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The role of hydrogen peroxide in natural aquatic media

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ABSTRACT. Chemical and biochemical processes defining the H_2O_2 cycle in natural waters and in biological subjects are examined. Assessments are given of the inflow of H_2O_2 into surface waters from the atmosphere, data are collated on the H_2O_2 content of sea and fresh water. Photochemical, catalytic and biochemical processes make the main contribution to H_2O_2 formation. The interaction between intracellular redox processes of aquatic microorganisms and the redox state of aquatic media is discussed. Biotic and abiotic processes for the decomposition of H_2O_2 in natural waters and the role of free radicals connected with the H_2O_2 cycle in the self-purification of natural aquatic media are examined. Data are presented which indicate the important role of H_2O_2 in the formation of the biological wealth of natural waters.

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I. Introduction

Truisms of the type "water is life" and "without oxygen the living world cannot exist" are well-known. Water is contained not only in the hydrosphere but is also one of the essential components of the atmosphere and is present in the lithosphere in large quantities and in the free state. In its turn molecular oxygen exists not only in the form of an atmospheric gas but is also partially dissolved in water and is contained in ground gases. The effects of water and oxygen on living processes are different. Organic life without water is impossible in principle, and strictly it originated on the Earth without oxygen.

It is known that oxygen is formed in photosynthesis by a 4-electron oxidation of water. In its turn oxygen is converted back into water by a 4-electron reduction. However in nature single-event 4-electron processes are only effected in specific biological and catalytic systems.[1,2] As a rule various intermediates are formed as a result of the oxidation of water or the reduction of O_2, among which is hydrogen peroxide, the stable intermediate product of 2-electron conversions of oxygen and water.

Even on the basis of such general considerations it may be proposed that hydrogen peroxide must accompany the oxidation–reduction conversions of oxygen and water. Even more, it is fairly difficult to purify water from traces of H_2O_2 without using special measures. The overall question is, in which processes and in what amounts is it formed and decomposed, and what is the "purpose" of hydrogen peroxide in the life processes of aerobic organisms and in the environment? The present review is devoted to the examination of these questions and their application to the problem of setting the quality of natural waters.

II. Methods for detecting hydrogen peroxide

The content of hydrogen peroxide in the environment is not large, so special, highly sensitive methods are required for its detection. Such methods have been developed only comparatively recently due to the recently awakened interest in studying the role of H_2O_2 in the environment. Methods of titrating H_2O_2 using the iodometric procedure[3] have a lower limit of sensitivity at the 10^{-5} M level. Sinel'nikov[4] has successfully reduced the level of sensitivity of the iodometric titration to 10^{-6} M, however the method is extremely laborious and is not widely used. Electrochemical methods of analysis of H_2O_2 are also not widely used at present, although the highly sensitive chromatographic method of analysis for the content of H_2O_2 in aqueous media is based on the use of an electrochemical transducer. Spectral methods, such as chemiluminescent, fluorometric and photometric methods, are the most widespread.

1. Chemiluminescent methods

Chemiluminescent methods have the greatest sensitivity. The oxidation reaction of luminol and other substances with hydrogen peroxide is used in these essentially kinetic methods for generating electronically excited species emitting quanta of light. The intensity of the chemiluminescence is proportional to the H_2O_2 concentration in the sample being analysed. The methods using luminol are the most widespread.[5] Luminol is very soluble only in a medium of pH $\gtrsim$ 10 so reaction is effected in an alkaline medium in the majority of chemiluminescent methods using it. Cobalt salts, haematin, potassium ferricyanide, etc. are used as catalysts of H_2O_2 decomposition in alkaline medium.

Various dissolved organic and inorganic compounds are present in natural aquatic objects. Some of them participate in the catalysis of H_2O_2 decomposition in alkaline medium (in which the analysis is carried out), others react with reactive intermediate particles or affect the properties of the catalyst used and the reaction kinetics. Because of this the chemiluminescent procedure does not always give correct results. It is necessary to construct calibration graphs of 'added

hydrogen peroxide *vs.* signal observed' for each new sample investigated. In addition, control analyses of water samples where hydrogen peroxide would be completely decomposed are necessary for background information. Catalase is usually used as the additive to decompose H_2O_2.

The enzyme peroxidase also serves as catalyst of the chemiluminescent emission of luminol when it is oxidised with hydrogen peroxide.[6] This reaction is effected in neutral or weakly alkaline media with an optimum in the region of pH 8–8.5. Impurities in natural waters may prove to have a marked inhibiting action because of the low solubility of luminol in neutral media.

