METHODS OF DETERMINING THE QUALITY OF WATER USING THE FUNCTIONAL CHARACTERISTICS OF ALGAE

by

L A Sirenko

(Institute of Hydrobiology of the Academy of Sciences of the Ukraine, Kiev)

In the study of questions relating to the quality of raw water and the biological productivity of water bodies algal indicators have an important place (11, 13, 20, 23-25, 30, 31). Analysis of the available scientific information shows that when studying algal indicators considerable attention is paid to the range of functional characteristics as well as to the numbers and morphological-systematic criteria. The most common ones used include the nature of the growth processes and the rate of multiplication (8, 10), survival (21, 27), intensity of primary production of organic matter and its use in bioenergetic processes (4, 5, 9, 18).

An example of functional algal tests in classical hydrobiology is a widely used current method– that of hydrobiological productivity and determination of the intensity of the primary production of organic matter (10, 26).

Despite the importance of these functional indicators in determining the quality of water and the nature of the production processes as a basis for preserving the ecological equilibrium of aquatic ecosystems, their use in the system of hydrobiological methods of monitoring the quality of surface water has not received proper consideration (22). At the same time there is an acute need for the development of dependable methods that are fast enough and use reliable equipment for the bioindication of water for the purposes of the biological monitoring of functional indicators of the primary photosynthesising component at the basis of the transformation of biogenic and organic substances in processes in inland water bodies. When using the opportunities offered by the wide use of functional algal criteria in determining indicators of the quality of raw water the following points should be borne in mind: firstly, the comparative technical simplicity of obtaining and artificially keeping test cultures of algae with the current progress in laboratory and mass culture technology; secondly, the fact that laboratory test cultures of a specific standard physiological condition can be obtained regardless of the time of year; thirdly, the fact that experiments can be automated with quantitative recording of indicators, using any desired system; fourthly, the high sensitivity of the response reaction of algae cells both to the content of basic biogenic elements and individual microelements in the environment and to toxic components.

The possible use of remote methods of monitoring bodies containing chlorophyll, using aeroplanes and satellites, is also of considerable significance.

Taking into account the above, we endeavoured to analyse the matter and the possible use of functional algal criteria in the system for the biological monitoring of aquatic objects and also to give some results in using these criteria.

Of the functional algal tests which are of interest in this matter we might provisionally mention two groups. The first group covers criteria based on recording changes over a specific period of time in individual water indicators as a result of the action in the water of the algal cells, both under normal conditions and in the presence of extreme factors.

The second group covers criteria based on consideration of changes in the physiological and biochemical characteristics of the algal cells which do not occur under normal conditions and under pathological conditions which can be due to the lack or excess of some element, the presence of toxic factors, changes in the temperature or light regimes.

For the usage of the characteristics of the first group in wider practice for the purposes of biological testing of the quality of water the following is required: firstly to equip a measuring apparatus with pick-up devices for recording and printing out changes in pH, the partial pressure of oxygen and temperature using existing pH-meter and oxygen meter models. Secondly, the apparatus must be thermostatically controlled and have facilities for the alternate creation of light and dark regimes. Thirdly, the functional reaction of the water studied must be recorded – test cultures of algae or specimens collected in nature – not momentary measurement but establishing the dynamics of the process over a specific time interval – eg 15-30 minutes – using an alternating system of light and dark exposure.

The nature of the light and dark curves of the reaction of the experimental body in comparison with the control, where there is no influence of the investigated factor, makes it possible to obtain sufficiently reliable rapid information on the biological indication of the water. Both the nature of the curve and the angle of its deflection from the control are very important in this case. When optimising the conditions for the growth of the algae, an increase in the level of the curve was observed over that of the standard test culture. In the presence of unfavourable factors in the water investigated – deficit in biogenic elements and pollution – the level of the curve is decreased in comparison with the test culture.

