fertiliser; CCsCf = Cultivation on Coir with Chemical fertiliser; CCsBf = Cultivation on Coir with Biological fertiliser.

¹De Kreij et al. (1999).

The concentrated solutions were injected directly into the irrigation water flow sent to the plants by using Dosatrons (Dosatron International, Tresses Bordeaux, France). Dosatrons use water pressure to work a motor piston which takes in the concentrated fertiliser and then injects the solution into the main water stream proportional to the main stream (Figure 2). The Dosatrons are, within limits, adjustable by hand. A double check on the amounts dosed was made by registering weight loss of all fertiliser stock containers.



Figure 2. Dosatron.

In the propagation phase, cucumbers were grown in 11 cm diameter and 0.7 L containers on fine Baltic milled white peat (Horticoop, Bleiswijk, The Netherlands) and on coir pith with 5% fibre (Dutch Plantin, Boekel, The Netherlands). Half of the substrate treatments received 3 kg micro-life starter per m³ substrate and then organic fertiliser (Table 2). The other half received conventional de novo fertilisers (Table 2). After propagation the bottom of the containers was removed to enable transplanting.

Table 2. Treatments and preparation of substrates before cultivation.

		Lime (Dolokal) (kg m ⁻³)	Fertiliser (PG-mix) (kg m ⁻³)	Biological starter (kg m ⁻³)	Added
PPsCf	Propagation Peat Chemical	5	1.5		B
PPsBf	Propagation Peat Biological	5		3	D
PCsCf	Propagation Coir Chemical				B
PCsBf	Propagation Coir Biological			3	D
CPsCf	Cultivation Peat Chemical	5	1.5		B
CPsBf	Cultivation Peat Biological	5			D
CCsCf	Cultivation Coir Chemical				A/B
CCsBf	Cultivation Coir Biological				C/D

A = 20 mmol L⁻¹ Ca(NO₃)₂ with double trace elements.

B = Coir start schedule at EC 3.0 dS m⁻¹ and with double trace elements.

C = F1 and F2 3:2 diluted to EC 2.0 dS m⁻¹ + double trace elements.

D = F1 and F2 2:3 diluted to EC 2.0 dS m⁻¹ + double trace elements and endoprotease 1:1000.

For cultivation 100×20×8 cm slabs of fine Baltic milled white peat (Horticoop, Bleiswijk, The Netherlands) and coir pith with 5% fibre (Dutch Plantin, Boekel, The Netherlands) were used. Propagation pots were pinned on plant holes with bamboo sticks. Nutrient solution was supplied with 2 L h⁻¹ drippers set to 180 s cycles (100 mL). Irrigation starts could be varied independently per treatment.

Prior to cultivation, peat is usually limed and fertilised and coir is usually washed with

calcium nitrate. This practise was mimicked with the organic fertilisers for treatments receiving biological fertilisers and resulted in several pre-treatments of substrates (Table 2).

As the water extract nutrient analysis (1:1.5) reports ammonium and nitrate but no organic nitrogen forms, a total nitrogen analyses was added.

The experiments were split into a propagation phase (P) and a cultivation phase (C). Within each phase there was a factorial test with substrate × fertiliser. The substrate factors were peat and coir (Ps, Cs) and the fertiliser factors were chemical and biological (Cf, Bf).

In the first experiment eight treatments were compared; in propagation there were four treatments (1) peat and chemical fertilisers (PsCf); (2) peat and biological fertilisers (PsBf); (3) coir and chemical fertilisers (CsCf); (4) coir and biological fertilisers (CsBf). Propagation was followed by cultivation on a slab of the same substrate material, but with half of the slabs receiving biological fertiliser and half of the slabs receiving chemical fertiliser. After 3 weeks cultivation the leaf area was measured.

In the second experiment four treatments were compared; the same four propagation treatments already mentioned were continued in the cultivation; PsCf; PsBf; CsCf; CsBf. Fruits were harvested for three weeks before ending the experiment.

Chemical treatments were repeated two times (PsCf, CsCf) and biological treatments were repeated 4 times (PsBf, CsBf). Each repetition held 9 plants in experiment 1 and 18 plants in experiment 2. Statistics were done with GENSTAT using an ANOVA for unbalanced design to account for uneven treatment replication (VSN International LTD, Hemel Hempstead, UK).

Climate and irrigation were set for cucumber with irrigation regime settings per treatment based on radiation sum and corrected on a drain set point of 30%.

Measurements included weekly drain analyses, regular slab analysis and plant analysis, two non-destructive leaf area estimates and trice weekly fruit weight and number. After final harvest root quantity and quality were assessed.

RESULTS

Treatments with biological fertilisers, compared to the chemical fertiliser treatments, reduced leaf growth, regardless whether biological fertilisers were added in propagation only or in cultivation only or both in propagation and cultivation (Table 3).

Table 3. Area of the second leaf from the lobes in cm², *n*=12, in the first cultivation.

