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Study report: GLP study

Study number: **ALGE 14 010**

Test substance: **PMV-01 (2014 TOX 026)**

Algal growth inhibition test *(Pseudokirchneriella subcapitata)*

According to OECD Guideline 201

vito applied bio molecular systems abs

Test facility:

VITO - ABS
Industriezone VLASMEER 7
B 2400 MOL (Belgium)
Contact:
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Sponsor:

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December 2014
2014/MRG /R /201

vito	**Study Report** **GLP study** **Environment assessment study** **Alga: Growth inhibition test** ***Pseudokirchneriella subcapitata***	**ALGE 14 010** Page 2 of 21 Date: 12-12-2014 9:28 print

TEST SUBSTANCE	CODE Test Substance
PMV-01	2014 TOX 026

TEST FACILITY MANAGER	STUDY DIRECTOR	QA
[redacted]	[redacted]	[redacted]
VITO - ABS *Vlaamse Instelling voor Technologisch Onderzoek* **Boeretang 200** **B2400 MOL** **Tel:** [redacted] **Fax:** [redacted] **e-mail:** [redacted]@vito.be	**VITO - ABS** *Vlaamse Instelling voor Technologisch Onderzoek* **Boeretang 200** **B2400 MOL** **Tel:** [redacted] **Fax:** [redacted] **e-mail:** [redacted]@vito.be	**VITO - ABS** *Vlaamse Instelling voor Technologisch Onderzoek* **Boeretang 200** **B2400 MOL** **Tel** [redacted] **Fax** [redacted] **e-mail:** [redacted]@vito.be
Date: 12/12/2014 **Signa** [redacted]	**Date:** 12/12/2014 **Signat** [redacted]	**Date:** 12/12/2014 **Signature:** [redacted]

Sponsor

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2860 Sint-Katelijne-Waver
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Personnel: [redacted]

10.1.c Wob juncto 63.2.a Vo 1107/2009 juncto 39.2.a Vo 178/2002

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STATEMENT OF GLP COMPLIANCE BY THE STUDY DIRECTOR

The study described in this report was carried out under my supervision and responsibility and in compliance with the OECD principles of Good Laboratory Practice.

I hereby attest to the authenticity of the study and guarantee that the study was performed according to the procedures described in this report. This study report is a complete and accurate representation of the data obtained.

There were no significant deviations which may have an adverse effect on the quality or integrity of this study.

The study director makes no GLP-compliance claim for the characterization of the test item, which is the responsibility of the sponsor.

Date: 12/12/14

Signature:
Study Director

[redacted]

[redacted]

STATEMENT QA

STUDY ALGE 14 010

Algal growth inhibition test *(Pseudokirchneriella subcapitata)* Test substance PMV-01 According to OECD Guideline 201

The data contained in this study report were audited by the quality Assurance Unit to assure compliance with the protocol, the standard operating procedure and the pertinent Good Laboratory Practice regulations of the OECD and EEC directives.

The audits took place, were reported to the study director and the management on the following dates:

Date	Audit	Reported findings to Study Director	Reported findings to Test Facility Management
14/08/2014	Study plan	14/08/2014	
20/10/2014, 21/10/2014, 23/10/2014	Inspection of the preliminary test (test system, preparation of the dilutions, evaluation of numbers the cells with the Fluoroskan, correct use of the appropriate PPE and CPE based on the VITO procedures, waste removal, registration of the instruments)	24/10/2014	03/11/2014
03/11/2014, 04/11/2014, 05/11/2014, 06/11/2014	Inspection of first final test (test system, preparation of the dilutions, evaluation of numbers the cells with the Fluoroskan, correct use of the appropriate PPE and CPE based on the VITO procedures, waste removal, registration of the instruments	13/11/2014	13/11/2014
27/11/2014	Draft report	27/11/2014	
09/12/2014	Final report	09/12/2014	

We declare that the report completely and accurately describes the used materials and methods and that the results and conclusions accurately reflect the raw data that were obtained during the study.

