

Observation Parameters in the Duckweed Growth Inhibition Test

Matthias Eberius, LemnaTec GmbH, Schumanstrasse 18, 52146 Würselen *

14. März 2001

The Colour of duckweed

The colour of the plant, in this case duckweed, is as important for the assessment of plant vitality as the number and area of fronds and their habitus. Often chlorotic and necrotic areas but also fronds coloured in a lighter green occur in biotesting besides reduction of fronds area and number. This shows that substances have a direct or indirect negative impact on the photosystem. But even the intensification of the green colour which occurs with low concentrations of triazines can indicate that the plant tries to compensate a toxic impact on its photosystem. This gradual classification of vitality by colour in biotests can be done in three ways if no image-analysis system is used.

Measurement of extracted chlorophyll

At the end of the test the duckweed has to be crushed in hot ethanol and extracted overnight. The next day chlorophyll will be quantified photometrically. This analysis needs additional work but if all conditions are held exactly, the results lead to a very sensible and reproducible toxic endpoint. As the measurement is destructive and no aliquots can be made no curves of growth are attainable. Only in combination with total frond area, deepened colour can be distinguished from a higher amount of biomass.

Manual report

According to test guidelines all changes in the test organisms should be noticed at any measurement of the parameters. This kind of qualitative description has a highly subjective part and significantly rises the time needed. But nevertheless, these data are not adapted in toxicological endpoints. Furthermore, later reanalysis or reconstruction of the real test event remains difficult, because of subjectively classification of fronds.

Visual frond classification

To avoid the equal assessment of healthy, damaged and dead fronds, ASTM and OPPTS-guidelines have decided that only healthy fronds with less than 50 percent chlorosis must be counted as living. This method enhances the counting method but on the other hand it results in another increase of time and a significant amount of subjective influences.

What the LemnaTec Scanalyser can achieve by image-analysis of colour

Image-analysis systems are by far superior to human eyes if reproducible detection and quantitative classification of colours is necessary. For this reason it is very important to transfer the colour information of every image pixel into ecotoxicological relevant information.

Distribution of colours of total frond area

To assess the vitality of the fronds the pixels are divided into colour-classes corresponding for example to healthy (green), lightly damaged (pale green), chlorotic (yellow) and necrotic (grey or brown) frond areas. Without additional work this method leads to an objective integrative assessment of chlorotic and necrotic structures in relation to the influence of the testing material (Fig.1).

* Tel.: +49(0)2405-412615, eberius@lemnatec.de

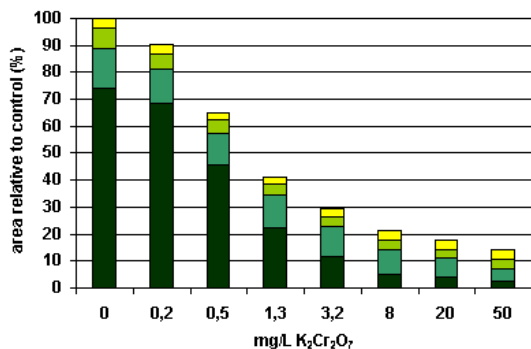


Fig. 1: Change in the areas of different colour classes with rising concentration of potassium dichromate.

Colour classes and single fronds

The LemnaTec Scanalyser is able to separate single fronds. The colour classes can be analogously determined for every single frond. This vast amount of information can be handled if the colour classification is combined with the distribution of frond area. The result is an objective and evident information if small or fully grown fronds are primarily damaged. Final reports can therefore be made on a highly and well documented basis. Fig. 2 shows the different steps from the original image to the frond area distribution combined with colour class information. Especially younger fronds and very large fronds are damaged by chlorosis and necrosis.

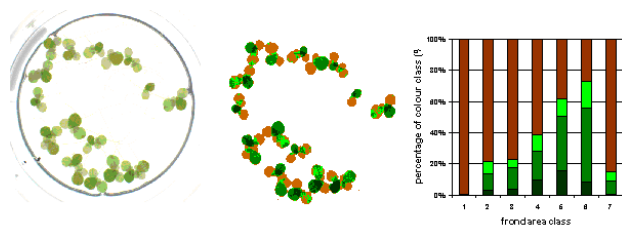


Fig. 2: Original image, colour class image and frond area distribution combined with colour classes. Brown colour represents chlorosis and necrosis.

Frond classification

Following the ASTM guideline image-analysis fully supports the classification of fronds in living and dead. For

this purpose only the percentage of colour classes has to be fixed according to the guideline. Without additional work the fronds can be classified in living and dead using data for the calculation of growth inhibition or rates of death.

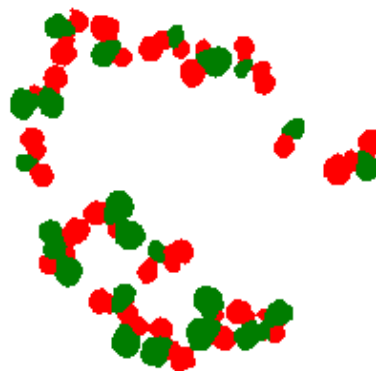


Fig. 3: Classification of fronds in living and dead (more than 50 percent chlorosis or necrosis)

Specific green-values

If colour-analysis is made in fine steps and the results are correlated with chlorophyll an image-analysis green-value can be assessed. This allows to use a value similar to chlorophyll contents without additional work and -more important- in a non destructive method. This makes it possible to use this sensitive endpoint in kinetic studies. Like chlorophyll, green-values integrate vitality in a single value.

Summary

Colour is an important parameter to assess vitality of duckweed in ecotoxicological test systems. The LemnaTec Scanalyser provides the unique possibility to use this colour information in compliance with international standards. Instructive colour quantification combined with analysis of frond area and frond number leads to comprehensive, objective and comfortable test documentation and toxicity assessment.