

IO cells



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*New Measuring Instrument for the
Indicative Investigation of Ballast Water*



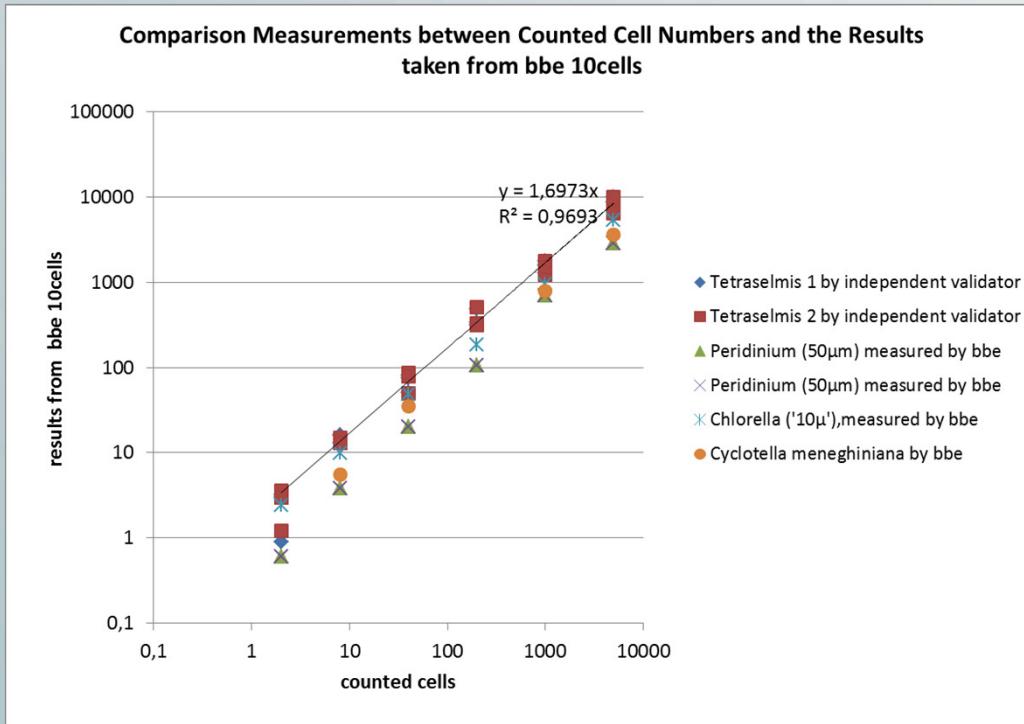


The Best First: the Advantages of bbe I0cells

- Resolution: 1-2 cells/ml
- Very simple operation
- Measurement within approx.1 min
- Based on fluorescence (f_{variable})
- No chemicals
- No ‘infection’ by the sample-taker

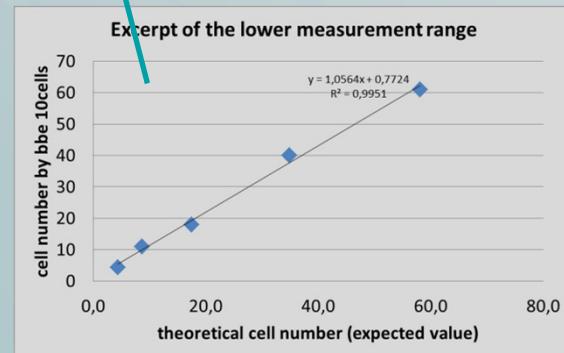
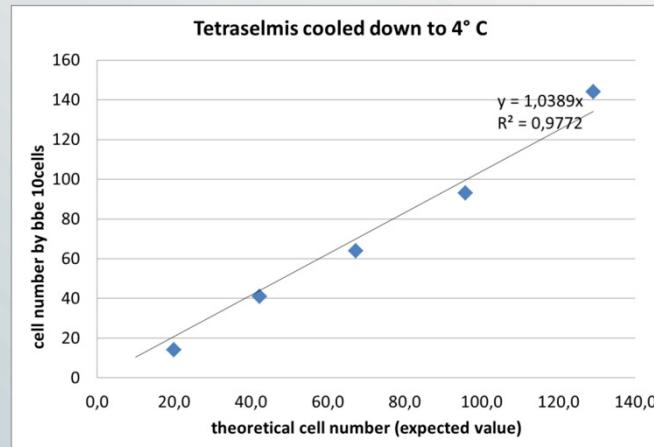
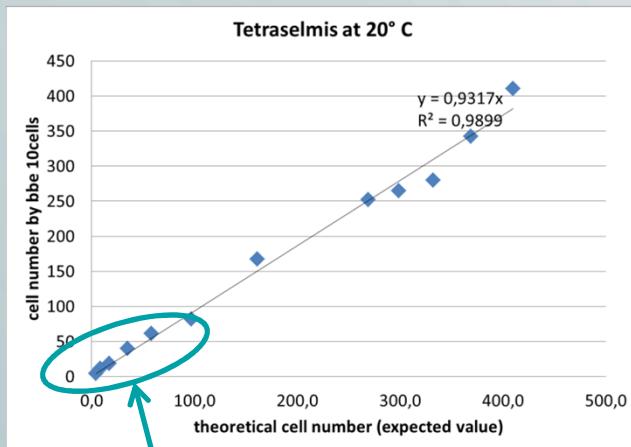