[redacted]

Date: 12/12/2014

[redacted]

10.1.c Wob juncto
63.2.d Vo 1107/2009

SUMMARY

The aim of this test was to evaluate the effect of the test substance PMV-01 (commercial product) on the growth rate of the green algal species *Pseudokirchneriella subcapitata* during 3 days of exposure. The test was performed according to the protocol described in OECD 201 (2006).

Test quality:	GLP
Test item:	PMV-01 (test substance 2014 TOX 026) [redacted] *Pepino mosaic virus*, CH2 strain, isolate 1906)
Test protocol:	OECD 201 (2006)
Test code:	ALGE 14 010
Test organisms:	*Pseudokirchneriella subcapitata*
Test concentrations:	The test substance PMV-01 is soluble in water. In the preliminary test the test substance was added directly to OECD medium at a concentration of 1000 mg/l. A 1/10 dilution series of this stock solution in OECD medium was tested (1000 – 100 – 10 -1 - 0.1 mg/l). No negative effects were seen. In the final test the following test concentrations were used: 1000-500-250-125-62.5 mg/l PMV-01.
Medium	OECD mineral medium (OECD 201, 2006).
Analyses:	the test substance is [redacted] *Pepino mosaic virus*, CH2 strain, isolate 1906. The viral load was measured using TaqMan RT-qPCR. Samples were taken for analyses of the viral load at the start and at the end of the final test.
Test vessels:	glass erlenmeyers 500 ml, air permeable cotton stoppers
Test conditions:	incubator (erlenmeyers randomly placed), continuous shaking (100 rpm), 23 ± 1 °C, continuous light (±3094 lux)
Test volumes:	100 ml control/test solutions and 100 µl algae (except in abiotic conditions), initial cell numbers: approx. 6559 x 10^3 cells per ml.
Set up:	Abiotic control (2 replicates) Blank control (6 replicates) Test solutions (3 replicates) Measurement of number of cells in parallel by coulter counter and fluoroskan on day 1 in controls, and by fluoroskan daily in all recipients after 24, 48 and 72 hours.
Dates:	3-6/11/2014

Results:

Test validation:	All validity criteria (growth rate parameters in controls) were met.
Results:	The RT-qPCR analyses showed that the viral load was below detection limit at all test concentrations. Results are expressed in mg/l of the test substance (nominal concentration). Algal growth rate was not inhibited in the concentration range up to 1 g/l PMV-01.

1. PURPOSE OF THE STUDY

The aim of this test was to evaluate the effect of the commercial product PMV-01 on the growth of the unicellular green algal species *Pseudokirchneriella subcapitata* during 3 days of exposure.
The method is based upon OECD guideline for testing of chemicals 201 (2006) and described in VITO SOP TALGE001v8.

2. TEST SUBSTANCE

2.1. Identification

VITO code	Sponsor code
2014 TOX 026	PMV-01

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TEST ITEM INFORMATION

Name:	PMV-01 [redacted] *epino mosaic virus*, CH2 strain, isolate 1906)
Batch number:	[redacted]
Expiring date:	[redacted]
Storage conditions:	Frozen (-20°C ± 2)
Purity:	na; active ingredient [redacted]
Formula:	Active ingredient: *Pepino mosaic virus*, CH2 strain, isolate 1906
Molecular weight:	na
Appearance:	Green solution with particles
CAS:	na
Solubility in water:	soluble
Stability:	7 d at 20°C 38 d at 4°C
H codes:	Not classified, no additional information on adverse effects available
Methodology for concentration analysis:	TaqMan RT-qPCR for viral load

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na = not applicable

Certificates of Analysis: annex 1.

2.2. Registration

All handling of the test substance was registered on forms BTEST001-Frm1v02 and Frm3v03. Forms will be kept in BIO1 - 321 and later in the archives.

3. TEST FACILITY

The test was performed at VITO ABS – Industriezone Vlasmeer 7, B2400 MOL Belgium - BIOL – ecotox facility (BIOL 0273 - 0277- 0202) and BIOL 0377 – 0365 - 0384 (analyses).

4. REFERENCE SUBSTANCE

No reference substance was used in the test. A reference substance ($ZnCl_2$) is periodically tested to monitor the sensitivity of the algal culture to toxic substances as a function of time.
Result of the most recent test ALGE 14 012 on 8-11/09/2014 showed E_rC_{50} = 0.1 mg/l $ZnCl_2$ after 72 hours of exposure. This is within the VITO historical data range (0.082-0.168 mg/l; n=15).