As is known (1, 6, 14, 32, 33), in the process of the life activity of algal cells the pH of the medium alters. Under normal conditions, changes in the pH of the medium and the pH of the culture medium in which the algae grow and in a water body are characterised by a marked daily rhythmicality: In light, as a result of the photosynthetic activity of cells – utilisation of carbon dioxide and carbonate ions – the pH reaction alters towards the alkaline end of the scale more quickly and more strongly, the more intensely the photosynthetic process proceeds . However, on the contrary in darkness, with increased cell respiration, acidification of the medium takes place due to release of carbon dioxide and bonding of alkaline metal ions in the form of carbonates. Thus, as a result of the operation of the biological system, a rhythmic change takes place in the pH towards the alkaline end of the scale (in the light) and then towards the acid end (in the dark). Under the influence of extreme factors which disrupt the normal life processes of the algae cells, the rhythmicality of the process alters, the fluctuations in pH are reduced, while with greater disruptions they stop or even reverse (eg in the light as a result of respiration activity there is a drop in the pH ie increased acidification). By recording the changes in pH with a measuring cell, by exposing it to light for 15-30 minutes and then putting it in the dark for the same period, one can obtain full details of the normal or pathological state of the system or sample of water to be investigated.

When recording changes in pH directly in a water body, a clear indication of the trend the processes are taking can be obtained for a longer light and dark period (1 to 4-6 hours) depending on the trophic state of the water-body. More rapid changes can be recorded in a single unit of time in eutrophic water bodies (pH change of 0.5-1). When taking measurements directly in a water body, the most sensitive changes are noted with an increase in the pH during the morning (from sunrise). The maximum swing towards the alkaline end of the scale occurs between 12.00 and 16.00. When recording in the dark, the fastest drop in the pH is noted under normal conditions immediately after the onset of darkness. The lowest pH level is reached in the pre-dawn period. Using the dynamics of the daily course of the pH change, the periods of extreme influences and the effects of these influences are clearly detected. To illustrate this point we shall give the results of an experiment to verify the sensitivity of this criterion.

Under laboratory experiment conditions the dynamics of change in the pH were measured on model suspensions of test cultures of the blue-green algae *Microcystis aeruginosa* Kutz. emend. Elenk and *M. pulverea* (Wood) Forti emend. Elenk. Similar results were obtained with cultures of the green algae *Chlorella vulgaris* and *Scenedesmus quadricauda.*

Research has shown that introducing into the medium compounds which are toxic and have algicidal activity sharply disrupts the daily pH and immediately indicates an unsatisfactory situation in the model culture (Figure 1). The sensitivity of the test was confirmed by using more than 400 samples of different types of organic compounds — heterocyclic compounds, derivatives of aliphatic acids, derivatives of aliphatic aldehydes and aliphatic hydrocarbons, derivatives of aromatic compounds, aromatic hydrocarbons, derivatives of phosphoric acid, derivatives of dithiocarbonic acid, sulpho derivatives, alcohols, ketones, derivatives of urea, complex ethers of mineral acids, derivatives of hydrazine, isothiocyanates, aliphatic amines, derivatives of condensed imidazoles, derivatives of phenyl biguanides (2, 3, 16, 19).

It has been found that if a compound had no effect on the life processes of the algae, then it did not affect the daily pH. Chemical compounds which are toxic to algae (Figure 1) and have an algicidal effect, disturbed the rhythm of the pH change in the medium to a greater extent, the greater the inhibiting effect they had. Evaluation of the presence or absence of the algicidal effect and the nature of the growth of the algae was made using a series of other indicators — alteration in numbers and biomass, the ratio between number of living and dead cells, the dynamics of change in organic matter, pigments, intensity of release of oxygen per unit time. In all cases, a close relationship was noted between the daily rhythmicality and change over a unit of time of the reaction of the pH of the medium and the degree of life activity of the test culture of the algae.

Similar results were obtained in field experiments in water bodies. Thus the change in the pH of the medium in the process of life activity of the test culture of the algae and the native phytoplankton under normal conditions and under the influence of extreme factors is worthy of inclusion in the functional algal test to obtain a rapid detailed characterisation of the investigated water.

