Evaluation of the *Vibrio fisherii* Luminescence Inhibition Test with Colour Correction to detect toxicity of dyes

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Introdution:

The *Vibrio fisherii* Luminescence Inhibition Test (DIN EN ISO 11348-3, 1998) is a well established, fast and easy assay to detect toxicity in waste water samples and water soluble substances. It is based on the fact that toxins weaken the normal luminosity of luminescent bacteria. The decrease in the amount of light emitted is proportional to the concentration of toxins and is measured with a luminometer. If a sample contains colourants false data can be obtained because of the absorption of the light emitted by the bacteria. In recent years luminescence measurement units (e.g. LUMISTox) became available which measure these light absorbance of coloured samples before measuring the light emitted by the bacteria. These units calculate the corrected light emission value in one single analysis.
During the first project phase it was checked whether the luminescence inhibition test with colour correction is a suitable tool for the fast toxicity screening of waste water samples containing dyes to control the success of treatment processes and of the toxicity of the dyes themselves.

Material and Method:

The investigations were performed with a LUMISTox 300 unit (provided by Hach Lange GmbH, Düsseldorf, Germany).

One limitation of the method is that dark coloured samples with optic densities above 1.8 at 490 nm (the maximum emission of light from the bacteria) can not be measured due to physical reasons. For this in the first step the maximum concentration of the model dyes use for the following investigations had to be determined which cause a maximum OD of 1.8.

Then the **limitations of the method** were checked. The standard positive substance $K_2Cr_2O_7$ was tested in several concentrations between 0.8 and 15 mg/l.Subsequent the same $K_2Cr_2O_7$ concentrations were supplemented with Direct Red R in a concentration of 40 mg/l and Reactive Black 5 in a concentration of 170 mg/l (which are the highest testable concentrations) and tested again.

In the second step the **toxicity of the dyes** were checked at the highest testable concentration (OD ≤ 1.8).

The third step was to **determine EC_{10} and EC_{50}** data of the model dyes which have turned out toxic before. For this concentration-response curves generated and the ECx values with the confidence limit were calculated using the statistical software ToxRat.

Results

Figure 1 shows the results of the concentration-response curves of $K_2Cr_2O_7$ and the calculated ECx. The two independent replicates are almost identical. The addition of Direct Red R in the highest testable concentration of 40 mg/l does not effect the results. By the addition of Reactive Black 5 in the highest testable concentration of 170 mg/l toxicity decreases, wich seems to be associated with the oxidation of the dye by $K_2Cr_2O_7$ which results in a decrease of $K_2Cr_2O_7$.

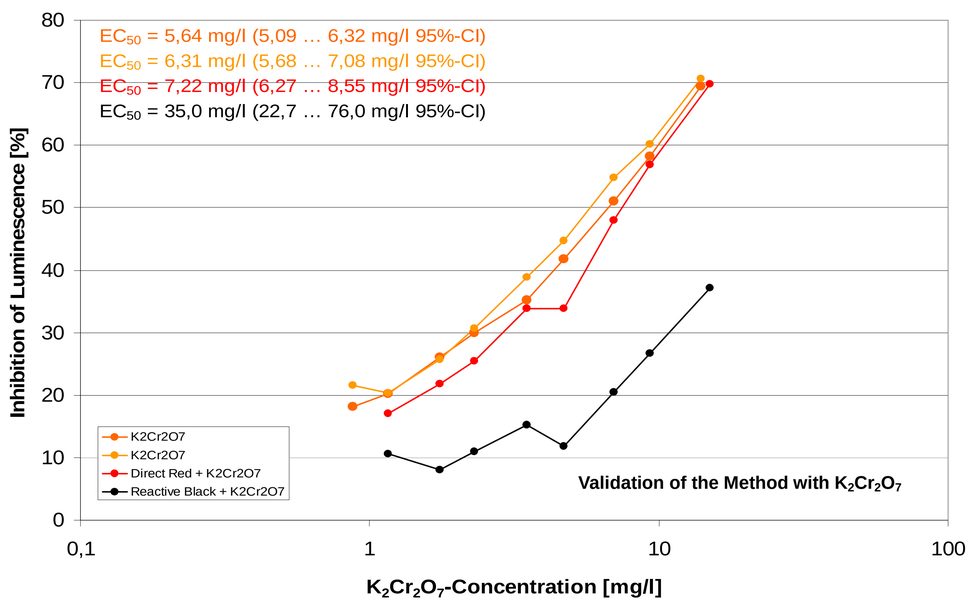


Figure 1: Concentration-response curves of $K_2Cr_2O_7$ and $K_2Cr_2O_7$ + dyes and the resulting EC_{50} values

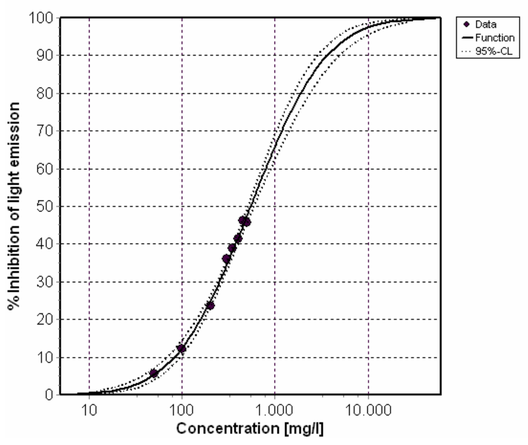
Table 1 shows the highest testable concentrations of 12 model dye substances. Higher concentrations cannot be tested due to the restriction that the OD has to be ≤ 1.8. This means that dark coloured samples can only be tested in high dilutions and resulting low concentration.
The inhibition of the light emission at the highest testable concentration is also presented. Only two out of the 12 dyes show no toxicity (less than 20% inhibition) in the test. This might be due to high dilution or low toxicity which cannot be evaluated with the test system. Six dyes show an inhibition between 20 and 50% and four dyes are quite toxic with inhibitions above 50%. This means that for 10 dyes out of the 12 model substances and concentrations below 1 g/l the success of waste water treatment processes concerning a reduction or elevations in toxicity can be evaluated with this test system.