5. ANALYTICAL METHODS

5.1. Standard measurements

pH meter: BIO 1334 (WTW portable)
Cell number: Coulter Counter BIO 1012; Fluoroskan: BIO 1289

pH and cell number were measured using standard laboratory equipment and standard calibration and verification procedures as described in the respective Standard Operating Procedures.

5.2. Analyses (virus load)

TaqMan RT-PCR (two-step) procedure for detection of *Pepino mosaic virus* in tomato leaf samples using Gutiérrez-Aguirre primers and probes. The method is described in annex 4.
The analytical protocol was provided by Scientia Terrae and a preliminary validation study was performed where the same samples (n=5) were measured in both the Scientia Terrae lab and the VITO facilities. The results of both labs were completely comparable. Further tests were performed at the VITO test facility.

6. TEST PERFORMANCE

6.1. Test system

6.1.1. Justification of the test system

Algal growth inhibition test is a widely accepted standardized test to evaluate the toxicity of products in water for unicellular producers (OECD, ASTM, ISO).
Pseudokirchneriella subcapitata is one of the species recommended by the authorities, as a representative of the trophic level of primary producers.

6.1.2. Characterization of the test system

NAME : *Pseudokirchneriella subcapitata*

CODE CCAP: 278/4 -Green micro algae. **Phylum:** *Chlorophyta* (Green Algae) **Class:** *Chlorophyceae* **Order:** *Not assigned* **Genus:** *Pseudokirchneriella* **Botanical name:** - *Pseudokirchneriella subcapitata* (Korshikov).

Lab keeping and breeding: SOP TALGV001v5.

10.1.c Wob juncto 63.2.a
Vo 1107/2009 juncto
39.2.a Vo 178/2002

Batch used in these experiments: 2103
Transfer from breeding compartment to pre-test vessel: 17 and 31/10/2014 for the preliminary and final test respectively.

6.2. Test description

6.2.1. Date of performance

ALGE 14 010
Exposure: 20-23/10/2014 (preliminary test) and 3-6/11/2014 (final test)

6.2.2. Principles of the test method (OECD 201)

The purpose of this test is to determine the effects of PMV-01 on the growth rate of unicellular freshwater microalgae. Exponentially growing unicellular algae are exposed for a period of 72 hours to a dilution series of the test substance, using 1g/l test substance as the highest test concentration. The cultures are allowed unrestricted exponential growth under nutrient sufficient conditions and continuous illumination. Growth and growth inhibition are quantified from measurements of the algal biomass as a function of time. The biomass is measured every 24 hours during the 72 hours of exposure.
The ecotoxicological response to be evaluated is reduction of growth rate of algal cultures. The response is evaluated as a function of the nominal concentration of test substance (mg/l).

6.2.3. Method of administration

The test solutions are administered as surrounding water. Exposure route is via cell membrane uptake.

6.2.4. Test concentrations

250 ml aliquots of the test substance were kept frozen until use. For the preliminary test the aliquot 2014 TOX 026/1 was used [redacted] and aliquot 2014 TOX 026/5 [redacted] was used for the final test.

A preliminary range finding test was set up. 1g of the test substance was added to 1 liter of mineral medium (OECD Algal medium: see table 2 for composition) and a 1/10 dilution series was used in the test (1000 – 100 – 10 – 1 – 0.1 mg/l). No adverse effects were seen on the growth parameters.

For the final test (ALGE 14 010 final - repeat) again 1g of the test substance was added to 1 liter of mineral medium (OECD Algal medium: see table 2) and a ½ dilution series was used in the test (1000 – 500 – 250 – 125 – 62.5 mg/l). Samples were taken at the start and at the end of the test for analyses of the viral load. These samples were frozen immediately until analyses.

6.2.5. Test conditions

Set up: Abiotic control (OECD medium: 2 replicates): to check for background biological growth
Blank (biotic) control (OECD medium + algal inoculum: 6 replicates): to establish the control growth parameters, needed for test validation (see validity criteria) and as a reference.
Test dilutions (3 replicates): to establish the inhibiting effect of different concentration of the test substance when compared to controls.

