

Management of *Fusarium oxysporum* f.sp *cubense* (Foc-TR4) from banana by anaerobic soil disinfestation (ASD)

PPO-AGV: Materials & methods, gas production and consumption & volatile fatty acids production

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1 Introduction

Applied Plant Research in Lelystad has, commissioned by Gert Kema, Plant Research International (PRI) and leader of the Panama Project, performed a trial to measure the efficacy of anaerobic soil disinfestation (ASD) with a 'Herbie' product against *Fusarium oxysporum* f.sp. *cubense* (Foc; TR 4), causing panama disease in bananas. The inoculum of conidiospores was provided by (PRI) at Wageningen. Also sand and clay soil were provided by PRI; two weeks before the experiment. After ASD the efficacy against Foc was also determined by PRI with a developed diagnostic tool (component of INREF Panama Project 3).

2 Materials and methods

The trial was performed under controlled conditions in 11- L polypropene containers with 8 L of soil each in fivefold. Sand and clay soil were mixed at a 1:1 ratio (v/v) and moistened to ensure sufficient water content for an optimal ASD-process; 15 to 20%. The mixed soil contained 6,3 kg sand and 4,7 kg clay per 8 L volume and was chemically and physically analyzed by BLGG AgroXpertus at Wageningen; www.blggagroxpertus.nl.

The soil was amended with an organic product (Herbie® 72) or not (control); www.thatchtec.nl. The product was applied at a dosage of 4 grams crude protein (CP) per L of soil, which equals 115 grams per container of 8 L soil and was mixed through the soil. After Herbie application, nylon mesh bags containing conidiospores of Foc-TR4 in soil were incorporated into the soil at a depth of 6 (top) to 9 (bottom) cm. The total soil layer was 15 cm high, so the inoculum was placed in the middle of the soil layer. The containers were then sealed off with a lid or not (control) and placed in a compartment at 24°C and approx. 70% RH. Exposure times to the treatments were 0, 7, 14, 21 and 28 days. Destructive sampling was performed after each exposure time. Lids were removed and inoculum was taken out and transported to Wageningen the same day for testing the efficacy. Soil humidity was measured before and after the trial.

Additionally several gases (O₂, H₂S, CH₄, CO₂, NH₃, N₂O) in the head space were measured before each destructive sampling. The gases except O₂ were measured with an infrared photo acoustic spectrophotometer for (greenhouse) gases (Innova 1412, LumaSense Technologies). O₂ was measured with a portable gas monitor based on electro-chemical sensor technology (type Impact Pro, Zellweger Analytics Ltd). In the compartment with the buckets four measurements were made each exposure time. After 7 days exposure time all the covered buckets were measured; 20 with amendment and 20 without amendment. After 14 days these numbers were 15 for both treatments. After 21 days 10 buckets of each treatment were measured and at the end after 28 days 5 buckets were measured per treatment.

Preparations for analysis of fatty acids concentrations in the soil were made directly after removing the lids from the containers. For the quantification of fatty acids, immediately after opening a container 10 subsamples were taken with a diam. 1.8 cm auger to make up a single sample. After mixing in a plastic bag, a subsample of 50 g was taken, 100 mL demineralized water was added, shaken for 30 min., and centrifuged at 5000 rpm for 25 min. Then 0.5 mL of supernatant was transferred to a 1.5 mL eppendorf tube. To each tube, 0.5 mL of 0.1 M ortho-phosphoric acid containing 0.5 mL 19.7 mmol/L isocaproic acid was added as internal standard. Samples were stored at -20°C until measurement. The volatile fatty acids were separated by GC using Grace EC-1000 length 30 m, ID 0.53 mm, 0.2 µm as column and He as carrier gas and detected by FID. Quantification was based on comparison with a chemical standard after internal standard correction.

Treatments are mentioned in table 1.

Table 1. Treatments in ASD trial against Foc-TR4

Treatment	Code
non-infested, non-amended and uncovered (total/negative control)	NINAU
infested, non-amended and uncovered (positive control)	INAU
infested, non-amended and covered (effect cover)	INAC
infested, amended and uncovered (effect product)	IAU
infested, amended and covered (effect product + ASD)	IAC

3 Results

3.1 Compartment conditions

3.1.1 Temperature

Average temperature throughout the trial was $23.7^{\circ}\text{C} \pm 0.3$.

3.1.2 Relative humidity air

Average air humidity throughout the trial was $72.7 \pm 0.5\%$.