Comparatively recently several sensitive chemiluminescent procedures for determining hydrogen peroxide in solution have been developed which are based on the luminescence of peroxyoxalates, which are the oxidation products of oxalate esters with hydrogen peroxide.[7–9]

Fluorimetric and photometric methods of determining H_2O_2, based on the peroxidase oxidation of specific substrates, are widespread. In the absence of kinetic chemiluminescent methods, the fluorescence and photometric methods may be regarded as stoichiometric. The decrease in the initial reagent or the concentration of final product of the peroxidase reaction are recorded. It is necessary to be confident that the stoichiometry of the reaction of H_2O_2 with the substrate is retained over a fairly wide range of conditions of carrying out the analysis.

The selective peroxidase oxidation of hydrogen donors with hydrogen peroxide and other organic peroxides has been known for a long time. Substrates include phenols, aromatic amines, triarylmethanes and certain heterocyclic compounds.

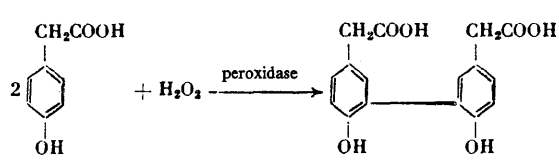
2. Fluorimetric methods

Fluorimetric methods of determining trace amounts of H_2O_2 have been carried out in two ways.

The first comprises oxidation of an H donor substrate, possessing marked fluorescent properties, with hydrogen peroxide into a non-fluorescent product. In this case the concentration of H_2O_2 is measured by the decrease in fluorescence intensity. Scopoletin (a fluorescent phenol) has been used as a donor for horse radish peroxidase when determining the H_2O_2 content of cell cultures.[10–12]

Zika and collaborators,[13–15] Kieber and Helz[3] and Holm and collaborators[16] have modified this procedure to be suitable for the analysis of the H_2O_2 content of natural waters. The procedure enables continuous automated measurements to be carried out.

In the second variant *p*-hydroxyphenylacetic acid is used as substrate for the peroxidase reaction. Oxidation of this substrate leads to the formation of a product possessing fluorescent properties.[9,17–26]



Fluorescence analysis is suitable only for relatively pure water since fresh and coastal sea waters contain a large quantity of interfering organic components.[27] An advantage of the method is the possibility of extended storage of the fluorescent product in the water sample being analysed.

3. Photometric methods

The photometric methods of determining H_2O_2 are the most widespread.[28,29]

In 1970 Motolla and collaborators[30] were the first to use the peroxidase oxidation of the leuco form of the dyestuff Crystal Violet to develop a highly sensitive analytical method for determining hydrogen peroxide.

This method was later used for the analysis of natural samples.[31] The procedure proposed[31] was inadequate due to the non-linear character of the dependence of the absorption of the oxidised form on H_2O_2 concentration in the sample and the extended (about an hour) time to carry out the analysis at a comparatively low sensitivity (10^{-5} M).

Optimisation of that method[30,31] has been carried out[25] by varying reagent concentrations, the order of mixing and the conditions of conducting the analysis. Overall the optimisation successfully increased significantly the sensitivity (10^{-7} M), reduced the time to carry out the analysis (3 min), reduced the consumption of reagents and secured linearity for the calibration graph. It became clear, in particular, that the growth rate and level of dyestuff coloration achieved (Malachite Green, Crystal Violet) depended on the relative content of peroxidase and humic compounds in the water. This is seemingly linked with the formation of a complex of the peroxidase with humic compounds and the participation of the reactive residues of the humic compounds in the reaction with the peroxidase–peroxide complex.

Under optimum conditions, using leuco-Crystal Violet (LCV) in the analysis of water samples for H_2O_2 content, 5 mg litre^{-1} leuco base and 20 mg litre^{-1} horse radish peroxidase were taken. Reaction was carried out in pH 4.5 acetate buffer (0.05 M). When carrying out serial measurements it is possible to prepare a "developing" solution containing a 10 times more concentrated mixture of leuco base and peroxidase in acetate buffer. In this way the analysis reduces to adding 1 ml mixture to 9 ml water sample to be analysed.

The advantages of the photometric methods of analysing samples of natural waters for H_2O_2 content are the simple design of the apparatus, the possibility of visual control (of Crystal Violet), the possibility of extended storage of "developed" test samples (F-substituted Malachite Green[32]) and the possibility of increasing the sensitivity of the method by the use of liquid chromatography.[23]

Among the disadvantages of the method are the need to conduct the reaction in weakly acidic media and the possible consequent appearance of unexpected factors affecting the results of analysis and the effect of turbidity of the water samples being analysed on the results of the photometric measurements (the need to filter or centrifuge test samples with a high content of suspended particles). In addition the need to construct calibration graphs for determining the apparent extinction coefficient of the dyestuff and the usually low solubility in water of the leuco base of the dyestuff lead to competition of the peroxidase–peroxide complex from H donors contained in the water sample being analysed.