Cell number at the start: Each recipient was inoculated with an equal number of algal cells at the beginning of the experiment, except for the 2 abiotic controls where no algae were added.

The number of cells in the inoculum suspension was 7849 x 10^3 cells/ml in the preliminary test, and 6559 x 10^3in the final test.

Test vessels: sterile glass erlenmeyers 500 ml, previously washed in a standard laboratory dishwasher and sterilized in the autoclave. During the test the vessels were covered with sterile cotton stoppers (air permeable).

Test volumes: 100 ml of each dilution or medium (control), 100 µl algae (not added to abiotic conditions).

Medium: Freshly prepared OECD Medium (SOP TALGV004v3) was used to provide the nutrients and minerals that are needed for unrestricted algal growth. The composition of the medium is listed in table 2.

Table 2: Composition of the OECD algal medium

Substance	mg/l
H_3BO_3	0,185
$MnCl_2,4H_2O$	0,415
$ZnCL_2$	0,003
$FeCl_3.6H_2O$	0,08
$CoCl_2.6H_2O$	0,0015
$Na_2MoO_4.2H_2O$	0,0037
$CuCl_2.2H_2O$	0,00001
$Na_2EDTA.2H_2O$	0,1
NH_4Cl	15
$MgCl_2.6H_2O$	12
$CaCl_2.2H_2O$	18
$MgSO_4.7H_2O$	7,4
KH_2PO_4	1,6
$NaHCO_3$	50

Conditions: test vessels were placed in incubator BIO 0765 at random places (daily changing), continuous shaking (100 rpm), Temperature 23-23.7°C during the final test (logger 73001399 – EBI 300; BIO 1594), continuous light (3094 lux).

6.2.6. Assessments, measurements and analyses (final test)

The number of algae in the inoculum is measured by coulter counter.
The correlation curve between coulter (cell number) and fluoroskan (fluorescent) signal was defined: a dilution series of the algal suspension is measured both by coulter and fluoroskan and the slope of the linear correlation curve is determined. This slope is used to convert the fluoroskan measurements into biomass equivalents.
After 24, 48 and 72 hours of exposure the fluorescent signal by the algal biomass is measured in an aliquot of each test vessel (800 µl in 48 well plates) by fluoroskan.

pH was measured in each dilution at the beginning and in each vessel at the end of the test (see annex 2: raw data). pH was approximately 8.5 (8.42-8.57) at the start of the test in all test conditions. This is within the acceptance range of 6-9 for algae. pH increased during the test to a mean value of 8.64 in controls.

At the final test samples for analyses were taken at the start of the experiment from each dilution and from one replicate of each concentration at the end of the test.

6.2.7. Calculations

Fluoroskan signals are transformed into coulter signals (biomass equivalents) by multiplying the fluoroskan signal with the slope of the correlation curve (see annex 2A: slope = 153.62). The offset value (-109.79) is not used in the calculations because this is a constant value that has no influence on the relative differences between test conditions and control.
Growth rate is calculated according to the method described in OECD guideline 201 (2006), using the biomass equivalent data. Results are expressed as a function of the nominal test substance concentration (mg/l).
Student t-test was used to evaluate statistic relevant differences when compared to controls ($p<0.05$).

6.3. Deviations

6.3.1. Deviations from the study protocol

The final test (ALGE 14 010 final) was performed as planned from 27-30/10/2014, but the test results were not valid (validity criteria were not met). The final test was therefore repeated (ALGE 14 010 final-repeat) using the same test conditions as described in the study protocol.

6.3.2. Deviations from OECD guideline 201

The composition of the medium is identical to the OECD medium as described in OECD 201 (2006) except for the concentrations of $FeCl_3.6H_2O$ (80 instead of 64 µg/l) and $MgSO_4.7H_20$ (7.4 instead of 15 mg/l). This slightly different medium is used for many years in the VITO lab. This has no further implication on the test results as growth parameters in controls are normal.

