## 3.3. Nitrification inhibition test

Significant inhibition of the nitrification process was demonstrated at a test substance concentration of 1000 mg/l and higher (Table 3.3 and Figure 3.2). Adjustment of pH was carried out at the test substance-substrate mixtures of 3200 and 10,000 mg/l. After adjustment of the pH, a change in color was visible. Since inhibition of nitrification was also apparent at lower concentrations (no pH adjustment), it can be stated that the inhibitory effects were not caused by the change in the pH.

The $EC_{50}$ of Citrex liquid is determined at 961 mg/l.

Table 3.3 Results of the nitrification inhibition test with Citrex liquid.

| | Control | 32 mg/l | 100 mg/l | 320 mg/l | 1000 mg/l | 3200 mg/l | 10000 mg/l |
|---|---|---|---|---|---|---|---|
| Δ $NH_4$-N (mg/l) | 42.6 | 42.9 | 42.4 | 41.7 | 18.4 | 2.9 | 0 |
| Inhibition (%) | | -0.5 | 0.7 | 2.3 | 56.9 | 93.2 | 100 |

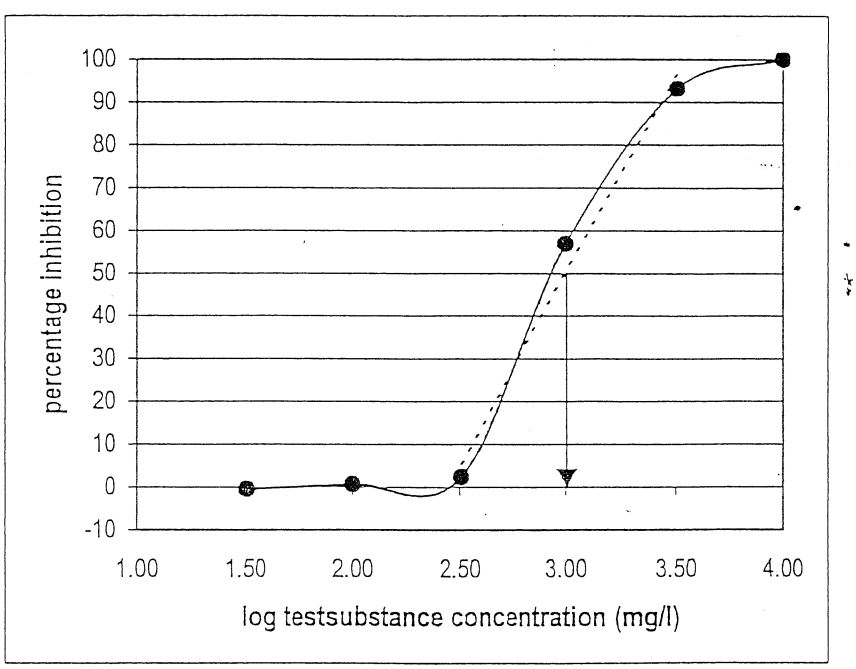



Figure 3.2 Nitrification inhibition curve of Citrex liquid.

# 4. Literature

*Postma, J.F., Valk de S., Dubbeldam, M., Maas. J.L., Tonkes, M., Kater, B.J.* (2001). Confounding factors in bioassays with freshwater and marine organisms. In preparation.

*Tidepool* (1995). ToxCalc user's guide. Comprehensive toxicity data analysis and database software. Version 5.0 for Microsoft Excel under Windows 3.1 or Apple Macintosh. Tidepool Scientific Software.

*NEN-ISO 8692*. Water quality- Fresh water algal growth inhibition test with *Scenedesmus subspicatus* and *Selenastrum capricornutum.*

*NEN-EN-ISO 8192*. Water quality. Test for the inhibition of oxygen consumption by activated sludge.

*NEN-EN-ISO 9509*. Water quality. Method for assessing the inhibition of nitrification of activated sludge micro-organisms by chemicals and waste waters.