Recently *N,N*-diethyl-*p*-phenylenediamine has been proposed as a peroxidase substrate. It possesses favourable analytical properties and a high solubility in water.[33]

As has been shown[31,34,35] when analysing natural waters using leuco Crystal Violet, the characteristic blue colour is determined by the presence in the water test sample of H_2O_2 exclusively and not organic peroxides. Nevertheless it is necessary to carry out experiments with catalase added to the water being analysed in order to allow for the possible contribution of organic peroxides to the observed colour and to determine the background absorption.

The emergence of coloured forms of leuco bases in the water samples may arise not only from the peroxidase reaction but also from the possible content of other oxidising agents in the medium. In order to verify such a possibility it is necessary to carry out a "cold" experiment without addition of peroxidase to the sample being analysed.

The photometric method of analysing low concentrations of H_2O_2 may be used in the kinetic mode. This is determined by the formation of a coloured compound (I) on reaction of H_2O_2 with peroxidase. In the presence of H_2O_2 and a suitable substrate the stationary concentration of compound (I) is determined by the rate of its formation (proportional to the H_2O_2 concentration in the medium) and its apparent transformation constant (proportional to substrate concentration). By measuring the concentration of compound (I) and knowing the rate constant for the reaction of H_2O_2 with peroxidase, it is possible to calculate the concentration of hydrogen peroxide in the medium.

Table 1. Content of H_2O_2 in natural waters.

Water sample	Place of sampling	$[H_2O_2]$, 10^7 M	Reference
Snow	Japan	20–40	[36]
Rain	Japan	100–250	[36]
	USA, California	3–40	[37]
	USA, Florida	9–75	[13]
	USA, North Carolina	0.01–1.5	[38]
	USA, North Carolina	1.5–45	[39]
	Gulf of Mexico	114–820	[40]
	Western Atlantic	84–206	[40]
	USA, Florida	284±38	[40]
	USA, California	9–880	[41]
	USA	3–720	[20]
Sea water	Gulf of Mexico	0.14–1.7	[12]
	Gulf of Mexico	1.7–2.9	[42]
	Gulf of Mexico	1.2–1.4	[43]
	Gulf of Mexico, at the coast	1.0–2.4	[44]
	Gulf of Mexico, far from the coast	0.9–1.4	[44]
	Biscayne Bay (Florida coast)	0.8–2.1	[45]
	Bahamas Bank	0.5–1.9	[45]
Estuaries	Pacific Ocean, Peruvian coast	0.08–0.7	[46]
	Chesapeake Bay	0.03–17	[47]
Fresh water	Volga, USSR	13–32	[4]
	Reservoirs, USSR	7–13	[48–50]
	USA, S.E.	0.9–3.2	[14]
Ground waters	USA	0	[14]

III. Distribution of hydrogen peroxide in the environment

Up to the middle sixties the attention of investigators was mainly drawn to hydrogen peroxide because of its practical use and as a convenient model reagent for studying mechanisms of catalytic oxidation–reduction processes. Even in the forties it was known that H_2O_2 was contained in rainfall in fairly large concentrations. Much work has appeared recently devoted to the detection of H_2O_2 in various natural waters. The results of some of these investigations are collected in Table 1.

1. Hydrogen peroxide in the atmosphere

The presence of H_2O_2 in the atmosphere was known even in the XIX century. The main mass of hydrogen peroxide is roughly in the thirteen kilometre layer of the troposphere. Hydrogen peroxide is present in the atmosphere in the gaseous state and in solution in drops of atmospheric moisture such as clouds, rain, mist and haze.

The ratio of the quantities of H_2O_2 in these forms is determined by three factors. Clouds occupy ~0.1 portion of the volume of the troposphere,[51] the coefficient of filling a cloud volume with drops of moisture is $\sim 10^{-6}$,[51] and the Henry constant, characterising the distribution of H_2O_2 between the aqueous and gaseous phases, is 1.2×10^5 mole litre^{-1} atm^{-1}.

$$[H_2O_2]_{(dis)} = K_H P_{H_2O_2}$$

It follows from these characteristics that

$$M_{H_2O_2(g)}/M_{H_2O_2(dis)} \simeq 1/(0.1 \times 10^{-6})(1.2 \times 10^5),$$

i.e. the main mass of H_2O_2 is in the gaseous phase.

