
**Water quality — Determination of the
inhibitory effect of water constituents on
the growth of activated sludge
microorganisms**

*Qualité de l'eau — Détermination de l'effet inhibiteur des constituants de
l'eau sur la croissance des micro-organismes de boues activées*



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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

International Standard ISO 15522 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

Annexes A, B and C of this International Standard are for information only.

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Introduction

Information generated by this method may be helpful in estimating the effect of a test material on mixed bacterial communities in aerobic biological wastewater treatment systems and in choosing suitable initial concentrations for aerobic biodegradability tests

The results of this test should be considered only as a guide to the likely toxicity of the test material, since activated sludge from different sources, or even from the same source taken at different times, may differ in bacterial composition and concentration. Also, laboratory tests cannot truly simulate environmental conditions. For example, no account is taken of longer term adaptation of the microorganisms to the test material or of materials which may adsorb onto biofilm or activated sludge in subsequent wastewater treatment and build up to a toxic concentration over a longer period of time.

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Water quality — Determination of the inhibitory effect of water constituents on the growth of activated sludge microorganisms

WARNING — Take appropriate precautions when handling sewage, as it may contain potentially pathogenic organisms. Handle with care all toxic test materials or those whose properties are unknown.

1 Scope

This International Standard specifies a method for assessing the potential toxicity of a test material to the growth of aerobic bacteria present in activated sludge. The inhibitory effect is restricted to those microorganisms capable of growth on the chosen organic test medium.

This method gives information on inhibitory effects on the microorganisms over incubation periods up to 6 h.

This method is applicable to water, wastewater and chemical substances which are soluble under the conditions of the test. Special care is needed with volatile or coloured materials and materials which form turbid suspensions or dispersions.

NOTE 1 Results with volatile test material should be interpreted with caution and are likely to underestimate any inhibitory effect because of the difficulties in maintaining the initial concentration in the test flasks.

NOTE 2 Coloured materials and materials of low water-solubility which form turbid suspensions or dispersions can be tested in many cases using colour/turbidity controls as blanks (see 9.4).

NOTE 3 Inhibitory effects on activated sludge microorganisms of test materials for which this test is not applicable can be determined by using an inhibition test based on respirometric measurements (see ISO 8192).

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO 5667-16, *Water quality — Sampling — Part 16: General guidance for the biotesting of water, waste water, and water ingredients — Sampling, pretreatment, performance and evaluation*.

ISO 8192, *Water quality — Test for inhibition of oxygen consumption of activated sludge*.

ISO 11733, *Water quality — Evaluation of the elimination and biodegradability of organic compounds in an aqueous medium — Activated sludge simulation test*.

3 Terms and definitions

For the purposes of this International Standard, the following terms and definitions apply.

3.1 growth

increase in the number of microbial cells during the test period, assessed by measurement of the biomass of a culture

NOTE Biomass can be determined by any suitable method, for example as turbidity [optical density (OD) or absorbance] with a spectrophotometer at 530 nm and expressed in relative turbidity units (OD₅₃₀).

3.2 growth curve

measured biomass growth graphically plotted against the incubation time

3.3 specific growth rate

μ
doubling of biomass (x) per time unit (t)

$$\mu = 1/x \cdot dx/dt$$

NOTE Growth rate is usually expressed in reciprocal hours (h⁻¹).

3.4 growth inhibition

difference in the growth at the end of the incubation time in the presence of organic test medium and test material, compared with that in a similar mixture without test material

NOTE Growth inhibition is expressed as a percentage.

3.5 inhibition curve

growth inhibition, in percent, plotted against the logarithm of the test material concentration

3.6 effective concentration EC value

concentration of the test material giving a calculated growth inhibition, or interpolated from the inhibition curve, of 50 % (EC₅₀), 20 % (EC₂₀) or 80 % (EC₈₀) compared with that of a similar mixture without test material

4 Principle

Flasks containing organic test medium and test material are inoculated with an overnight culture of activated sludge microorganisms and incubated on a shaking device at 22 °C ± 2 °C. The total test duration is normally 6 h, including an exposure time of about 4,5 h. The biomass of these cultures and of blank controls without test material is determined using an appropriate method. Measurement of turbidity in a spectrophotometer at a wavelength of 530 nm and expression in relative units (OD₅₃₀) is recommended. The growth inhibition, in percent, at the end of incubation is calculated by comparison with blank controls and is plotted, for example in a semilogarithmic curve against the test material concentration, to derive the EC values. The sensitivity of the activated sludge microorganisms can be checked against a reference substance (see 6.7).

5 Test environment

Incubation shall take place in the dark or in diffuse light in an enclosure which is maintained at 22 °C ± 2 °C and which is free from vapours toxic to microorganisms.