# Annex 1 raw data of algae toxicity test

## Algen reproductie test-(Groeisnelheid (µ))

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 06-Nov-01 | Test ID: | 323666GD | Sample ID: | 1909 |
| End Date: | 09-Nov-01 | Lab ID: | AQUASENSE_-Amsterdam | Sample Type: | AFW-Afvalwater |
| Sample Date: | | Protocol: | - | Test Species: | RS-Raphidocelis subcapitata |
| Comments: | | | | | |

| Conc-ug/L | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| B-Control | 0.0591 | 0.0657 | 0.0651 | 0.0608 | 0.0603 | 0.0635 |
| 0.1 | 0.0566 | 0.0620 | 0.0626 | | | |
| 0.32 | 0.0617 | 0.0592 | 0.0584 | | | |
| 1 | 0.0592 | 0.0601 | 0.0601 | | | |
| 3.2 | 0.0616 | 0.0597 | 0.0618 | | | |
| 10 | 0.0609 | 0.0585 | 0.0638 | | | |
| 32 | 0.0489 | 0.0551 | 0.0593 | | | |

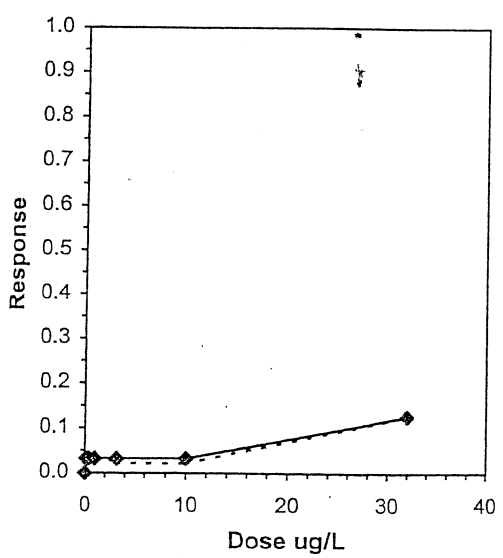
| | | | Transform: Untransformed | | | | | 1-Tailed | | | Isotonic | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-ug/L | Mean | N-Mean | Mean | Min | Max | CV% | N | t-Stat | Critical | MSD | Mean | N-Mean |
| B-Control | 0.0624 | 1.0000 | 0.0624 | 0.0591 | 0.0657 | 4.366 | 6 | | | | 0.0624 | 1.0000 |
| 0.1 | 0.0604 | 0.9675 | 0.0604 | 0.0566 | 0.0626 | 5.510 | 3 | 1.012 | 2.655 | 0.0053 | 0.0604 | 0.9679 |
| 0.32 | 0.0598 | 0.9578 | 0.0598 | 0.0584 | 0.0617 | 2.917 | 3 | 1.313 | 2.655 | 0.0053 | 0.0604 | 0.9679 |
| 1 | 0.0598 | 0.9577 | 0.0598 | 0.0592 | 0.0601 | 0.857 | 3 | 1.318 | 2.655 | 0.0053 | 0.0604 | 0.9679 |
| 3.2 | 0.0610 | 0.9778 | 0.0610 | 0.0597 | 0.0618 | 1.898 | 3 | 0.691 | 2.655 | 0.0053 | 0.0604 | 0.9679 |
| 10 | 0.0611 | 0.9787 | 0.0611 | 0.0585 | 0.0638 | 4.335 | 3 | 0.664 | 2.655 | 0.0053 | 0.0604 | 0.9679 |
| *32 | 0.0545 | 0.8725 | 0.0545 | 0.0489 | 0.0593 | 9.543 | 3 | 3.969 | 2.655 | 0.0053 | 0.0545 | 0.8725 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates normal distribution (p > 0.01) | 0.99161 | 0.884 | -0.2688 | -0.0105 |
| Bartlett's Test indicates equal variances (p = 0.20) | 8.48734 | 16.8119 | | |

| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU | MSDu | MSDp | MSB | MSE | F-Prob | df |
|---|---|---|---|---|---|---|---|---|---|---|
| Bonferroni t Test | 10 | 32 | 17.8885 | | 0.00532 | 0.08526 | 2.2E-05 | 8E-06 | 0.04547 | 6, 17 |