Results from Dilutions of Algae Species in the Range of 10µm - 50µm Cell Size





Hardly any Influence of the Temperature on bbe 10cells Measurements



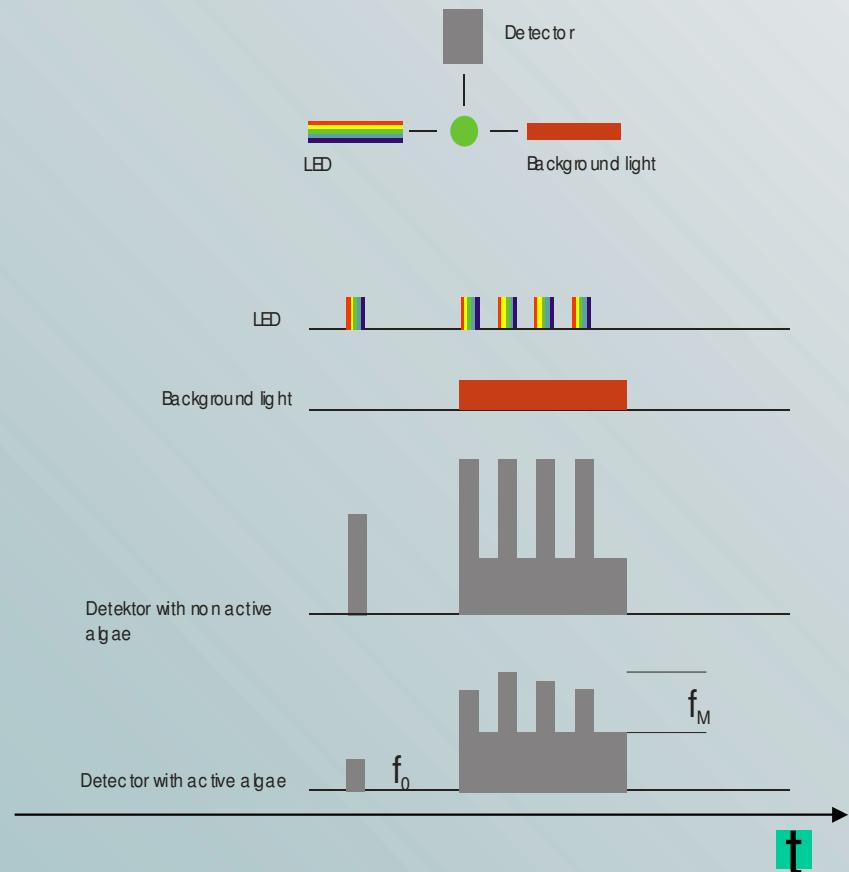
The deviation of the slopes is less than 10% at 16°C temperature difference, and therefore within the deviation limits for repetitions at 20°C



PAM AlgaeLabAnalayser

- Measurement of the chlorophyll concentration by chlorophyll fluorescence
- Algae class differentiation
- Weak relation to cell numbers
- Genty