The validity criterion as defined by OECD:

> *Coefficient of variance for the mean specific growth rate for the individual days (days 0-1, 1-2, 2-3) ≤ 35%.*

has been adapted in the VITO lab to:

> *Coefficient of variance for the mean specific growth rate for the individual days (days 1-2, 2-3) ≤ 35%.*

The number of cells in controls at day 1 is too low to yield a significant fluorescent signal, leading to a virtual lag phase. In a validation study (N°11, jan 2013: algen fluo/coulter) it was clearly demonstrated that this is not due to a lower algal growth rate, but to the fluorescent signal in response to the cell number at these low concentrations. Therefore these data are omitted from the calculations. This has no impact on the final results as was shown by the validation study.

6.4. Standard operating procedures concerning the test and test organisms

- TALGE001v8 (growth inhibition test on algae)
- TALGV001v5 (algal culture)
- TALGV002v4 (Breeding medium (Jaworski))
- TALGV004v3 (OECD algal mineral medium)

- TTOES062v4 (pH measurement)
- TTOES055v3 (Milli Q)
- TTOES047v4 (Coulter counter)
- TTOES057v2 (luxmeter)
- TTOES058v2 (Fluoroskan)
- analytical protocol (Scientia Terrae)

7. RESULTS

7.1. pH

pH was measured at the start and at the end of the test (annex 2A - raw data).
pH was not a confounding factor.
The mean increase in pH in control vessels was 0.07 units which is within acceptable limits (<1.5).

7.2. Viral load

The viral load was measured but was below detection limit at all test concentrations. Raw data are presented in annex 3. Therefore results are expressed in nominal concentration of PMV-01.

7.3. Test results

Raw data of fluoroskan signal and calculated biomass equivalents: annex 2A.
Calculated values on specific growth rate: annex 2B.

7.3.1. Validity of the test (annex 2B)

In controls:

a) Biomass should increase at least 16x from 0 to 72 hours, i.e. a minimum value for specific growth rate of 0.92/day.
This was indeed the case: biomass increased 26.8x.

b) Coefficient of variance for the mean specific growth rate for the individual days (days 1-2, 2-3) ≤ 35%.
This was indeed the case: VC was 27.56%.

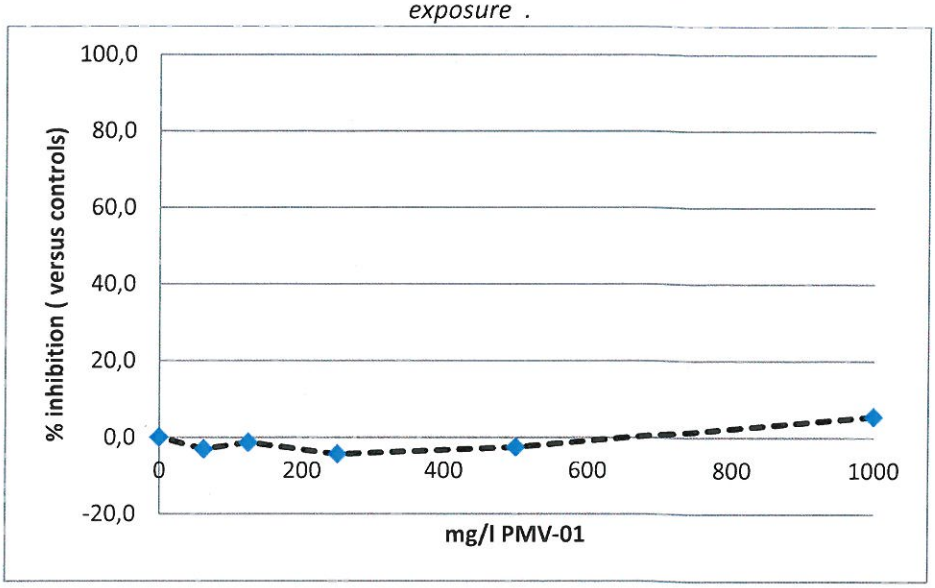
c) Coefficient of variance for the specific growth rate for day 0 - 3 for replicates ≤ 7%.
This was indeed the case: VC was 1.98%.

Based on the validity criteria the results of the test ALGE 14 010 (final repeat) are accepted and reported here.