Equally important information can be obtained by recording the dynamics of change in the oxygen content of the medium per unit time. As can be seen from Figure 2, the test culture of the algal *M. aeruginosa,* initially exposed for a sharper reaction to darkness for 2-3 hours, actively photosynthesises under normal conditions on exposure to light. When the light is turned off, the release of oxygen gives way to absorption of the oxygen in the respiration process. When introducing a disrupting factor into the medium (derived imidazole with a strong algicidal effect) the photosynthetic effect is reduced or even gives way to the absorption of oxygen in light, with an increased effect when the light is increased under dark conditions.

Investigations carried out to check the sensitivity of this functional test show that, recording for a period of 15-30 minutes, the release of oxygen in light and, thereafter, its absorption in the dark, can give rapid and sufficiently marked indications of the quality of the medium into which the test culture is placed. In our opinion this functional test is of particular interest for detailed biological indication of water quality. (This rapid test can be carried out in qualitative form directly under field conditions with, for example, green filamentous algae of the genus *Cladophora* (28)). As regards practical use, it can be carried out in conjunction with a determination of the quantity of chlorophyll in the suspended load. Calculation of the quantity of oxygen per unit of chlorophyll under normal and extreme conditions gives a quantitative value of the inhibition of the functional activity of the algal cells, ie makes it possible to determine criteria for biological indication.

As can be seen from Figure 3 (Experiments carried out by A V Kareshkevich), introduction into the medium with the test culture of different chemical compounds showed a different effect on the release of oxygen in light. The chemical compounds which did not affect the cells of the algae or stimulated their life processes had a similar effect on the intensity of oxygen release. Substances which had an algicidal effect markedly inhibited the photosynthetic release of oxygen.

Verification of the sensitivity of the test with different waste waters showed that waste water, which did not contain substances toxic to algae and had a sufficient amount of biogenic elements and organic substances, not only did not inhibit the photosynthetic release of oxygen but even stimulated it. Waste water which did contain toxic substances, however, significantly suppressed the photosynthetic release of oxygen and stimulated its absorption in the photo-respiration process, ie was biologically inferior.

Thus the characteristics of the intensity of light and dark changes in the oxygen in the medium as a result of the life processes of the cells of the algae under normal and pathological conditions in the procedure described above can be used for very rapid indication of water quality indicators.

From the functional algal tests of the second group, which show the physiological condition of the cells and change under the influence of extreme conditions, the following are worthy of attention for the biological indication of water: changes in the native fluorescence of chlorophyll in algal cells; chlorosis and carotinogenesis as functional characteristics of the biological synthesis of the photosynthetic apparatus of cells; cytochemistry of individual oxidation-reduction enzymes associated with the transformation of energy in the cell; the electrophysiological characteristics of the cell membranes, etc.

Recording the native fluorescence of chlorophyll as a criterion of the degree of viability of algal cells attracted the attention of researchers a relatively long time ago (7). At the present time it is used to determine the physiological condition of algal cells (17) and also of the population as a whole (15). It has been shown that in ultra-violet rays the spectrum of the native luminescence of the algal cells changes from bright red, dull claret or pink-red, orange-pink or blue-green to olive-green. These shades are characteristic of dead cells and detritus. Intermediate shades of luminescence are characteristic for different degrees of moribund cells.

The use of a laboratory microspectrofluorimeter, with a probe system for the quantitative recording of the intensity of native fluorescence and its spectral structure (15), shows that the methods of luminescent spectral analysis can be used in a system of biological monitoring to track the state of a population of blue-green algae in a water body as the most abundant and dominant group in algocenoses of different ecological zones.