### Linear Interpolation (200 Resamples)

| Point | ug/L | SD | 95% CL(Exp) | Skew |
|---|---|---|---|---|
| IC05 | 14.129 | | | |
| IC10 | 25.666 | | | |
| IC15 | >32 | | | |
| IC20 | >32 | | | |
| IC25 | >32 | | | |
| IC40 | >32 | | | |
| IC50 | >32 | | | |



## Algen reproductie test-(Groeicurve opp. (A))

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 06-Nov-01 | Test ID: | 323666GD | Sample ID: | 1909 |
| End Date: | 09-Nov-01 | Lab ID: | AQUASENSE_-Amsterdam | Sample Type: | AFW-Afvalwater |
| Sample Date: | | Protocol: | - | Test Species: | RS-Raphidocelis subcapitata |
| Comments: | | | | | |

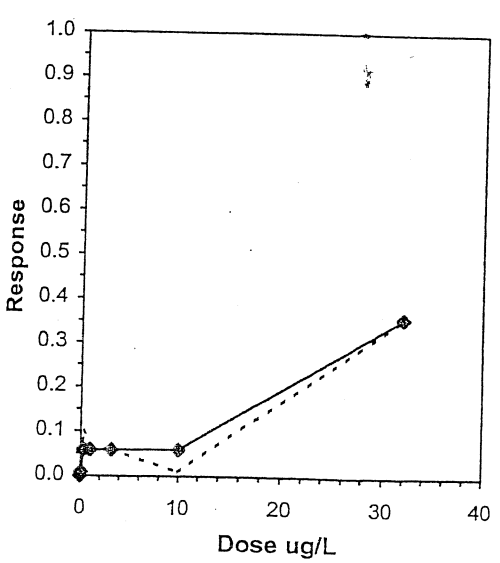
| Conc-ug/L | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| B-Control | 1.2E+07 | 1.4E+07 | 1.4E+07 | 1.1E+07 | 1.2E+07 | 1.3E+07 |
| 0.1 | 1.2E+07 | 1.3E+07 | 1.2E+07 | | | |
| 0.32 | 1.3E+07 | 1.1E+07 | 9583169 | | | |
| 1 | 1.2E+07 | 1.1E+07 | 1.2E+07 | | | |
| 3.2 | 1.3E+07 | 1.1E+07 | 1.2E+07 | | | |
| 10 | 1.2E+07 | 1.1E+07 | 1.4E+07 | | | |
| 32 | 5182241 | 8014229 | 1.1E+07 | | | |

| | | | Transform: Untransformed | | | | | 1-Tailed | | | Isotonic | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-ug/L | Mean | N-Mean | Mean | Min | Max | CV% | N | t-Stat | Critical | MSD | Mean | N-Mean |
| B-Control | 1.3E+07 | 1.0000 | 1.3E+07 | 1.1E+07 | 1.4E+07 | 8.885 | 6 | | | | 1.3E+07 | 1.0000 |
| 0.1 | 1.2E+07 | 0.9911 | 1.2E+07 | 1.2E+07 | 1.3E+07 | 3.282 | 3 | 0.107 | 2.655 | 2759750 | 1.2E+07 | 0.9911 |
| 0.32 | 1.1E+07 | 0.8988 | 1.1E+07 | 9583169 | 1.3E+07 | 15.438 | 3 | 1.219 | 2.655 | 2759750 | 1.2E+07 | 0.9415 |
| 1 | 1.2E+07 | 0.9353 | 1.2E+07 | 1.1E+07 | 1.2E+07 | 4.125 | 3 | 0.780 | 2.655 | 2759750 | 1.2E+07 | 0.9415 |
| 3.2 | 1.2E+07 | 0.9415 | 1.2E+07 | 1.1E+07 | 1.3E+07 | 9.101 | 3 | 0.705 | 2.655 | 2759750 | 1.2E+07 | 0.9415 |
| 10 | 1.2E+07 | 0.9905 | 1.2E+07 | 1.1E+07 | 1.4E+07 | 12.358 | 3 | 0.115 | 2.655 | 2759750 | 1.2E+07 | 0.9415 |
| *32 | 8051981 | 0.6427 | 8051981 | 5182241 | 1.1E+07 | 35.877 | 3 | 4.306 | 2.655 | 2759750 | 8051981 | 0.6427 |