7.3.2. Algal growth inhibition

Figure 1 shows the concentration effect curve after an exposure period of 72 hours as a function of the nominal concentration of the test substance PMV-01 (data in Annex 2B.2). There was no significant inhibitory effect on the algal growth rate in concentration range up to 1g/l.

Figure 1: Concentration effect curve for ALGE 14 010 (final repeat): effect on growth rate after 72 h of exposure .



7.4. Interpretation of the results

The test substance has no adverse effects on the algal growth rate in the test range up to 1000 mg/l PMV-01 (nominal concentrations).

8. ARCHIVES

The next items will be kept in archives at the VITO ABS archives for at least 5 years after the issue of the final report.

- *Dossier*: study protocol, draft versions of the study report, copies of the study report and attached documents, the original raw data,
- *General*: logbooks of the instruments.
- *Test substance*: product registration forms

The archive is located at VITO - BIO L 110 - Industriezone Vlasmeer 7 – B 2400 MOL (Belgium).

After the period of 5 years the sponsor will be contacted to decide on prolongation of retention, relocation or disposal.

9. PRINCIPAL INVESTIGATORS

Not applicable.

[redacted]

[redacted]

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[redacted]

ANNEX 2A: RAW DATA ALGE 14 010 (FINAL REPEAT)

Fluorescent data

ALGE14010 f repeat	erlenmeyertest	OECD protocol
2014TOX026/5	3-6/11/2014	

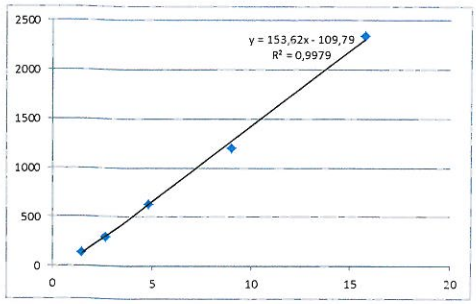
day 0
pre-culture (fluoro)

	coulter	2341	1203	633	299	143
	fluoroskan	15,7225	8,995	4,8125	2,6425	1,4675
		15,57	8,92	4,79	2,61	1,47
		15,6	8,99	4,85	2,64	1,47
		15,74	9,05	4,85	2,68	1,47
		15,98	9,02	4,76	2,64	1,46

Correlation	
slope	offset
153,62	109,79

daily measurements

test substr	mg/l	0	0	62,5	125	250	500	1000	mg/l
day 0	fluoroskan	0,26	0,46						
		0,26	0,46						
			0,44						
			0,46						
			0,48						
			0,47						
day 1	fluoroskan	0,26	1	1,1	1,08	1	1	0,94	
		0,26	1,12	1,03	0,92	1,06	0,94	1,01	
			1,2	1	1,09	1,05	0,93	1,03	
			1,14						
			1,14						
			1,12						
day 2	fluoroskan	0,27	4,51	5,48	5,07	5,54	5,47	3,89	
		0,26	4,48	4,83	4,03	5,55	4,11	3,73	
			4,76	5,24	5,41	5,49	4,4	3,78	
			5,98						
			5						
			5,68						
day 3	fluoroskan	0,39	11,68	14,3	14,5	13,84	14,93	11	
		0,36	11,64	13,49	11,41	14,09	12,04	9,62	
			12,57	13,31	13,12	15,01	13,55	10,41	
			13,45						
			11,78						
			13,24						



Biomass data (calculated from fluorescent data)