3.2 Soil analyses

3.2.1 Chemical & physical soil analysis

Chemical analysis is shown (in Dutch) in appendix 1. The texture of the mixed soil was 7% clay (<2 μm), 17% silt (2-50 μm) and 71% sand (50-2000 μm). The internationally used texture triangle is presented in appendix 2. Organic matter content was 4.3 % and pH 6.5.

3.2.2 Soil humidity/dry matter content

Soil humidity of the sand soil after application of 40 L water was 19% and 81% dry matter.

Soil humidity of the clay soil after application of 80 L water was 18.5% and 81.5% dry matter.

Soil humidity of the mixed soil used was calculated as 18.75% and 81.25% dry matter.

Soil humidity before and after the treatments is presented in table 2.

Table 2. Soil humidity in non-infested (NI), amended (A), non-amended (NA), covered (C) or uncovered (U) soil.

Treatment; 5 replicates	% dry matter	% humidity
NINAU – day 0	81.25	18.75
INAU – day 28	89.23	10.77
INAC – day 28	82.04	17.96
IAU – day 28	88.04	11.96
IAC – day 28	81.36	18.64

In open containers (INAU and IAU) soil humidity dropped during 28 days with some 40% in comparison with closed containers.

3.3 Gases

Average gas data measured are mentioned in appendix 3.

In figure 1 O_2 decline is presented. In the treatment with Herbie O_2 dropped to 1.9% after 7 days and ultimately to 1.5% after 28 days, indicating breakdown of organic matter through microbial activity.

These levels are somewhat higher than expected where O_2 dropped to <1.3% in a trial at 20°C and to approximately 0.5% at 16°C . Possibly at higher temperatures polypropene containers transmit more oxygen from the compartment into the containers than at lower temperatures. The virtually impermeable film (VIF) used in the field has a much lower oxygen transmission rate (OTR) than polypropene which diminishes this effect to happen.

Also without Herbie amendment O_2 declines to the same level as with Herbie. An explanation is the relatively high amount of organic matter in the original soil mixture.

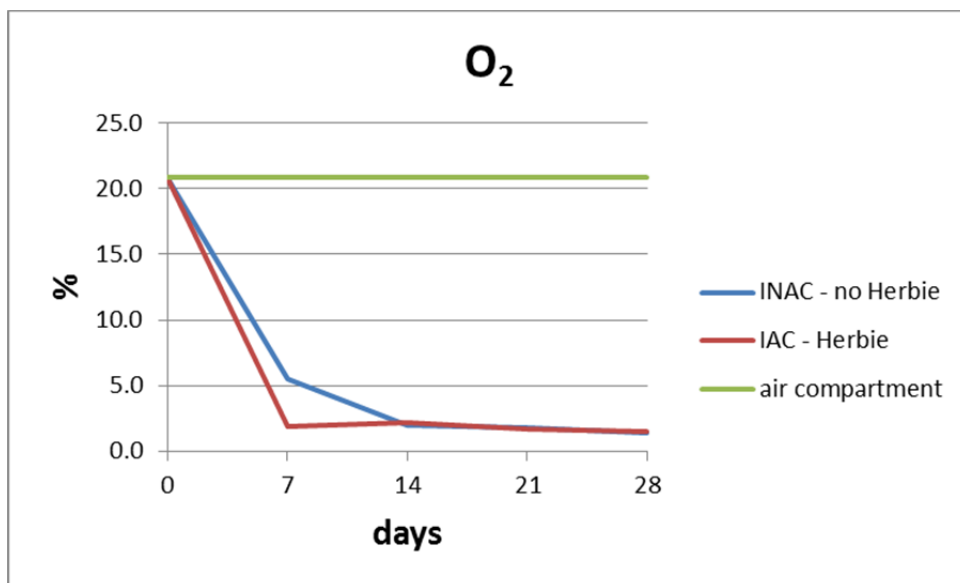


Figure 1. O₂ concentration during ASD trial against Foc from banana

Figure 2 shows the production of CO₂ which behaved largely inverse to the observed O₂ pattern. The production of CO₂ increased from 600 ppm to 140000 in the Herbie treatment and to some 60000 without amendment.