| Auxiliary Tests | | | | | Statistic | | Critical | | Skew | Kurt |
|---|---|---|---|---|---|---|---|---|---|---|
| Shapiro-Wilk's Test indicates normal distribution (p > 0.01) | | | | | 0.98136 | | 0.884 | | 0.01373 | 0.61593 |
| Bartlett's Test indicates equal variances (p = 0.20) | | | | | 8.58164 | | 16.8119 | | | |
| **Hypothesis Test (1-tail, 0.05)** | **NOEC** | **LOEC** | **ChV** | **TU** | **MSDu** | **MSDp** | **MSB** | **MSE** | **F-Prob** | **df** |
| Bonferroni t Test | 10 | 32 | 17.8885 | | 2759750 | 0.22029 | 7.9E+12 | 2.2E+12 | 0.01626 | 6, 17 |

### Linear Interpolation (200 Resamples)

| Point | ug/L | SD | 95% CL(Exp) | | Skew |
|---|---|---|---|---|---|
| IC05 | 0.282 | 5.345 | 0.000 | 21.082 | 0.6552 |
| IC10 | 13.057 | | | | |
| IC15 | 16.739 | | | | |
| IC20 | 20.420 | | | | |
| IC25 | 24.102 | | | | |
| IC40 | >32 | | | | |
| IC50 | >32 | | | | |



# Annex 2 raw data of respiration inhibition test

| | | | |
|---|---|---|---|
| EcoLIMSnr | 323666 | Test temperature | 23 |
| Project code | 1909 | Source activated sludge | DWR RWZI Oost Amsterdam |
| Standard | NEN-EN-ISO 8192 | Sampling date | 2001-10-17 |
| Type material | chemical substance | Dry weight sludge (g/l) | 5.7 |
| Date of performance | 2001-10-17 | | |

measured data

| | control | 1 mg/l | 10 mg/l | 100 mg/l | 1000 mg/l | 3200 mg/l | 10000 mg/l |
|---|---|---|---|---|---|---|---|
| Δ T (min) | 2.5 | 3 | 3 | 2.5 | 4 | 8 | 10 |
| Δ $O_2$ (mg/l) | 3.88 | 4.71 | 4.81 | 4.17 | 5.75 | 5.97 | 2.30 |
| Oxygen consumption rate ($mg\ O_2.l^{-1}.h^{-1}$) | 93.12 | 94.20 | 96.20 | 100.08 | 86.25 | 44.78 | 13.80 |
| Inhibition (%) | | -1.2 | -3.3 | -7.5 | 7.4 | 52 | 85 |

# Annex 3 raw data of nitrification inhibition test

| | | | |
|---|---|---|---|
| EcoLIMSnr | 323666 | Test temperature | 23 |
| Project code | 1909 | Source activated sludge | DWR RWZI Oost Amsterdam |
| Standard | NEN-EN-ISO 9509 | Sampling date | 2001-10-17 |
| Type material | chemical substance | Dry weight sludge (g/l) | 5.6 |
| Date of performance | 2001-10-18 | | |

measured data

| | Control | 32 mg/l | 100 mg/l | 320 mg/l | 1000 mg/l | 3200 mg/l | 10000 mg/l |
|---|---|---|---|---|---|---|---|
| $NH_4$-N (mg/l T=0 | 53.1 | 53.1 | | | | | 53.1 |
| $NH_4$-N (mg/l T=4 | 10.5 | 10.2 | 10.7 | 11.4 | 24.7 | 50.2 | 57.9 |
| Δ $NH_4$-N (mg/l) | 42.6 | 42.9 | 42.4 | 41.7 | 18.4 | 2.9 | 0 |
| Inhibition (%) | | -0.5 | 0.7 | 2.3 | 56.9 | 93.2 | 100 |