The total quantity of atmospheric H_2O_2 in our estimation based on the vertical profile of H_2O_2 distribution in the troposphere is ~2 megaton.

The formation of H_2O_2 in the atmosphere is photochemical in nature.[52]

$$O_3 + h\nu \rightarrow O_2 + O('D), \text{ at } \lambda < 310 \text{ nm};$$

$$O(^1D) + H_2O \rightarrow 2OH, \; k_2 = 2.2 \times 10^{-10} \text{ cm}^3.\text{sec}^{-1}$$

Both species $O(^1D)$ and OH react with methane.

$$O(^1D) + CH_4 \rightarrow OH + CH_3, \; k_3 = 1.4 \times 10^{-10} \text{ cm}^3.\text{sec}^{-1},$$

$$OH + CH_4 \rightarrow H_2O + CH_3, \; k_4 = 2.3 \times 10^{-12} \exp(-1700/T) \text{ cm}^3.\text{sec}^{-1}.$$

In the subsequent chain of conversions the first stable oxidation products of methane, viz. CH_2O, H_2O, O_3 and H_2O_2, are formed.[52]

$$CH_3O + O_2 \rightarrow CH_2O + HO_2, \; k_5 = 3.9 \times 10^{-14} \exp(-900/T) \text{ cm}^3.\text{sec}^{-1},$$

$$HO_2 + HO_2 \rightarrow H_2O_2 + O_2, \; k_6 = 2.3 \times 10^{-13} \exp(-600/T) \text{ cm}^3.\text{sec}^{-1},$$

$$HO_2^{\cdot} + HO_2 \xrightarrow{M} H_2O_2 + O_2, \; k_7 = 1.7 \times 10^{-33} \exp(-1000/T) \text{cm}^6.\text{sec}^{-1},$$

where M is any particle in the medium (N_2, O_2, H_2O etc.) participating in the termolecular reaction to draw off the excess energy.

The oxidation of other hydrocarbons in the atmosphere leads to the formation of H_2O_2 by side reactions. Since methane is the main organic compound in the atmosphere the photoinitiated oxidation of methane may be considered to be the main source of H_2O_2.

The consumption of gaseous hydrogen peroxide in the atmosphere occurs by three routes.[52]

$$OH + H_2O_2 \rightarrow H_2O + HO_2, \; k_8 = 1.9 \times 10^{-12} \exp(-160/T) \text{ cm}^3.\text{sec}^{-1},$$

$$H_2O_2 + h\nu \rightarrow 2O\dot{H}, \; \lambda \leqslant 400 \text{ nm},$$

$$H_2O_{2(g)} \rightarrow H_2O_{2(dis)}.$$

Within the framework of a one-dimensional model of the atmosphere the following equation holds true for each height.

$$[H_2O_2] = \frac{k_7[H^{\cdot}O_2]^2}{k_3[OH] + a_9 I_\lambda + \varkappa_{10}},$$

where a_9 is the capture cross section for a photon with a defined λ,

I_λ is the intensity of solar radiation of wavelength λ at height H, $\varkappa_{10}$ is the apparent rate constant of "rain out" hydrogen peroxide by its absorption into atmospheric moisture and removal from the atmosphere with rain.

The significance of the terms in the denominator can be determined as a function of height. The last of the components has the greatest significance for tropospheric conditions, i.e. $[H_2O_2]_g$ is high in the troposphere if $[HO_2^{\cdot}]$ or $[\dot{O}H]$, which is linked linearly with $[HO_2^{\cdot}]$, are large and the content of atmospheric moisture is low. Since the formation of OH occurs at a rate proportional to $[O_3]$, $[H_2O]$ and the intensity of UV irradiation, then the greater these factors are the greater will be $[H_2O_2]$. $[H_2O_2]$ will be greatest in a well-illuminated and humid atmosphere with minimum aerosol content. The presence of aerosols of natural or anthropogenic origin may lead to a reduction of $[\dot{O}H]$ and correspondingly of $[H\dot{O}_2]$.[53] As is evident from the equation a 3–5 fold reduction of $[HO_2^{\cdot}]$ occasions a 10–25 fold reduction of $[H_2O_2]$.