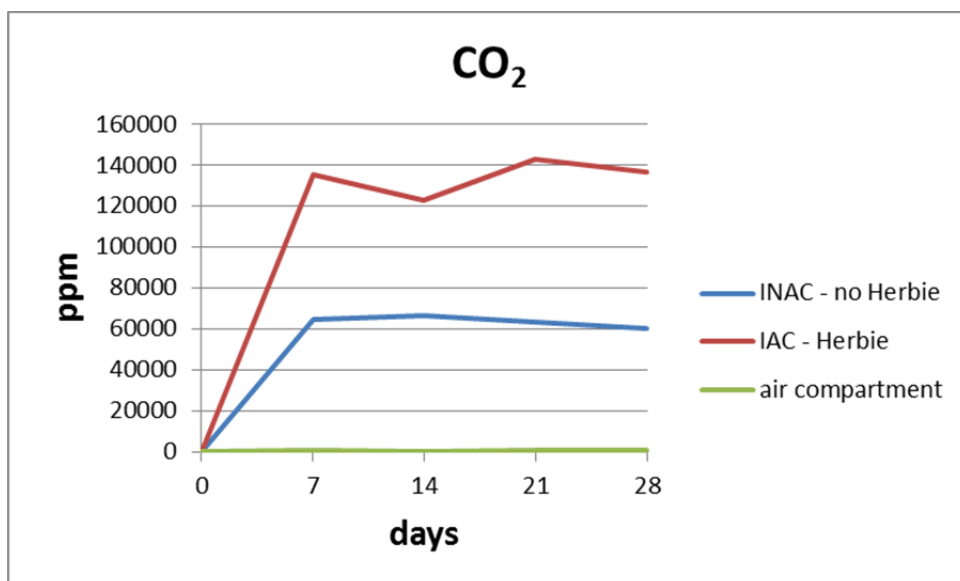


Figure 2. CO₂ concentration during ASD trial against Foc from banana

Figure 3 shows the production of N₂O. A peak of 2700 ppm was noticed in the non-amended treatment, whereas in the Herbie treatment the maximum was 430 ppm.

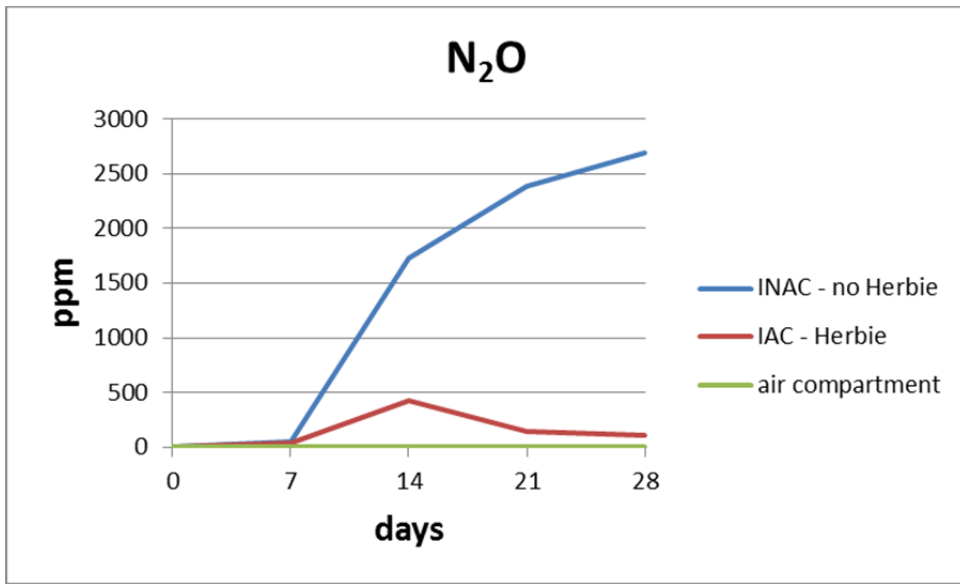


Figure 3. N₂O concentration during ASD trial against Foc from banana

Figure 4 presents ammonia (NH₃) production during the trial. Production of NH₃ is limited to maximally 6 ppm after 7 days in Herbie amended soil.