Theoretically: active algae = $f(\text{chl}) \times \text{Genty}$

Principle of the Genty-Measurement to Detect the Activity of the Algae

$$\text{Genty} = 100 * \frac{f_m - f_o}{f_m} [\%]$$

Non-Active Algae – fluorescence response independent from the background light, the same proportion of the excitation energy decays in form of fluorescence

Active Algae - energy distributed in chemical reaction, thermal loss and fluorescence



UV Treatment: How much Energy is Needed

| Day 1 treatment | Chlorophyll ALA | | | |
|-------------------|--------------------|------------------------|----------------------------|--|
| | Sample/ Replica | Tetraselmis suicica | Microcystis aeroguinosa | Thalassiosir a sp./ Peridinium sp |
| | µg/L | µg/L | µg/L | µg/L |
| 0% Dosis | | | | |
| 1. Replica | 1,54 | 0,23 | 2,30 | 4,07 |
| 2. Replica | 1,67 | 0,21 | 2,38 | 4,26 |
| 3. Replica | 1,75 | 0,23 | 2,47 | 4,45 |
| 50% Dosis | | | | |
| 1. Replica | 1,99 | 0,25 | 1,74 | 3,98 |
| 2. Replica | 2,16 | 0,22 | 1,61 | 3,99 |
| 3. Replica | 2,22 | 0,32 | 1,70 | 4,24 |
| 100% Dosis | | | | |
| 1. Replica | 2,05 | 0,23 | 1,09 | 3,37 |
| 2. Replica | 2,25 | 0,30 | 1,50 | 4,05 |
| 3. Replica | 2,19 | 0,27 | 1,57 | 4,03 |
| 150% Dosis | | | | |
| 1. Replica | 1,48 | 0,00 | 0,82 | 2,30 |
| 2. Replica | 1,84 | 0,00 | 1,21 | 3,05 |
| 3. Replica | 1,84 | 0,00 | 1,39 | 3,23 |
| 200% Dosis | | | | |
| 1. Replica | 1,58 | 0,00 | 0,94 | 2,52 |
| 2. Replica | 1,63 | 0,00 | 0,99 | 2,62 |
| 3. Replica | 1,40 | 0,00 | 0,82 | 2,22 |
| 400% Dosis | | | | |
| 1. Replica | 0,77 | 0,00 | 0,44 | 1,21 |
| 2. Replica | 0,91 | 0,00 | 0,35 | 1,26 |
| 3. Replica | 0,85 | 0,00 | 0,57 | 1,42 |

| Day 5 darkness | Chlorophyll ALA | | | |
|-------------------|--------------------|------------------------|----------------------------|--|
| | Sample/ Replica | Tetraselmis suicica | Microcystis aeroguinosa | Thalassiosir a sp./ Peridinium sp |
| | µg/L | µg/L | µg/L | µg/L |
| 0% Dosis | | | | |
| 1. Replica | 0,66 | 0,09 | 1,36 | 2,11 |
| 2. Replica | 1,6 | 0,25 | 1,98 | 3,84 |
| 3. Replica | 1,69 | 0,23 | 2,04 | 3,97 |
| 50% Dosis | | | | |
| 1. Replica | 1,03 | 0,28 | 1,25 | 2,55 |
| 2. Replica | 0,66 | 0,15 | 0,97 | 1,78 |
| 3. Replica | 1,24 | 0,29 | 1,22 | 2,75 |
| 100% Dosis | | | | |
| 1. Replica | 1,1 | 0,16 | 1,11 | 2,37 |
| 2. Replica | 1,14 | 0,26 | 1,24 | 2,64 |
| 3. Replica | 1,16 | 0,24 | 1,18 | 2,58 |
| 150% Dosis | | | | |
| 1. Replica | 0,88 | 0,08 | 0,96 | 1,92 |
| 2. Replica | 1,05 | 0,16 | 1,04 | 2,25 |
| 3. Replica | 1,13 | 0,22 | 1,19 | 2,54 |
| 200% Dosis | | | | |
| 1. Replica | 0,77 | 0,13 | 0,89 | 1,78 |
| 2. Replica | 0,79 | 0,14 | 0,84 | 1,77 |
| 3. Replica | 0,68 | 0,25 | 0,66 | 1,59 |
| 400% Dosis | | | | |
| 1. Replica | 0,35 | 0,00 | 0,32 | 0,67 |
| 2. Replica | 0,56 | 0,00 | 0,12 | 0,68 |
| 3. Replica | 0,25 | 0,00 | 0,21 | 0,46 |