The overall quantity of hydrogen peroxide passing from the atmosphere with precipitation to the surface of the oceans and land in a year may not exceed the quantity of $O(^1D)$ atoms generated in that time. However a global calculation of that sort is fairly complex. It is possible to estimate the maximum quantity of H_2O_2 falling onto the Earth from the atmosphere by proceeding from an average figure for precipitation of ~1000 mm in a year and the concentration of H_2O_2 in the precipitation. The latter figure varies within wide limits, from zero to 10^{-4} M[55] (see also Table 1). Taking a value of $[H_2O_2]$ ~10^{-5} M as being characteristic, we find ~200 g H_2O_2 falls each year on each square metre of land and ocean surface. To this figure must be added the hydrogen peroxide dissolved in natural waters and moisture of the soil. To make any global estimate of the quantity of H_2O_2 passing into water in this way is impossible since this quantity will be determined by the mixing layer renewing the contact surface of water and air. The problem of the dynamics of gases dissolving in water has been specially considered.[56] The time for H_2O_2 to reach the equilibrium concentration $[H_2O_2]_{dis}$ in drops of atmospheric moisture is several seconds.[56] Taking a value of $K_H = 1.2 \times 10^5$ mole litre^{-1} atm^{-1}, we obtain $[H_2O_2]_{dis} \sim 10^{-4}$ M. However this value characterises $[H_2O_2]$ in a thin surface layer. Diffusion transfer even in a metre thick water layer would require ~10^7 sec at a diffusion coefficient of ~10^{-5} cm^2 sec^{-1}. A more effective mechanism for the transfer of H_2O_2 from the surface is wind and wave mixing of the surface layers of the water. In order to assess the maximum quantity of H_2O_2 which may pass into natural waters by solution from the atmosphere it is possible to use the expression

$$\frac{dN}{dt} = \frac{\bar{v}n}{4} S\alpha,$$

where $\bar{v}$ is the root mean square velocity of gaseous particles, n their concentration, S the contact surface of gas with liquid and α the coefficient of accommodation.

Putting the values $t = 3.15 \times 10^7$ sec (1 yr), $S = 10^4$ cm^2 (1 m^2), $n = 2.2 \times 10^{10}$ cm^{-3},[51] $\bar{v} = 4.2 \times 10^4$ cm sec^{-1} and $\alpha = 0.18$[57] into the integrated form of this equation, we obtain $N = 1.23 \times 10^{24}$ ~2 mole, i.e. 70 g m^{-2}, a number comparable with the estimate given above for the quantity of H_2O_2 passing into natural waters with rainfall. One must not overlook the hypermaximal character of the given estimate made using the maximum concentration of H_2O_2 in the gas being absorbed by a constantly renewed water surface. Seemingly it is reasonable to speak of two channels, close in scale, for the inflow of H_2O_2 from the atmosphere, viz. with rainfall and by dissolving gaseous H_2O_2. It is interesting to note that if decomposition and other chemical conversions of hydrogen peroxide do not occur in natural waters then $[H_2O_2]$ of ~10^{-4} M, i.e. of the order of a saturating H_2O_2 concentration, would have accumulated in a stirred surface layer of thickness 20 m in a year.

Both fluxes of hydrogen peroxide from the atmosphere into natural waters, as already mentioned above, are highly sensitive to anthropogenic contamination of the atmosphere by aerosols and are reduced ten-fold by serious contamination. This is linked with the chemical origin of H_2O_2, viz. its formation on recombination of $HO_2^{\cdot}$ radicals. The reduction in the quantity of H_2O_2 in precipitation may be even greater due to autocatalytic oxidation of sulphite ions by hydrogen peroxide taking place in the droplets of clouds.

$$H^+ + HSO_3^- + H_2O_2 \rightarrow 2H^+ + SO_4^{2-} + H_2O.$$

Analysis of the various processes for oxidising HSO_3^- with oxygen or ozone and the catalysis of these processes by ions of iron and manganese showed that the oxidation of HSO_3^- with hydrogen peroxide is more significant.[58,59] It was noted previously,[60] that as $[SO_2]_g$ increased then $[H_2O_2]$ in raindrops was reduced. In other investigations an inverse proportional relationship was noted between the concentrations of sulphate ions and of hydrogen peroxide in rain water.[61,62] There are also other data obtained in aerochemical investigations of the chemical composition of cloud droplets,[63] in which such a correlation was not detected.