The practical use of the method of luminescence microscopy, both by visually calculating the number of cells with different luminescence in the field of vision of the microscope and by quantitatively recording the intensity and spectral range of the luminescence, shows that this functional criterion can be used for very rapid evaluation of the condition of the algae in relation to the effect of extreme factors in the medium. It can be used in three different ways, first, by microscopic examination of fresh samples of water and bottom deposits in a luminescent microscope to determine the physiological condition of the native phytoplankton and phytobenthos; second, used in testing standard algal cultures, for example, unialgal cultures of *Microcystis, Chlorella, Scenedesmus,* etc. When carrying out biological indication using this method a specific amount of the test culture, with a known ratio of living, dead and moribund algae cells, is introduced into the liquid to be investigated. Within 12-24 hours a further microscopic examination is made with a differentiated calculation of the ratio of cells of differing physiological condition, which gives an idea of the presence in the water-course under investigation of factors which have a negative effect on the life processes of the algae; third, in our opinion special attention should be given to the possibility of a preparation from a test culture of algae

by applying it on to filters. Knowing the ratio of algal cells in different states of life on the filter under normal conditions and as a result of the action of some extreme factor, we can obtain very rapid information on the biological indication of the water investigated.

As can be seen from the data in Table 1, with the algicidal effect there is a significant change in the ratio of the number of live and dead cells in a test culture of algae, despite the fact that calculation of the number of cells did not give such a clear picture using a light microscope due to the impossibility of differentiating live and dead cells. With a photo multiplier tube (FEU-22), calculation of the number of cells in different physiological states can be replaced by rapid and effective measurement of the overall intensity and spectral luminescence characteristics (Table 2) (In the experiments with algicides N M Stetsenko took part). According to their physiological condition, the algal cells differ significantly in brightness and in the spectral nature of the luminescence.

The test of intravital luminescental microscopy can have particular value when using it to indicate the quality of water of phytobenthic samples. As is known, bottom deposits and bottom layers of water are distinguished by the presence of higher amounts both of biogenic elements and polluting compounds due to the processes of sedimentation and accumulation in silts. Disruption of the normal life process and the disappearance of bottom biocenoses is highly dangerous for the internal processes in a water body as it weakens the self-purification capacity, lowers the biological productivity and increases the possible occurrence of secondary pollution.

Modern equipment makes it possible to automate the recording process, in particular by determining the overall brightness of the native luminescence of a surface unit of the silty deposits of a water body, for example above and below the discharge of a given outlet, ie, in the presence or absence of the action of an extreme factor.

The combined recording of the changes in the native chlorophyll fluorescence of individual species of algae, taking into account their sensitivity to specific factors (12, 29), would appear to make it possible to obtain even more detailed information.

Of course, under these circumstances a large amount of work still needs to be carried out to determine the adequacy of the response of the algal cells to the effect of the individual extreme factors. However, no doubt is raised by the fact that even in the current state of the problem, the use of luminescence microscope examination of live algae in nature (phytoplankton, phytobenthos) and the use of test cultures makes it possible to obtain precise and rapid information on the indication of the quality of the water investigated.

Table 1. Change in the ratio of live and dead cells in a test culture of the alga *M. aeruginosa* under the influence of various chemical compounds introduced into the medium to produce an algicidal effect.

	Active substances	Conc. mg/l	Ratio of cells % Live	Dead	Presence of algicidal effect
1.	Meta-xylylbiguanide hydrochloride recrystallised	5	41.2	58.8	Yes
		10	27.8	72.7	Yes
2.	Meta-xylylbiguanide hydrochloride crystallised	5	45.4	54.6	Yes
		10	30.0	70.0	Yes
3.	Meta-xylylbiguanide hydrochlorine freshly prepared	5	73.0	27.0	No
		10	71.7	28.3	No
4.	Meta-xylylbiguanide hydrochloride industrial	5	62.0	38.0	Slight
		10	59.0	41.0	Slight
5.	Phenylbiguanide hydrochloride industrial	5	56.9	43.1	Slight
		10	47.6	52.4	Slight
6.	N-phenylsulphonyltrichloracetamide	5	66.7	33.3	No
		10	66.7	33.3	No
7.	N-p-chlor-meta-nitro-phenyl-sulphonyltrichloracetamide	5	71.9	28.1	No
		10	80.4	19.6	No
8.	N-p-tolylsulphonyltrichloracetamide	5	74.4	25.6	No
		10	75.3	24.7	No
9.	N-p-bromphenylsulphonyltrichlor-acetamide	5	16.7	83.3	Yes
		10	2.3	97.7	Yes
10.	Tris(tolylamino)phosphazo-N-p-tolyliminotrochloracetyl	5	77.2	22.8	No
		10	84.4	15.6	No
11.	Control	—	84.7	15.3	