ALGE14010 f repeat	erlenmeyertest	OECD protocol
2014TOX026/5	3-6/11/2014	

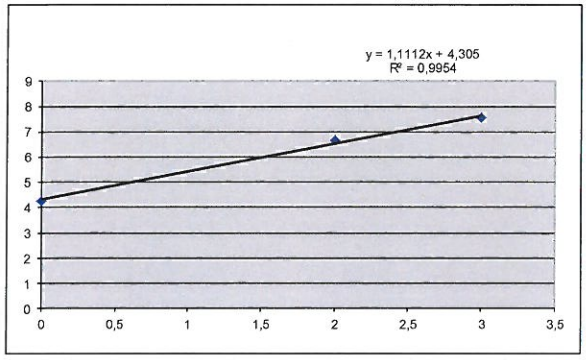
	name	time (h) mg/l	cell concentration 0	24	48	72	pH start	pH end	mean values pH start	mean values pH end
blanks	abiotic control	0	40				8,57	7,94		
blanks	abiotic control	0	40					7,96		
									8,57	7,95
controls	biotic control	0	71	154	693	1794	8,57	8,38		
	biotic control	0	71	172	688	1788		8,65		
	biotic control	0	68	184	731	1931		8,7		
	biotic control	0	71	175	919	2066		8,8		
	biotic control	0	74	175	768	1810		8,46		
	biotic control	0	72	172	873	2034		8,84	8,57	8,64
replicate 1	test concentration 1	1000		144	598	1690	8,48	8,53		
replicate 2	test concentration 1			155	573	1478		8,21		
replicate 3	test concentration 1			158	581	1599		8,59	8,45	8,44
replicate 1	test concentration 2	500		154	840	2294	8,48	8,33		
replicate 2	test concentration 2			144	631	1850		8,76		
replicate 3	test concentration 2			143	676	2082		8,59	8,48	8,56
replicate 1	test concentration 3	250		154	851	2126	8,45	8,98		
replicate 2	test concentration 3			163	853	2165		9,18		
replicate 3	test concentration 3			161	843	2306		9,31	8,45	9,16
replicate 1	test concentration 4	125		166	779	2227	8,42	8,8		
replicate 2	test concentration 4			141	619	1753		8,57		
replicate 3	test concentration 4			167	831	2015		9,04	8,42	8,80
replicate 1	test concentration 5	62,5		169	842	2197	8,43	9,54		
replicate 2	test concentration 5			158	742	2072		8,92		
replicate 3	test concentration 5			154	805	2045		8,71	8,43	9,06

ANNEX 2B: CALCULATED VALUES

2B.1: control of validity criteria: orange = validity criteria, green = measured values

total growth FACTOR day 1-3	26,8447653	> 16
mean growth factor (per day)	3,13217956	> 2,8
mean specific growth factor	1,096	> 0,92

day	counts	ln counts	
0	70,92123333	4,26156987	
1			
2	779	6,65749405	
3	1904	7,55164071	R^2>0,95



Specific growth rate (day 0->3) in control replicates

0	
1,0769	
1,0758	
1,1014	
1,1240	
1,0798	
1,1187	
1,096	
0,022	
1,983	< 7%

Mean specific growth rate per day in controls

%VC controls

day1-2	1,424	
day 2-3	0,959	
MEAN	**1,191**	
SD	0,3283443	
%VC	**27,561**	<35%

2B.2: Specific growth rates

day	1000	500	250	125	62,5	0	
3 - x1	1,06	1,16	1,13	1,15	1,14	1,0769	
3 - x2	1,01	1,09	1,14	1,07	1,12	1,0758	
3 - x3	1,04	1,13	1,16	1,12	1,12	1,1014	
3 - x4						1,1240	
3 - x5						1,0798	
3 - x6						1,1187	
3 - mean	1,036	1,124	1,144	1,111	1,130	**1,096**	
SD	0,022	0,036	0,014	0,040	0,013	**0,022**	
%VC						**1,983**	< 7%
% inhibition growth rate							
day	1000	500	250	125	62,5	0	
3 - x1	3,57	-5,72	-3,41	-4,83	-4,41	1,75	
3 - x2	7,65	0,83	-3,96	2,46	-2,63	1,85	
3 - x3	5,25	-2,77	-5,88	-1,79	-2,22	-0,48	
3 - x4						-2,54	
3 - x5						1,49	
3 - x6						-2,06	
3 - mean	5,5	-2,6	-4,4	-1,4	-3,1	0,0	
3 - SD	2,0	3,3	1,3	3,7	1,2	2,0	

ANNEX 3: RAW DATA TAQMAN RT-PCR

Experiment: 141113_PepMV_RW Selected Filter: FAM (465-510)