The problem of relating the compositions of raindrops and cloud droplets is not simple. There are differences in their lifespans (~1 h for clouds and ~100 sec for rain) and dimensions, the radius of a cloud droplet is 10 μm and of a raindrop is 300–1000 μm. A change in the composition of a falling raindrop compared to cloud occurs through absorption of various gaseous constituents and capture of aerosol particles. However in the lifespan of a raindrop the equilibrium concentrations of absorbed substances are reached only in the thin surface layers of a drop due to the low rate of diffusion. We are unaware of a quantitative solution to the problem of relating the composition of a raindrop to the composition of a cloud allowing for the absorption of gases in the atmosphere near the ground. There is considerable interest in the investigation of the H_2O_2 content in fossil ice in Greenland and Antarctica.[8] It turned out that in the Northern Hemisphere H_2O_2 was detected in significant concentrations in ice, the age of which was estimated at tens of thousands of years. This observation is the basis for the statement that hydrogen peroxide is an inherent component of atmospheric precipitation, a natural "companion" of O_2 and H_2O.

It also follows from these data,[8] that in the ice of Antarctica, in contrast to the Arctic, the H_2O_2 content falls rapidly with the age of the ice. It is not excluded that this is linked with a fall of H_2O_2 under the influence of solar UV radiation. The results of measuring H_2O_2 in the fossil ice of Antarctica indicates indirectly the natural occurrence of an "ozone hole" over Antarctica in times past. Attention has primarily been paid to these "holes" only since the early eighties.[64,65] The changes in UV radiation in the troposphere affect the photodissociation reaction which leads to a change in the equilibrium concentrations of the atmospheric oxidising agents, viz. O_3, $\dot{O}H$, $HO_2^{\cdot}$, H_2O_2.[66]

Measurements of the H_2O_2 content of snow revealed the correlation that the greater the atmospheric contamination by smoke the lower was the H_2O_2 concentration in melted snow.[67] This observation is in agreement with the hypothesis given above that an increase in aerosol contamination is associated with a reduction in the stationary concentration of $\dot{O}H$ and of $HO_2^{\cdot}$ and a large

reduction in H_2O_2 concentration in the gaseous phase. When the atmosphere is contaminated by both aerosols and sulphur dioxide it is possible that the oxidation of HSO_3^- remains incomplete in drops due to the decrease of $[H_2O_2]_g$ and correspondingly of $[H_2O_2]_{dis}$. In this event rainfall may contain unreacted oxidation substrates, such as sulphite ion and formaldehyde. This leads to the fact that in many areas substances which react efficiently with H_2O_2 are discovered in the water instead of H_2O_2. Nevertheless it can be proposed that the flux of oxidation equivalents in the form of hydrogen peroxide dissolved in water is reduced depending on the industrial development of a region. Moreover, it is possible for predominantly reduction equivalents (metal ions in the reduced form, sulphite ions, other reduced forms of sulphur) to reach the Earth's surface. This problem has not been studied adequately.

2. Hydrogen peroxide in the sea

The content of H_2O_2 in natural waters was determined for the first time in 1966 by Van Baalen and Marler[12] in the Gulf of Mexico. These investigations stimulated authors to study the ecological role of blue-green algae. The H_2O_2 content of the sea proved to be $\sim 10^{-7}$ M. It was suggested that atmospheric precipitation, metabolites of aquatic organisms and photochemical processes are the sources of H_2O_2.

After a long interruption the study of the H_2O_2 content of the sea was continued in the eighties by Zika and collaborators.[45,68] It was shown that the H_2O_2 content of the water of the Gulf of Mexico fell from $(1.2-1.4) \times 10^{-7}$ M in the surface layer to 5×10^{-9} M at a depth of 100 m.[43,69]

A detailed study of the distribution of hydrogen peroxide in the waters of the Gulf of Mexico was undertaken in 1985.[44] In oligotrophic regions weakly saturated with organic substances the H_2O_2 content in the water varied within the limits $(1.2-1.6) \times 10^{-7}$ M, while at a depth of 40 m, in a layer of convective mixing, the H_2O_2 concentration remained approximately constant, and below this it was reduced. In the coastal zone insignificant diurnal changes of H_2O_2 concentration were observed, viz. from 1.7×10^{-7} M at 8 am to 1.9×10^{-7} at 2 pm in May and from 1.9×10^{-7} M at 6 am to 2.9×10^{-7} M at 6 pm in August. Below the 20 m layer of efficient mixing the concentration was reduced to 0.3×10^{-7} M and did not undergo diurnal variations.

A tenfold and greater increase in concentration in layers at depths up to 50 m and over was shown after rain.[40]

An investigation of the H_2O_2 content in the ocean along the coast of Peru has been described.[46] A correlation was recorded between the H_2O_2 content of the water and biological activity and photochemical reactions.

The possible role of microorganisms in the formation of H_2O_2 in sea water has been established.[70] The ability to produce H_2O_2 not only by the action of sunlight but also in the dark was discovered in certain forms of algae. Not all algae possess this property. The sea diatom *Thalassiosira weissflogii* does not produce H_2O_2 in the dark or in the light.