Table 2. Intensivity of native fluorescence of cell chlorophyll in relation to the degree of viability, based on the example of natural populations of *M. aeruginosa.*

No	Degree of viability	Colour of colony under microscope		Visual intensity rating	Relative intensity rating
		Visible	UV		
1.	Young newly formed colonies	Dark green	Bright purple	5	576±21
2.	Young colonies but already dividing	Green	Purple	4	470±9
3.	Colonies at a stationary growth stage	Light green	Red, dull red	3	373±14
4.	Old regenerating colonies	Dark olive	Orange-red	2	172±17
5.	Moribund colonies	Olive	Pale orange	1	55±6
6.	Dead	Flesh-coloured	Pale green	0	18±3

Table 3. Change in the ratio of live and dead cells in a test culture of *M. aeruginosa* under the influence of chemical compounds with an algicidal action; effect of dilution.

Active substance	Dilution	Cell ratio in %	
		Live	Dead
1. Ethylthozilate-1,2-dimethylnaphth (2,3-d)-imidazole-4,9 dione	1 : 1	0.4	99.6
	1 : 10	27.3	72.7
	1 : 100	74.5	25.5
2. Ethylethylsulphate-1,2-dimethyl naphthoquinono-1,4-(2,3-5,4) imidazole	1 : 1	2.3	97.7
	1 : 10	15.0	85.0
	1 : 100	51.4	48.6
3. Control		82.0	18.0

Over the past few years greater use has been made of electrophysiological tests based on recording the biopotentials of algae cells. In these tests most use is made of the huge cells of the Characeae algae as this is a highly sensitive test on membranotropic agents, making it possible to carry out a series of successive measurements with the reactions being automatically recorded.

In conclusion we can make the following points:

1. To evaluate the condition of the energy flow which ensures the functioning of aquatic ecosystems, algae, as test subjects, are of prime significance. This is due to the fact that algae are primary producers, since they are at the base of the majority of the food chains in water bodies. Because of the characteristic plasticity of their metabolic processes, in most cases they are distinguished by their high sensitivity to the content of biogenic elements, organic substances and toxic pollutants in the environment. In this connection, both planktonic and benthonic forms of algae can be used as test subjects to evaluate the degree and nature of pollution and eutrophication of water bodies by determining their functional characteristics.

2. Of the functional characteristics of algae worthy of attention from the point of view of biological testing and for the purposes of approval and unification, we must mention:

   a. recording the changes in the medium per unit time, using indicators such as the pH reaction and oxygen content in the light and the dark. This functional test is simple to carry out, makes it possible to use automatic methods and is sufficiently fast and sensitive. The temporal characteristics (15-30 minutes) of changes in the mentioned factors in light and dark reflect the normal and pathological functioning of the test objects, under the influence of a given factor.

   b. use of luminescent microscopic examination of algal samples from nature or in test cultures to determine the ratio of live and dead cells and differences in the spectral characteristics of luminescence.

3. Research needs to be extended on determining the possible and future use of various functional algal tests, using the modern methods and equipment of plant physiology and of the biochemistry and biophysics of plant cells. Special attention should be given in this respect to enzymes associated with bio-energetic processes, calorimetric characteristics and the bioelectric features of membranes.

4. Special attention in developing methods of biological indication should be given to harmonising, standardising and developing equipment and expressing the results obtained in a uniform manner.