| 9mJ/cm² | | | | |
|---------------------------|------------------------|----------------------------|--|-------|
| 200 J/Liter = 100% | | | | |
| Resolution 0,1µg/l | | | | |
| =50cells Tetras. | | | | |
| =400cells Talassio | | | | |
| Day 20 growth | | | | |
| Sample/ Replica | Tetraselmis suicica | Microcystis aeroguinosa | Thalassiosir a sp./ Peridinium sp | |
| | µg/L | µg/L | µg/L | |
| 0% Dosis | | | | |
| 1. Replica | 46,72 | 2,66 | 0 | 49,39 |
| 2. Replica | 31,06 | 3,28 | 0 | 34,35 |
| 3. Replica | 33,92 | 3,27 | 0 | 37,19 |
| 50% Dosis | | | | |
| 1. Replica | 1,51 | 0,03 | 0 | 1,55 |
| 2. Replica | 1,66 | 0,04 | 0 | 1,7 |
| 3. Replica | 0,07 | 0,01 | 0 | 0,08 |
| 100% Dosis | | | | |
| 1. Replica | 0,07 | 0 | 0 | 0,07 |
| 2. Replica | 0 | 0 | 0 | 0 |
| 3. Replica | 0,02 | 0,01 | 0 | 0,02 |
| 150% Dosis | | | | |
| 1. Replica | 0 | 0 | 0 | 0 |
| 2. Replica | 0,25 | 0 | 0 | 0,25 |
| 3. Replica | 1,21 | 0,01 | 0 | 1,23 |
| 200% Dosis | | | | |
| 1. Replica | 0 | 0 | 0 | 0 |
| 2. Replica | 0 | 0 | 0 | 0 |
| 3. Replica | 0,1 | 0,03 | 0 | 0,13 |
| 400% Dosis | | | | |
| 1. Replica | 0,00 | 0,00 | 0,00 | 0,00 |
| 2. Replica | 0,00 | 0,00 | 0,00 | 0,00 |
| 3. Replica | 0,00 | 0,00 | 0,00 | 0,00 |



| Day 1 | Chlorophyll ALA | Aktivität ALA | 10cells |
|--------------------|--------------------|---------------|----------|
| Sample/ Replica | Total Chl | PAM Aktiv. | 10cells |
| | µg/L | % | cells/ml |
| 0% Dosis | | | |
| 1. Replica | 4,07 | 51,81 | 1466 |
| 2. Replica | 4,26 | 52,01 | 1796 |
| 3. Replica | 4,45 | 53,7 | 2098 |
| 50% Dosis | | | |
| 1. Replica | 3,98 | 28,37 | 525 |
| 2. Replica | 3,99 | 33,96 | 537 |
| 3. Replica | 4,24 | 26,16 | 401 |
| 100% Dosis | | | |
| 1. Replica | 3,37 | 31,05 | 236 |
| 2. Replica | 4,05 | 28,21 | 427 |
| 3. Replica | 4,03 | 31,17 | 484 |

| Day 1 | Chlorophyll ALA | Aktivität ALA | 10cells |
|--------------------|--------------------|---------------|----------|
| Sample/ Replica | Total Chl | PAM Aktiv. | 10cells |
| | µg/L | % | cells/ml |
| 150% Dosis | | | |
| 1. Replica | 2,30 | 17,13 | 183 |
| 2. Replica | 3,05 | 23,89 | 294 |
| 3. Replica | 3,23 | 24,59 | 537 |
| 200% Dosis | | | |
| 1. Replica | 2,52 | 21,13 | 208 |
| 2. Replica | 2,62 | 14,14 | 198 |
| 3. Replica | 2,22 | 13,33 | 143 |
| 400% Dosis | | | |
| 1. Replica | 1,21 | 8,11 | 34 |
| 2. Replica | 1,26 | 6,41 | 25 |
| 3. Replica | 1,42 | 10,56 | 37 |