The distribution of H_2O_2 in estuarine water has been studied.[47] The investigation was carried out in November. A diurnal variation was detected in the H_2O_2 content of water, the maximum hydrogen peroxide concentration of 9×10^{-7} M being reached at 6 pm. Seasonal variations were detected in the H_2O_2 content of the water from 0.35×10^{-7} M in February to 1.3×10^{-6} M in July.

The H_2O_2 content of water in the northwest portion of the Black Sea has been measured.[71] The H_2O_2 concentration did not exceed 1.5×10^{-7} M. Substances with a reducing nature, reacting efficiently with H_2O_2, were recorded in certain samples in place of H_2O_2.

The distribution of H_2O_2 concentration in the water of the White Sea has been studied using chemiluminescent methods.[72] Colleagues of the Pacific Ocean Oceanological Institute of the Far Eastern Division of the USSR Academy of Sciences have carried out investigations in many regions of the world's oceans.[73] The H_2O_2 concentration in these investigations varied within the limits 20 to 470 nM.

Methods for modelling mathematically the H_2O_2 distribution in sea water in time and space have been discussed.[74]

3. Hydrogen peroxide in inland fresh water

The first measurements of the H_2O_2 content of inland natural waters were made by V E Sinel'nikov using chemiluminescent and iodometric methods.[4,48,49,75–77] The investigations were mainly carried out on the Volga over several years at the end of the sixties and the beginning of the seventies. The H_2O_2 content in Volga water varied within the limits of 3×10^{-7} to 3×10^{-6} M. As a result it was concluded that 10^{-6} M was a typical value for the H_2O_2 content of uncontaminated sections of the water.[48]

According to these investigations microorganisms, particularly algae, play a major role in the formation of H_2O_2 in natural waters.[78] A significant increase (up to 10^{-5} M[79]) in H_2O_2 concentration was detected in the zone of contamination of reservoirs by domestic effluent, containing readily oxidisable organic compounds. The content of organic peroxides accompanying H_2O_2 was measured by iodometric titration using catalase to destroy hydrogen peroxide. Their concentration did not exceed 10% of the H_2O_2 concentration.

Later, in the early eighties, a study was undertaken of hydrogen peroxide in fresh natural waters.[31,34] Samples of water which were used for farm irrigation, samples of river water, drainage from fields and unpurified sewage were taken. They were irradiated under laboratory conditions with an artificial light source simulating sunlight. The concentration of H_2O_2 formed was $(2-7) \times 10^{-6}$ M for the majority of samples studied, while in water samples taken from biologically clean ponds it was 3×10^{-5} M.

A diurnal variation was detected in the H_2O_2 content of the surface water layer of a pond on a sunny day of 2.9×10^{-7}, 8.8×10^{-7} and 1.5×10^{-8} M at 8 am, 1 pm and midnight respectively.[8]

Many measurements of the H_2O_2 content of various water samples were carried out in this country in the eighties. The main results of these measurements have been collated.[80–83] Investigations were carried out using chemiluminescent and peroxidase photometric methods.

In the same regions of the Volga where Sinel'nikov had carried out measurements 10–15 years previously, an intensification of the diurnal variation and the initiation of seasonal variations were detected[82] in the H_2O_2 content of the water. In May the H_2O_2 concentration in Volga water near Volgograd varied between 10^{-6} and 3×10^{-6} M. Towards the middle of June the H_2O_2 content of the water began to undergo sudden diurnal changes. In sunny weather the maximum H_2O_2 content occurred at 4 pm and the minimum in the early morning hours. In overcast weather hydrogen peroxide was, in general, not detected. Shortly afterwards a period arrived when H_2O_2 generally disappeared from Volga water. On the contrary, titration of natural water with small additions of hydrogen peroxide enabled the detection of the presence in it of compounds of a reducing nature which reacted efficiently with H_2O_2. The authors thereby recognised a change in the state of the medium from normal

oxidising, when H_2O_2 was present in the water, to quasi-reducing, when a reducing agent was recorded in place of H_2O_2 and was titrated by adding H_2O_2. This change of the "sign" of the redox state of the medium was apparently produced for the first time in the mid-seventies and led to the catastrophic change in the ecosystem of the Volga (see Section VIII, No. 2).[80]

The results of measuring the H_2O_2 content of the waters of the Volga,[82] the Dnieper[84] and other waters of this country show that the seasonal change of the dynamic redox state of aquatic media has in recent years become the rule and not the exception.