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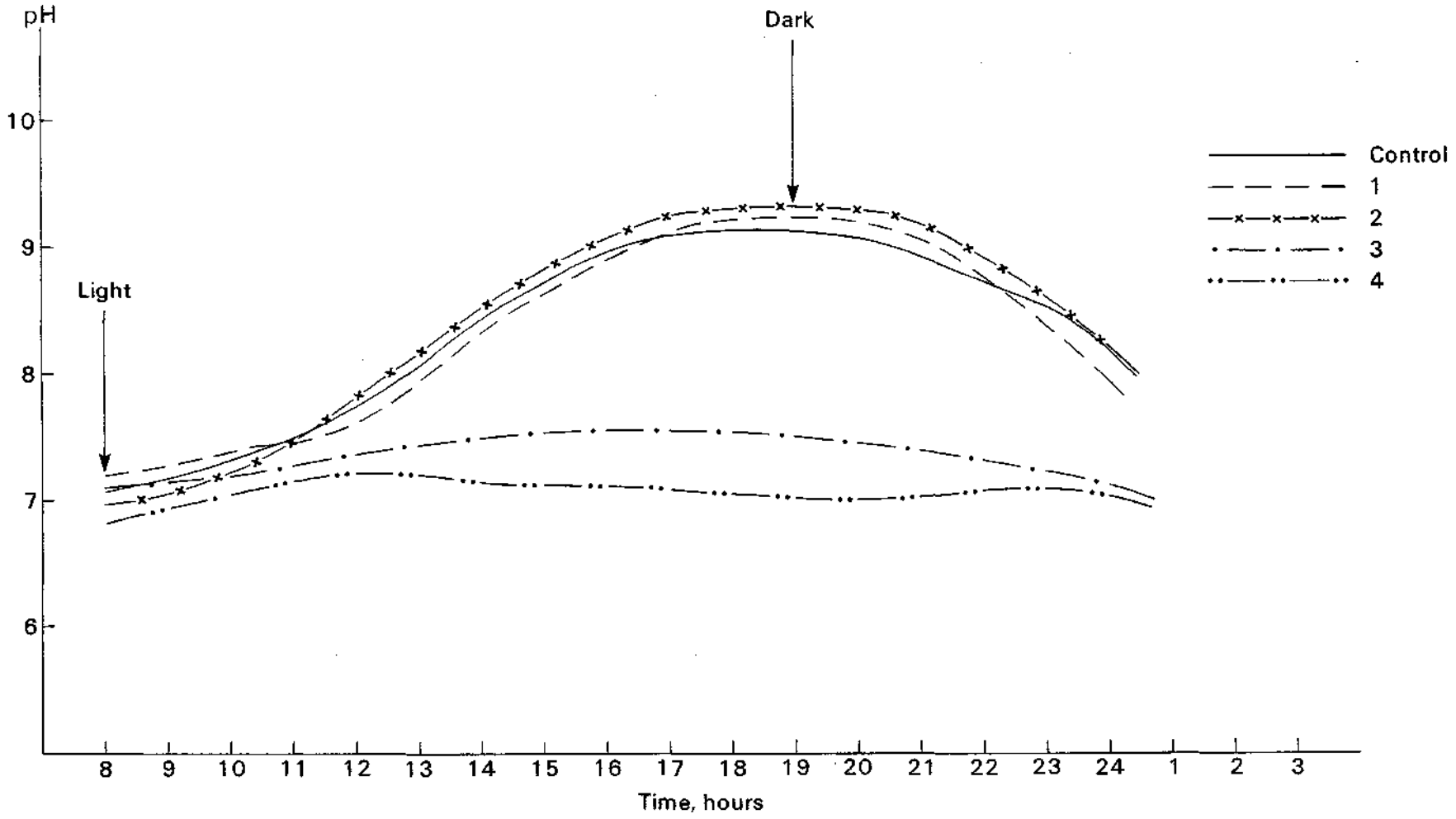
pH
10
9
8
7
6
Dark
Light
Control
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4