Code	Propagation biological fertiliser		p=0.05	Propagation chemical fertiliser		p=0.05
	cm ²	%CPsBf		cm ²	%CPsBf	
CPsCf	332	128	d	372	138	e
CPsBf	259	100	ab	271	100	b
CCsCf	326	125	d	368	136	e
CCsBf	247	95	a	292	107	c

Area is estimated as measured squared width. CPsCf = Cultivation on Peat with Chemical fertiliser; CPsBf = Cultivation on Peat with Biological fertiliser; CCsCf = Cultivation on Coir with Chemical fertiliser; CCsBf = Cultivation on Coir with Biological fertiliser. *p*=0.05; if treatments do not share the same letters, the difference is of statistical significance with a probability of >95%.

Cucumber production measured as total fruit weight is highest for the peat bags with chemical fertilisers. The total fruit weight on coir of both chemical and biological fertilisers was 10% lower than for peat bags with chemical fertilisers (Table 4). The total fruit weight on peat with biological fertilisers suffered from a poor start and recovered from 20% to just over 50% of the yield on peat with chemical fertiliser. The rooting with chemical fertilisers at the bottom of the slabs appeared to be a factor 2-10 more intensive than with biological fertilisers (data not shown).

The peat bags with biological fertilisers produced less than the other bags as the material became too wet (>80%-v/v). The interval between start-time and stop-time was decreased with 3 h to avoid the too low air contents related to high water levels (Niu et al., 2012). As a consequence treatments were compared without factorial analysis.

Table 4. Fruit yield in kg m⁻², *n*=2 (PsCf, CsCf) and *n*=4 (PsBf, CsBf), second experiment.

	10-Oct	p=0.05	17-Oct	p=0.05	29-Oct	p=0.05
CPsCf	0.31	c	1.30	c	2.29	c
CPsBf	0.06	a	0.61	a	1.24	a
CCsCf	0.21	b	1.08	b	2.07	bc
CCsBf	0.17	b	0.89	b	2.06	b

CPsCf = Cultivation on Peat with Chemical fertiliser; CPsBf = Cultivation on Peat with Biological fertiliser; CCsCf = Cultivation on Coir with Chemical fertiliser; CCsBf = Cultivation on Coir with Biological fertiliser. *p*=0.05; if treatments do not share the same letters, the difference is of statistical significance with a probability of >95%.

The EC in the biological fertiliser solution was about 65% of that for chemical fertiliser solution (Table 1). In the second cultivation the EC in the biological fertiliser solution was increased to 82% of the chemical solution and nitrogen supply was 30% higher than the nitrogen supply in the chemical treatment.

The EC in the root environment of treatments with biological fertiliser was only half that of the root environment of treatments with chemical fertilisers (Table 1). The lower root EC in the root environment of treatments with biological fertilisers did not increase leaf area, though it seemed to increase leaf angle (i.e., improve light interception, photographs not shown). The amount of cation and anion charges has to be equal so the apparent lack of anion charges in Table 1 indicates that other anion charges are present, presumably bicarbonate and organic anion charges.

The pH was at times too high (8.5) and buffered solidly by the high bicarbonate levels of over 20 mmol L⁻¹ (Table 1). To force the pH down, endoprotease was dosed to convert proteins into amino acids and thus lower the pH. Four days after applying endoprotease the pH had gone down from 8.2 to 7.2 for treatments with biological fertiliser (data not shown).

Uncommonly high levels of ammonium found were 4-8 mmol L⁻¹ at pH 7.5-8.3 (Table 1). Related to the accumulation of ammonium and the elevated pH is the accumulation of bicarbonates. The maximum values found were 20-25 mmol L⁻¹ at pH 8.5 (Table 1).

Indications of an active microbial life and an associated poorly aerobic environment in the irrigation lines and substrates in treatments with biological fertilisers were fungi on the bamboo support sticks after propagation, slimes hanging from drippers and the marked local rooting in peat slabs with biological fertiliser but not with coir and biological fertiliser (Figure 3).



Figure 3. Rooting pattern of a peat slab with biological fertiliser.

DISCUSSION

Biological and conventional de novo nutrient solutions were designed to resemble

each as close as possible. Nevertheless differences in irrigation water content and root environment were found for EC, pH, ammonium content, bicarbonate and nitrate.

The substrate EC was initially 35% and later 18% lower at equal element supply in biological treatments because most nitrogen is present in uncharged form as organic-N (urea or proteins). Obviously all nitrogen forms present must be measured and taken into account to evaluate the true nutrient potential offered. Therefore growers using biological fertiliser must regularly order the analysis of N-ammonium and N-nitrate and incidentally may want to know the quantities of N-urea, N-amino acid and N-protein, the last three usually analysed together as N-Kjeldahl. As sources of the N-Kjeldahl are all eventually released as ammonium, the N-Kjeldahl reading is an indication of the potential ammonium still in the system. Should the lack of nitrate when using biological fertilisers be solved, it is possible the lower EC will result in extra fresh mass growth due to increased water uptake (Sonneveld and Voogt, 1990).

