

DIN 38412-59:2022-12 (E)

German standard methods for the examination of water, waste water and sludge -
Test methods using water organisms (group L) - Part 59: Algal growth inhibition test
on microplate with unicellular green fresh water algae (L 59)

Contents	Page
Foreword	4
1 Scope	6
2 Normative references	6
3 Terms and definitions	6
4 Interferences	9
5 Principle	9
6 Designation	10
7 Reagents, media and dilutions	10
8 Test organism	14
9 Apparatus and materials	14
10 Sample preparation	16
11 Procedure	16
11.1 Preparation of 10-fold concentrated growth medium	16
11.2 Preparation of the growth medium for the preculture and for the inoculum	17
11.3 Preparation of the preculture and the inoculum	17
11.4 Preparation of test batch and control	18
11.5 Incubation	19
11.6 Measurements	19
12 Calculations	20
12.1 General	20
12.2 Calculation of growth rate and percentage inhibition	20
12.3 Calculation of the mean coefficient of variation of the section-by-section specific growth rate	22
12.4 Calculation of the coefficient of variation of the growth rates in the controls after 72 h	22
12.5 Determination of EC(r)x (e.g. EC(r)10 and EC(r)50)	23
12.6 Determination of the lowest ineffective dilution (LID)	23
13 Validity criteria	23
14 Expression of results	24
15 Test report	24
16 Performance data	25
Annex A (informative) Performance data	26

Annex B (informative) Sources of supply for the test organisms	29
Annex C (informative) Example of the calculation of growth rate and growth rate inhibition on a 24-well microplate and determination of validity criteria	31
C.1 Example data	31
C.2 Example for the calculation of the average specific growth rate 1 for the control replicates (A1 to A3) after 72 h (= 3 d) incubation [Equation (1)]	35
C.3 Example for the calculation of the average specific growth rate 1 for the positive control replicates (D1 to D3) after 72 h (= 3 d) incubation [Equation (1)]	35
C.4 Example for the calculation of the average specific growth rate 2 for the replicates of the test batches (B4 to B6) after 72 h (= 3 d) incubation [Equation (2)]	36
C.5 Example of the calculation for the percentage inhibition of the growth rate of the positive control after 72 h (= 3 d) incubation [Equation (3)]	37
C.6 Calculation of the percentage inhibition of the growth rate of the sample dilution SD 1 : 2 after 72 h (= 3 d) incubation [Equation (3)]	37
C.7 Determination of validity criteria (Clause 13)	37
Annex D (informative) Representation of growth curves	41
Annex E (informative) Preparation of the preculture	42
Bibliography	43
Tables Table 1 -- Concentration of nutrients in the stock solutions and in the test batch [1]	12
Table 2 -- Example for the preparation of blanks, controls and test batches on 24-well microplates	18
Table 3 -- Example for the arrangement of blanks, controls and test batches on a 24-well microplate	19
Table A.1 -- Results of the validation test for EC(r)50 and LID when using waste water samples as well as 3,5-dichlorophenol (3,5-DCP) and potassium dichromate (K₂Cr₂O₇) for <i>Desmodesmus subspicatus</i>	27
Table A.2 -- Results of the validation trial for EC(r)50 and LID when using waste water samples as well as 3,5-dichlorophenol (3,5-DCP) and potassium dichromate (K₂Cr₂O₇) for <i>Raphidocelis subcapitata</i>	28
Table C.1 -- Arrangement of blank, control and test batches on a 24-well microplate	31
Table C.2 -- Fluorescence units after 0 h, 24 h, 48 h and 72 h incubation	32
Table C.3 -- Average specific growth rate after 0 h, 24 h, 48 h and 72 h incubation	33
Table C.4 -- Inhibition of specific growth rate, in % at 0 h, 24 h, 48 h and 72 h incubation	34
Table C.5 -- Section-by-section specific growth rate 1 for control replicates A1 to A3 and section-by-section coefficient of variation CV %R and mean coefficient of variation CV %Rm	39
Table C.6 -- Coefficient of variation CV %72h of the growth rate in controls A1 to A3 after 72 h	39