Sample Name	Concentration of Test Solution [mg/l]	E	Inhibition of Luminescence [%]
Disperse Red 1	limited solubility saturated solution	0,023	61,3
Direct Red R	40	1794	3,4
Reactive Red 4	251,5	1526	25,9
Acid Red 299	limited solubility 504	1861	50,19
Disperse Blue 1	1106	1867	66,1
Direct Blue 1	145	1626	31,4
Reactive Blue 19	1002	1865	32,1
Acid Blue 62	680	1895	57,5
Direct Black 38	160	1594	20,6
Reactive Black 5	170	1862	9,2
Disperse Yellow 3	260	1800	48,2
Reactive Yellow 81	limited solubility 801	1794	24,5

E: Optical Density (extinction)
no toxicity detectable weak toxicity detectable toxic

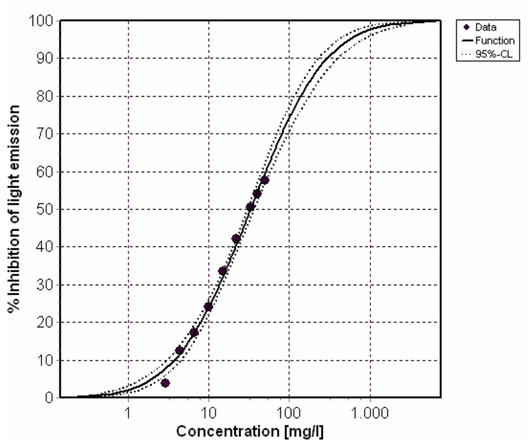
Table 1: Inhibition of luminescence [%] at the higest testable concentration (OD ≤ 1.8)

Concentration-Response Relationship for 4 dyes:
The figures 2 - 4 show the concentration-response relationship of 3 dyes which have shown high toxicity with the highest testable concentration. The EC10, EC20 and EC50 values with the confidence limits are presented. To evaluate the success of a waste water treatment we recomend to use the EC_{10} value. If there is a significant reduction of the EC_{10}, which can be used as a now effect concentration (NOEC), a significant detoxification of the sample can be stated.



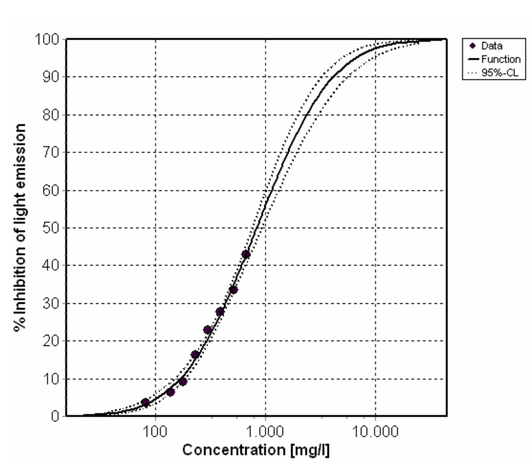
Acid Red 299 (NY1)
CAS-No.: 57741-47-6
stock solution: 1006 mg/l
highest concentration tested: 504 mg/l

EC10: 83.2 mg/l (69.2 … 96.5 mg/l 95%-CI)
EC20: 159.2 mg/l (142.9 … 174.1 mg/l 95%-CI)
EC50: 547.6 mg/l (511.7 … 593.2 mg/l 95%-CI)
(probit analysis using linear max. Likelihood reg-



Disperse Blue 1
CAS-No.: 2475-45-8
stock solution: 2212 mg/l
highest concentration tested: 1106 mg/l

EC10: 3.7 mg/l (3.0 … 4.4 mg/l 95%-CI)
EC20: 7.9 mg/l (6.9 … 8.9 mg/l 95%-CI)
EC50: 33.1 mg/l (30.4 … 36.4 mg/l 95%-CI)
(probit analysis using linear max. Likelihood regres-



Acid Blue 62
CAS-No.: 4368-56-3
stock solution: 1336 mg/l
highest concentration tested: 666 mg/l

EC10: 169.7 mg/l (150.1 … 187.7 mg/l 95%-CI)
EC20: 294.1 mg/l (274.9 … 312.1 mg/l 95%-CI)
EC50: 837.1 mg/l (765.3 … 933.3 mg/l 95%-CI)
(probit analysis using linear max. Likelihood reg-

References

DIN EN ISO 11348-3 Water Quality—Determination of the inhibitory effect of water samples on the light emission of *Vibrio fisherii* (Luminescent bacteria test) - Part 3: Method using freeze-dried bacteria (April 1999).

Dr. Lange Info No. 13: Corrcting absorptive inhibition in the luminescent bacteria test by means of a combined luminometric/photometric procedure (January 1998).

Klein, B. (1990): Möglichkeiten und Grenzen der Farbkorrektur im Leuchtbakterientest mit Hilfe von Absorptions-Korrektur-Küvetten. Z. Wasser-Abwasser-Forsch. 23, 70-74.