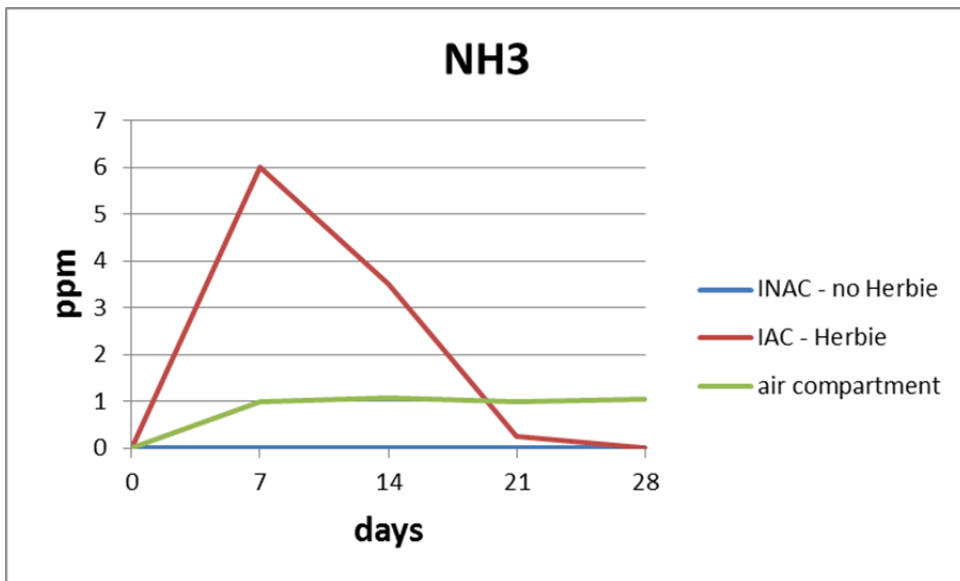


Figure 4. NH₃ concentration during ASD trial against Foc from banana

Figure 5 presents CH₄ production during the trial. Methane is produced in the Herbie treatment and increased to > 40000 ppm after 28 days.

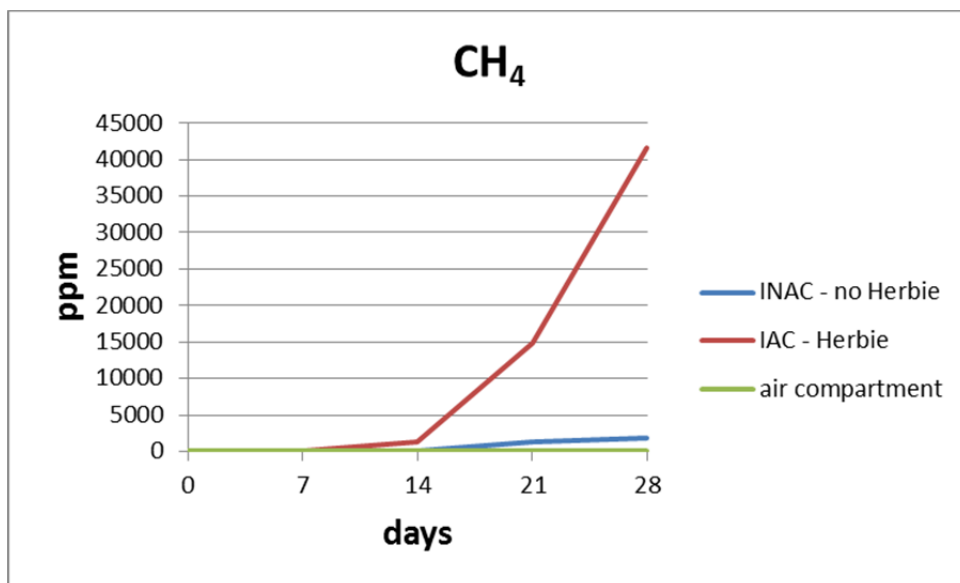


Figure 5. CH₄ concentration during ASD trial against Foc from banana

Figure 6 shows H₂S production during the trial. Starting at 21 days in amended soil H₂S increased to 300000 ppm after 28 days.