The fast rise of pH to values above 8.2 concurrent with high ammonium concentrations is thought to be dangerous for plants as ammonium is noticeably converted to ammonia at pH values above 7.5. Not only does this result in nitrogen losses as ammonia gas, but ammonia is also highly toxic for roots (Amberger-Ochsenbauer et al., 2012).

Ammonium levels of 4-8 mmol L⁻¹ would normally result in quick uptake of ammonium by the plant and a consequent steep drop in root environmental pH (Fisher et al., 2014; Imas et al., 1997; Sonneveld and Voogt, 2009). The low availability of anion species, predominantly caused by the lack of nitrate, is thought to hamper the uptake of extra cations, especially ammonium.

The accumulation of ammonium is uncommon when using conventional de novo fertilisers according to a standard nutrient recipe. In natural soil the conversion of ammonium to nitrate is facilitated by aerobic bacteria, which first transform ammonia to nitrite (ammonia oxidising bacteria) and then to nitrate (nitrite oxidising bacteria). This process requires pHs above 6.5, because bacteria can only oxidise the ammonia molecule and not the ammonium molecule. Reasons for the unexpectedly low conversion could be: a) a lack of oxygen due to poor aeration (too wet substrate); b) a lack of oxygen due to a higher oxygen use by more abundant/more active micro-organisms; c) a low level of nitrifying bacteria in the substrates used; d) high concentrations of organic carbon hampering the activity of nitrifying bacteria; e) organic substances (like volatiles) which inhibit growth or activity of nitrifying bacteria. Poor aeration and or a higher oxygen use seem the more likely options.

Thus it is advised to convert proteins and ammonium into nitrate during the biological fertiliser production or on greenhouse locations before dosing. It is further suggested to use a bacterial conversion unit, i.e., bioreactor, to do so. Reasons to use a separate unit are: 1) a stable population of specific bacterial species is required; 2) a controlled environment at 20-30°C is needed; 3) a stable and high supply of air is needed to match bacterial activity and; 4) the pH must remain stable at a value above 7.0 (Matsumoto et al., 2012).

Bicarbonate values were actually much higher than the equilibrium values for the accompanying pH i.e., 20 mmol L⁻¹ instead of 4.5 mmol L⁻¹. The 20 mmol L⁻¹ level is also twice the level in the original biological fertiliser. The increase could be the result of supersaturation with bicarbonate or, which is more likely; the carbon dioxide content in the slabs was about 5 times higher than ambient. The carbon dioxide content and the bicarbonate concentration are linked proportionally by the bicarbonate reaction equilibria. The higher carbon dioxide levels may be caused by the higher activity of micro-organisms and a slower transport away from the substrate by the probable poorer aeration.

The lower leaf area and yield with biological fertilisers is thought to be the result of the too low nitrate level which effectively hampers the vacuoles from filling with electrolytes to create cell stretching (Dodd et al., 2003, 2004; Ghanem et al., 2011).

The yield difference between peat and coir with biological fertilisers is believed to be caused by a lack of oxygen in the peat treatment CPsBf as visible in rooting pattern (Figure 3). It is also plausible the combination of peat and organic fertiliser leads to a reduction of the oxygen level in the root environment, much further than in peat with conventional de

novo fertilisers, by an increase in microbial activity level (Bonachela et al., 2010). Our reasoning is that the level of microbial activity in a substrate is primarily governed by the amount of digestible organic matter available per unit volume per unit time i.e., equilibrium between substrate and microbial population. By adding biological fertilisers the supply of digestible organic matter is substantially raised, allowing the microbial population to increase, together with the use of oxygen and the production of carbon dioxide. The microbial respiration is of practical consequence for the total slab respiration of roots and micro-organisms together as the microbial respiration is believed capable of at least matching the root respiration level (Kuzyakov and Domanski, 2002; Hernández and Hobbie, 2010; Niu et al., 2012). The increase in respiration caused by the organic fertiliser is true for coir as well but coir was not as wet as the peat which was too fine for the irrigation regime used.

In conclusion, plant growth is limited by the poor availability of nitrate as the conversion of ammonium to nitrate is not fast enough to keep up with plant requirements. This results in 20-30% lower leaf areas in the initial stages when plants not yet intercept all incoming light. The lower leaf area then reflects in initially at least 10% lower production. The production is lower still when substrate oxygen supply is limiting the bacterial conversion of ammonium to nitrate. It is advised to look into ways of converting organic nitrogen and ammonium nitrogen into nitrate before dosing the solution to the plants. It is also advised to keep slabs drier than when using chemical fertilisers to allow for higher substrate oxygen supply. Advantages of the new fertilisers are the dosing via conventional dosing equipment and drip lines as well as the possible regulation of the nitrogen supply and pH with endoprotease. The element analysis must be adapted to incidentally include urea-N, amino-N and protein-N. The EC set points in climate and nutrient regulation are 25-40% lower as urea-N, amino-N and protein-N do not contribute to the EC via a counter ion.

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