Effects on UV-treated Ballast Water

| Day 5 darkness | Chlorophyll ALA | Aktivität ALA | 10cells | Day 5 | Chlorophyll ALA | Aktivität ALA | 10cells |
|--------------------|--------------------|---------------|----------|--------------------|--------------------|---------------|----------|
| Sample/ Replica | Total Chl | PAM Aktiv. | 10cells | Sample/ Replica | Total Chl | PAM Aktiv. | 10cells |
| | µg/L | % | cells/ml | | µg/L | % | cells/ml |
| 0% Dosis | | | | | | | |
| 1. Replica | 2,11 | 52,48 | 710 | 1. Replica | 1,92 | 33,1 | 18 |
| 2. Replica | 3,84 | 45,13 | 568 | 2. Replica | 2,25 | 44,48 | 62 |
| 3. Replica | 3,97 | 43,89 | 759 | 3. Replica | 2,54 | 46,6 | 82 |
| 50% Dosis | | | | | | | |
| 1. Replica | 2,55 | 27,89 | 96 | 1. Replica | 1,78 | 29,15 | 14 |
| 2. Replica | 1,78 | 41,23 | 36 | 2. Replica | 1,77 | 27,31 | 25 |
| 3. Replica | 2,75 | 24,94 | 105 | 3. Replica | 1,59 | 44,09 | 42 |
| 100% Dosis | | | | | | | |
| 1. Replica | 2,37 | 31,14 | 36 | 1. Replica | 0,67 | 6,12 | 7 |
| 2. Replica | 2,64 | 28,52 | 75 | 2. Replica | 0,68 | 4,25 | 5 |
| 3. Replica | 2,58 | 35,06 | 200 | 3. Replica | 0,46 | 7,58 | 9 |



Effects on UV-treated Ballast Water

| Day 20 under light | Chlorophyll ALA | Aktivität ALA | 10cells | |
|-----------------------|--------------------|---------------|----------|--|
| Sample/ Replica | Total Chl | PAM Aktiv. | 10cells | |
| | µg/L | % | cells/ml | |
| 0% Dosis | | | | |
| 1. Replica | 49,39 | 18,32 | 2201 | |
| 2. Replica | 34,35 | 13,03 | 1960 | |
| 3. Replica | 37,19 | 16,97 | 1752 | |
| 50% Dosis | | | | |
| 1. Replica | 1,55 | 6,43 | 54 | |
| 2. Replica | 1,7 | 2,96 | 51 | |
| 3. Replica | 0,08 | -- | 4 | |
| 100% Dosis | | | | |
| 1. Replica | 0,07 | -- | 2 | |
| 2. Replica | 0 | -- | 1 | |
| 3. Replica | 0,02 | -- | 2 | |

| Day 20 | Chlorophyll ALA | Aktivität ALA | 10cells | |
|--------------------|--------------------|----------------------|----------|--|
| Sample/ Replica | Total Chl | PAM Aktivit ät | 10cells | |
| | µg/L | % | cells/ml | |
| 150% Dosis | | | | |
| 1. Replica | 0 | -- | 1 | |
| 2. Replica | 0,25 | 6,72 | 0 | |
| 3. Replica | 1,23 | 32,03 | 18 | |
| 200% Dosis | | | | |
| 1. Replica | 0 | -- | 0 | |
| 2. Replica | 0 | -- | 0 | |
| 3. Replica | 0,13 | 0 | 6 | |
| 400% Dosis | | | | |
| 1. Replica | 0,00 | -- | 0 | |
| 2. Replica | 0,00 | -- | 1 | |
| 3. Replica | 0,00 | -- | 0 | |



Advantages and Disadvantages of Indirect Methods of Algae Counting in the Range of 10-50µm – Personal Opinion

| | 10cells | PAM | ATP | FDA |
|--|---------|-----|-----|-----|
| Cell counting | - | - | - | - |
| Resolution | ++ | - | o | o |
| Time needed | ++ | ++ | o | ? |
| Costs | o | o | o | o |
| Use of chemicals | ++ | ++ | o | - |
| Simple measurement, no. of steps needed | ++ | ++ | - | - |
| Transportable | ++ | ++ | ++ | ++ |
| Affected by temperature | ++ | ++ | - | - |



We have found an extremely suitable method to be able to measure ballast water concentrations (algae) in the range of 10µm-50µm upto far below 10 cells/ml.

This idea has now been implemented by the new I0cells instrument.

Many thanks for your attention!