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1
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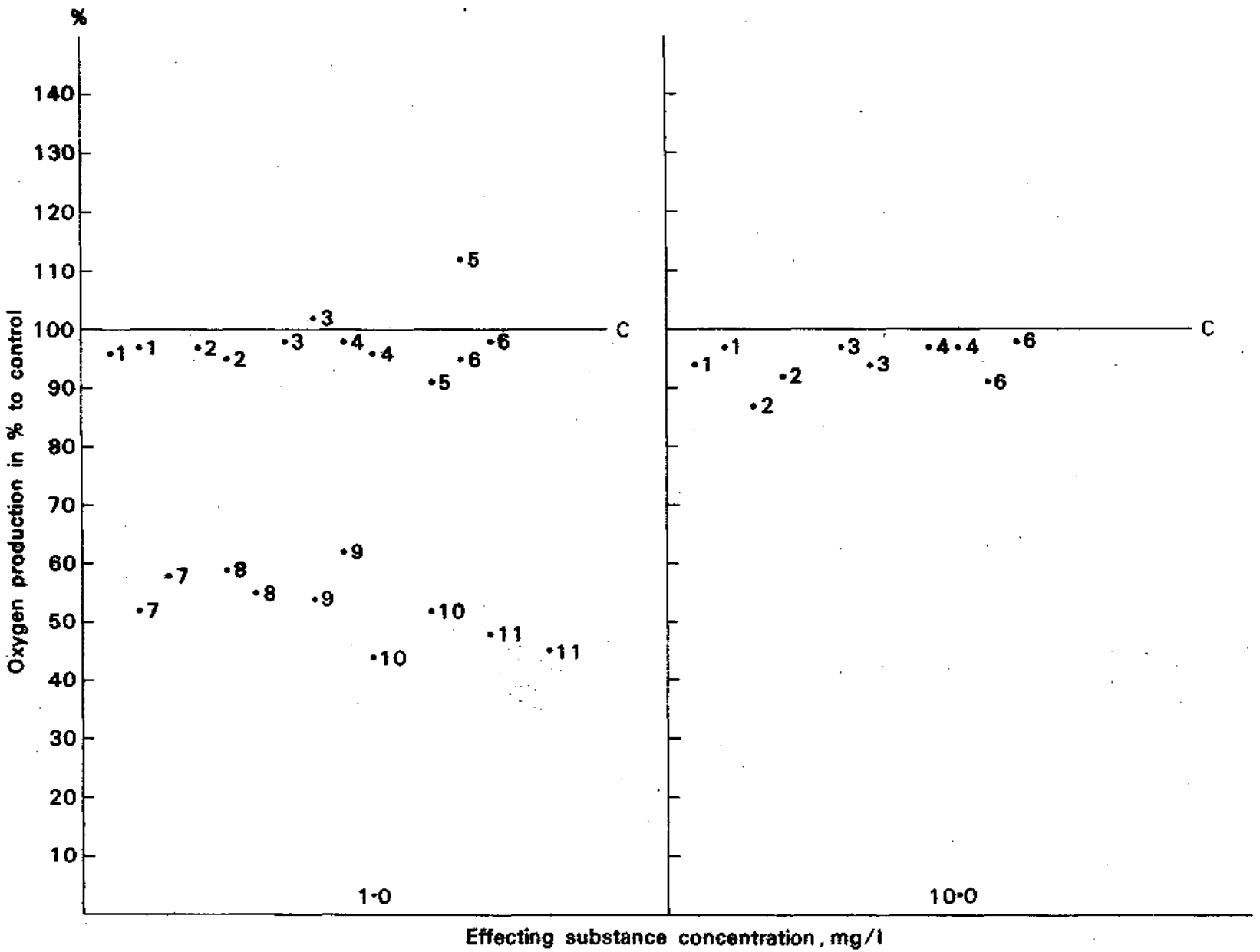
Time, hours