Include	Color	Pos	Name	Cp	Status
TRUE	255	A7	ALGE Stop 1000	35.00	>- Late Cp call (last five cycles) has higher uncertainty
TRUE	255	A8	ALGE Stop 1000	35.00	>- Late Cp call (last five cycles) has higher uncertainty
TRUE	255	A9	ALGE Stop 1000	35.00	>- Late Cp call (last five cycles) has higher uncertainty
TRUE	65280	B4	ALGE Start Co		
TRUE	65280	B5	ALGE Start Co		
TRUE	65280	B6	ALGE Start Co		
TRUE	255	B7	ALGE Stop 500	35.00	>- Late Cp call (last five cycles) has higher uncertainty
TRUE	255	B8	ALGE Stop 500	35.00	>- Late Cp call (last five cycles) has higher uncertainty
TRUE	255	B9	ALGE Stop 500	35.00	>- Late Cp call (last five cycles) has higher uncertainty
TRUE	255	C4	ALGE Start 1000	35.00	>- Late Cp call (last five cycles) has higher uncertainty
TRUE	255	C5	ALGE Start 1000	35.00	>- Late Cp call (last five cycles) has higher uncertainty
TRUE	255	C6	ALGE Start 1000	35.00	>- Late Cp call (last five cycles) has higher uncertainty
TRUE	255	C7	ALGE Stop 250	35.00	>- Late Cp call (last five cycles) has higher uncertainty
TRUE	65280	C8	ALGE Stop 250		
TRUE	255	C9	ALGE Stop 250	35.00	>- Late Cp call (last five cycles) has higher uncertainty
TRUE	255	D4	ALGE Start 500	35.00	>- Late Cp call (last five cycles) has higher uncertainty
TRUE	65280	D5	ALGE Start 500		
TRUE	255	D6	ALGE Start 500	35.00	>- Late Cp call (last five cycles) has higher uncertainty
TRUE	255	D7	ALGE Stop 125	35.00	>- Late Cp call (last five cycles) has higher uncertainty
TRUE	255	D8	ALGE Stop 125	35.00	>- Late Cp call (last five cycles) has higher uncertainty
TRUE	255	D9	ALGE Stop 125	35.00	>- Late Cp call (last five cycles) has higher uncertainty
TRUE	255	E4	ALGE Start 250	34.21	
TRUE	255	E5	ALGE Start 250	35.00	>- Late Cp call (last five cycles) has higher uncertainty
TRUE	255	E6	ALGE Start 250	35.00	>- Late Cp call (last five cycles) has higher uncertainty
TRUE	65280	E7	ALGE Stop 62.5		
TRUE	255	E8	ALGE Stop 62.5	35.00	>- Late Cp call (last five cycles) has higher uncertainty
TRUE	255	E9	ALGE Stop 62.5	35.00	>- Late Cp call (last five cycles) has higher uncertainty
TRUE	65280	F4	ALGE Start 125		
TRUE	255	F5	ALGE Start 125	35.00	>- Late Cp call (last five cycles) has higher uncertainty
TRUE	255	F6	ALGE Start 125	35.00	>- Late Cp call (last five cycles) has higher uncertainty
TRUE	255	F7	Pos Co	26.05	
TRUE	255	F8	Pos Co	25.98	
TRUE	255	F9	Pos Co	25.90	
TRUE	65280	G4	ALGE Start 62.5		
TRUE	65280	G5	ALGE Start 62.5		
TRUE	255	G6	ALGE Start 62.5	35.00	>- Late Cp call (last five cycles) has higher uncertainty
TRUE	65280	G7	NTC		
TRUE	65280	G8	NTC		
TRUE	65280	G9	NTC		
TRUE	65280	H4	ALGE Stop Co		
TRUE	65280	H5	ALGE Stop Co		
TRUE	65280	H6	ALGE Stop Co		

ALGE Start Co: control test solution at the start of the experiment
ALGE Stop Co: control test solution at the end of the experiment
ALGE Start x: test dilution x at the start of the experiment
ALGE Stop x: test dilution x at the end of the experiment
Pos Co: positive control
NTC: negative control

10.1.c Wob juncto 63.2.a en d Vo 1107/2009 juncto 39.2.a Vo 178/2002

[redacted]

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