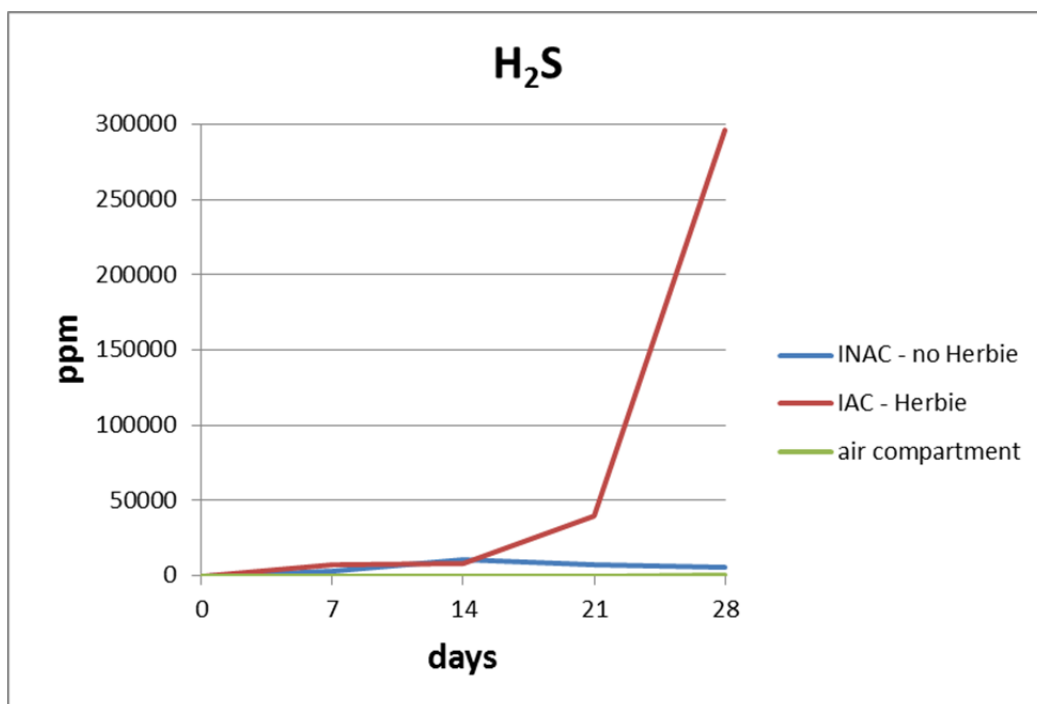


Figure 6. H₂S concentration during ASD trial against Foc from banana

3.4 Volatile fatty acid (VFA) production

Appendix 4 shows the production of all measured VFA's during ASD process for 28 days. The figures 7, 8 and 9 show the average production of acetic, propionic and butyric acid respectively in graphs. Iso butyric acid and (iso)valeric acid values are < 1 mmol/L soil and therefore not presented in graphs.

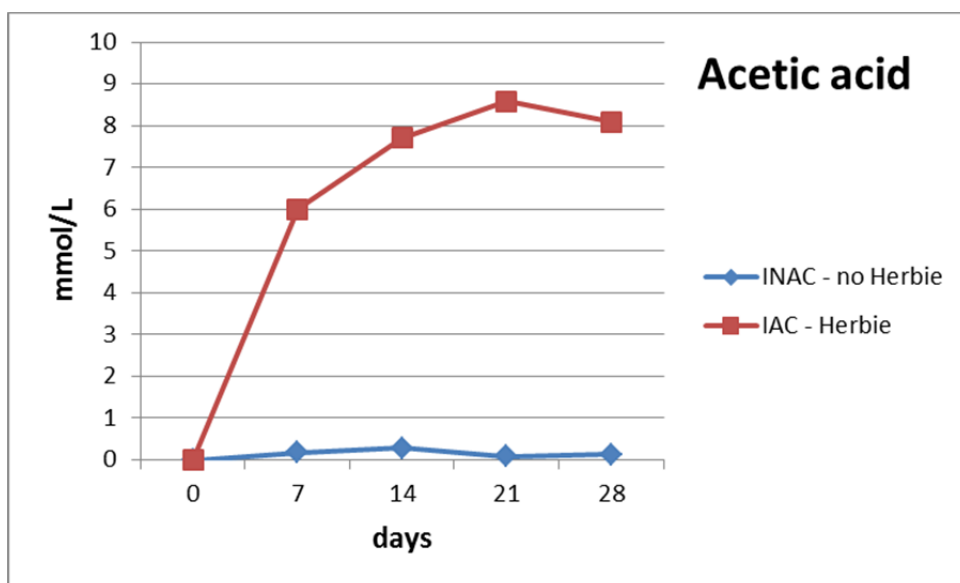


Figure 7. Acetic acid concentration during ASD trial against Foc from banana

Figure 7 shows that after 21 days of ASD the highest average concentration of acetic acid is reached; 8.6 mmol/L soil.