FIG 1


%
140
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120
110
100
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80
70
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C
Oxygen production in % to control
1·0
10·0
Effecting substance concentration, mg/l

FIG.2

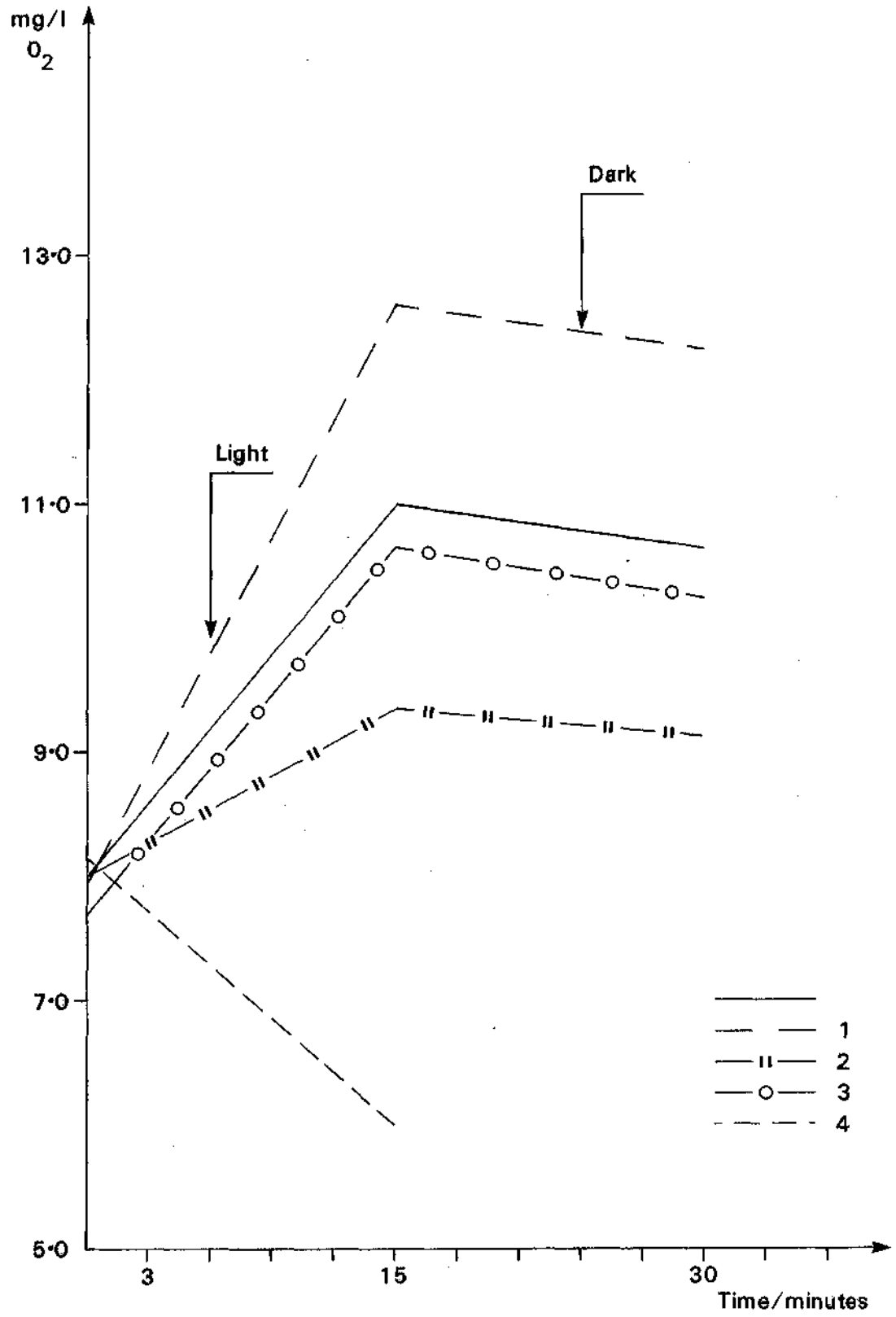

FIG.3