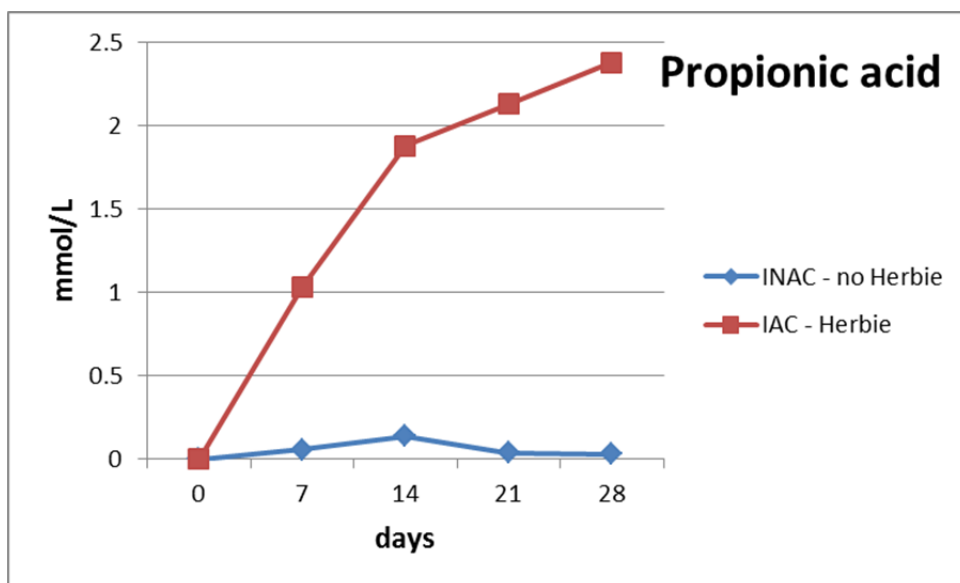


Figure 8. Propionic acid concentration during ASD trial against Foc from banana

Figure 8 shows that after 28 days of ASD the highest average concentration of propionic acid is reached; 2.4 mmol/L soil.

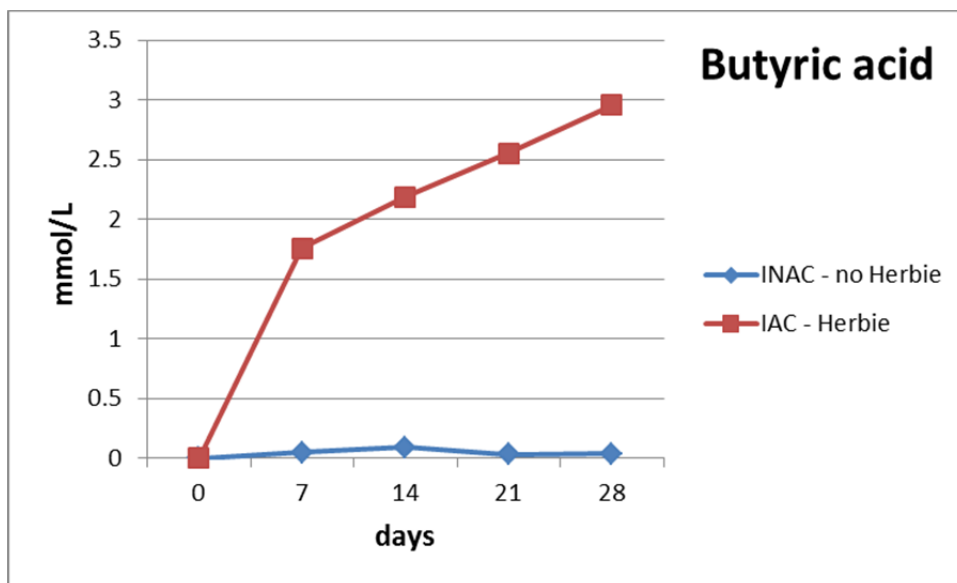


Figure 9. Butyric acid concentration during ASD trial against Foc from banana

Figure 9 shows that after 28 days of ASD the highest average concentration of butyric acid is reached; 3.0 mmol/L soil.

Appendix 1 Analysis of soil used for trial ASD against Foc



Akker-/tuinbouw
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Uw klantnummer: 6518834

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BLGG AGROXPERTUS



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T klantenservice: +31 (0)88 876 1010
E klantenservice@blgg.agroxpertus.nl
I blgg.agroxpertus.nl

Onderzoek: Runia 224105 Banaan
Onderzoek-/ordernr: 711946/003319570
Datum monstername: 02-04-2014
Datum verslag: 17-04-2014
Subsidieverlener: BLGG AgroXpertus, Korfingsregeling
Postbus 170, 6700 AD WAGENINGEN

Resultaat	Eenheid	Resultaat	Gem.*	Streeftraject	laag	vrj laag	goed	vrj hoog	hoog
Resultaat hoofdelement									
N-totale bodemvoorraad	mg N/kg	1490							
C/N-ratio		17	17	13 - 17					
N-leverend vermogen	kg N/ha	56	62	93 - 147					
fysisch									
S-totale bodemvoorraad	mg S/kg	390							
C/S-ratio		64		50 - 75					
S-leverend vermogen	kg S/ha	20	12	20 - 30					
P plant beschikbaar	mg P/kg	3,8	5,0	1,3 - 2,6					
P-bodemvoorraad (P-AI)	mg P ₂ O ₅ /100 g	93	62	30 - 46					
P-buffering		24		17 - 27					
Pw	mg P ₂ O ₅ /l	72							
K plant beschikbaar	mg K/kg	59		70 - 110					
K-getal		14	16						
K-bodemvoorraad	mmol+/kg	4,2		3,0 - 4,2					
Ca plant beschikbaar	kg Ca/ha	447		236 - 552					
Ca-bodemvoorraad	kg Ca/ha	7285		5245 - 7865					
Mg plant beschikbaar	mg Mg/kg	110	83	50 - 85					
Na plant beschikbaar	mg Na/kg	21	10	35 - 50					
Zuurgraad (pH)		6,5	5,1	5,6 - 6,1					
Organische stof	%	4,3	4,0						
C-anorganisch	%	0,16							
Koolzure kalk	%	0,8		2,0 - 3,0					
Klei	%	7							
Silt	%	17							
Zand	%	71							
biologisch									
Klei-humus (CEC)	mmol+/kg	129	81	> 75					
CEC-bezetting	%	100	71	> 95					
Bodemleven	mg N/kg	26		60 - 80					

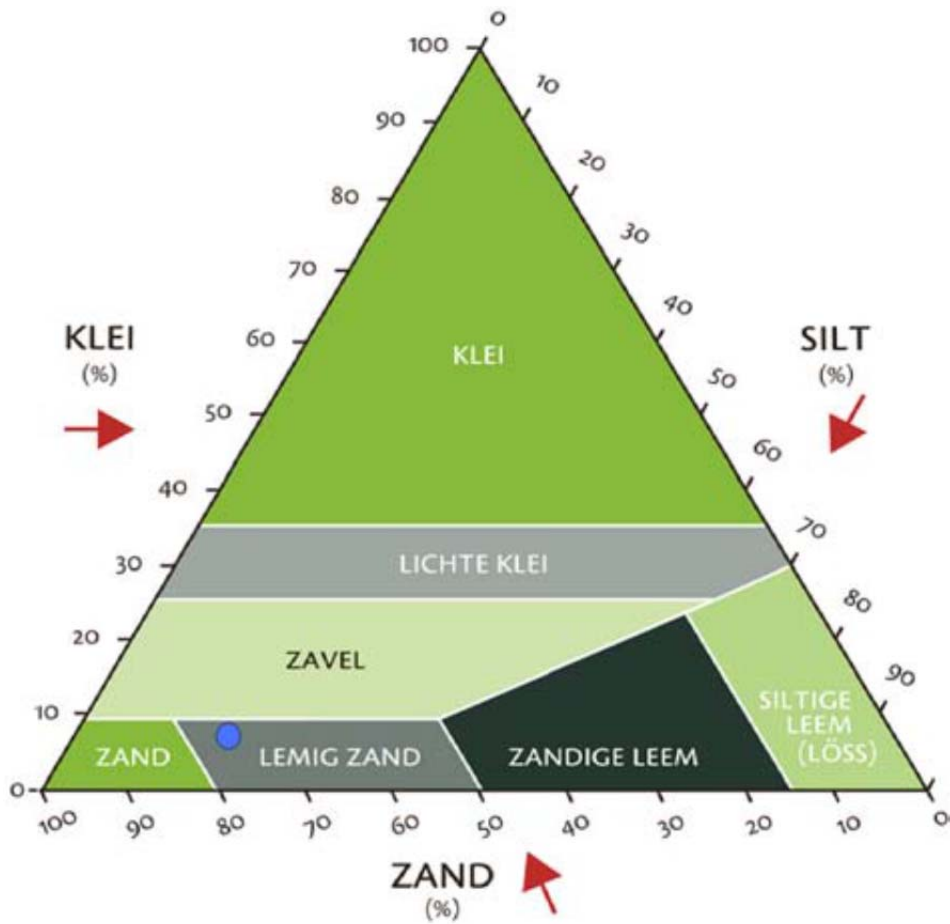
* Dit zijn regiemiddelen. Meer informatie staat bij onderdeel Gemiddelde.

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Totaal aantal pagina's: 5
711946, 17-04-2014



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Appendix 2 Texture of soil used for trial



Appendix 3 Average gas data

# buckets measured	Code	PPM CO2	PPM N2O	PPM NH3	PPM CH4	PPM H2S	PPM water vapour	% O2
20	INAC_7_average	64980	51	0	0	2660	16	5.5
15	INAC_14_average	66477	1728	0	66	10565	16	2.0
10	INAC_21_average	63570	2389	0	1317	7599	17	1.8
5	INAC_28_average	60430	2685	0	1833	5871	18	1.4
20	IAC_7_average	135475	43	6	0	7288	16	1.9
15	IAC_14_average	122953	430	4	1353	8081	15	2.2
10	IAC_21_average	142700	148	0	14929	40070	16	1.7
5	IAC_28_average	136500	115	0	41660	296000	17	1.5
4	air compartment_7	600	0	1	21	0	16	20.9
4	air compartment_14	576	0	1	25	22	18	20.9
4	air compartment_21	645	0	1	24	0	17	20.9
4	air compartment_28	598	0	1	24	171	18	20.9

Appendix 4 Volatile fatty acids

		Acetic acid	Propionic acid	iso Butyric acid	Butyric acid	iso Valeric acid	Valeric acid
3-1	inac7	0.52	0.17	0.02	0.14	0.03	0.04
3-2	inac7	0.09	0.02	0.01	0.03	0.02	0.02
3-3	inac7	0.16	0.06	0.02	0.03	0.05	0.07
3-4	inac7	0.08	0.02	0.00	0.02	0.01	0.02
3-5	inac7	0.05	0.01	0.00	0.02	0.01	0.01
3-1	inac14	0.62	0.28	0.07	0.18	0.13	0.12
3-2	inac14	0.17	0.08	0.03	0.07	0.07	0.11
3-3	inac14	0.13	0.05	0.02	0.04	0.04	0.05
3-4	inac14	0.48	0.25	0.08	0.12	0.08	0.12
3-5	inac14	0.06	0.03	0.01	0.03	0.02	0.04
3-2	inac21	0.08	0.02	0.00	0.02	0.01	0.02
3-4	inac21	0.08	0.03	0.01	0.02	0.03	0.04
3-1	inac21	0.10	0.03	0.00	0.02	0.01	0.02
3-3	inac21	0.08	0.07	0.04	0.07	0.07	0.12
3-5	inac21	0.05	0.03	0.01	0.03	0.03	0.05
3-1	inac28	0.23	0.05	0.01	0.06	0.02	0.04
3-2	inac28	0.21	0.04	0.00	0.06	0.02	0.02
3-3	inac28	0.10	0.03	0.01	0.02	0.02	0.02
3-4	inac28	0.07	0.01	0.01	0.02	0.01	0.02
3-5	inac28	0.06	0.01	0.00	0.01	0.01	0.01
5-1	iac7	5.55	0.97	0.06	1.47	0.05	0.01
5-2	iac7	5.89	0.96	0.08	2.28	0.10	0.11
5-3	iac7	6.08	1.05	0.08	1.61	0.08	0.08
5-4	iac7	6.15	1.12	0.08	1.70	0.08	0.07
5-5	iac7	6.34	1.03	0.06	1.73	0.05	0.03
5-1	iac14	8.06	1.89	0.16	2.24	0.10	0.06
5-2	iac14	7.27	1.98	0.20	2.11	0.14	0.11
5-3	iac14	8.39	1.94	0.16	2.31	0.09	0.05
5-4	iac14	7.52	1.96	0.21	2.24	0.13	0.09
5-5	iac14	7.29	1.61	0.15	2.03	0.09	0.06
5-1	iac21	7.18	1.77	0.20	2.08	0.10	0.08
5-2	iac21	7.76	1.90	0.23	2.33	0.11	0.08
5-3	iac21	9.78	2.36	0.27	2.91	0.12	0.08
5-4	iac21	9.03	2.38	0.26	2.72	0.13	0.10
5-5	iac21	9.16	2.23	0.24	2.72	0.11	0.07
5-1	iac28	8.38	2.29	0.33	2.82	0.14	0.11
5-2	iac28	7.39	2.15	0.33	2.72	0.16	0.12
5-3	iac28	8.91	2.59	0.37	3.23	0.15	0.13
5-4	iac28	8.74	2.65	0.38	3.17	0.16	0.14
5-5	iac28	7.01	2.21	0.18	2.84	0.15	0.11