IV. Processes forming hydrogen peroxide in natural waters

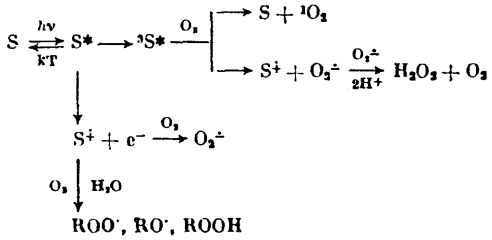
The fact that part of the H_2O_2 concentration in water undergoes diurnal changes correlating with solar activity suggests that hydrogen peroxide is formed predominantly by photochemical reactions. At the same time the formation of H_2O_2 in aquatic media is linked with the activity of algae and bacteria. The general considerations expressed at the beginning of the review also indicate the possible role in the formation of hydrogen peroxide of catalytic oxidation processes of readily oxidisable compounds with oxygen. We will discuss the most probable channels of introduction into aquatic media in more detail.

1. Photochemical processes in natural waters leading to the formation of H_2O_2

The photochemistry of natural waters is one of the most successfully developed areas of studying chemical processes occurring in the environment.[85,88]

Electronically excited species are formed by the action of solar radiation ($\lambda \geqslant 300$ nm) in water as primary products. These include S*, $^1O_2$ (S are organic compounds, photosensitisers, dissolved in water),[89–92] free OH radicals,[87,93–97] $O_2^{\cdot-}$,[14,34,98–101] peroxy radicals ROO·[93,102] and solvated electrons.[92,103–106] The reaction of the reactive intermediates with one another and with compounds dissolved in water may lead to the formation of H_2O_2 in an aerobic medium.[15]

The general scheme for the photochemical conversions of organic substances (S) in natural waters as a result of direct photolysis may be represented as follows.[107,108]

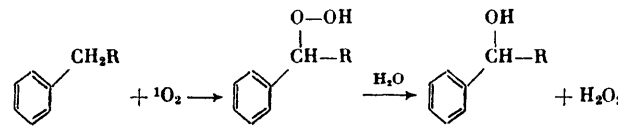


Organic substances dissolved in natural waters are mainly humic compounds. The efficiency of forming H_2O_2 in aquatic solutions containing humic and fulvic acids, and also various amino acids, has been studied and it was discovered that the rate of forming H_2O_2 was greater in humic than in fulvic acids.[31,34] Tryptophan was the most productive of the amino acids, absorbing light in the longest wave region of the UV spectrum.

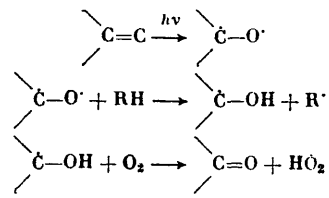
Test samples of surface and ground water with various contents of humic compounds have been irradiated with sunlight[14] and it was discovered that the rate of H_2O_2 formation correlated with the concentration of compounds absorbing light in the near UV region.[15]

The presence of $O_2^{\cdot-}$ in natural waters has been confirmed by adding superoxide-dismutase and also acceptors of $O_2^{\cdot-}$ such as dichlorophenolindophenol, cytochrome C and nitrotetrazolium blue.[14,34,98,99,109]

It was shown that $O_2^{\cdot-}$, as a precursor of H_2O_2, is also formed on photolysis of tryptophan.[34,110,111] Flavins also differ from other compounds of biological origin by having a high efficiency of H_2O_2 formation.[112] The generation of H_2O_2 is accelerated in the presence of electron donors. A high rate of hydrogen peroxide formation through the intermediate formation of $O_2^{\cdot-}$ radicals was observed in photochemical conversions of pyruvate, glyoxalate and other keto-acids especially in the presence of H donors. The accelerating effect of electron donors (H atom) on the rate of formation of H_2O_2 may be linked with their participation in reaction with $^1O_2$ [113] with the formation of $O_2^{\cdot-}$ radicals or with oxidising agents S^+ with subsequent oxidation of the resulting radical–donor by molecular oxygen. Hydrogen peroxide may be formed as the product of the reaction of $^1O_2$ with certain organic compounds avoiding the step of forming $O_2^{\cdot-}$ radicals.[114]



Carbonyl compounds readily take part in a photochemical reaction of a different type. In particular, humic acids form reactive biradicals in the excited state.[115,116]



Many of the resulting radicals undergo various conversions in an aerobic medium in the presence of variable valency metal ions, often with the intermediate formation of H_2O_2 (see Section VII).

Both organic and inorganic compounds may undergo photochemical conversion in natural waters and as a result various reactive intermediates are formed. In an aerobic medium this may lead to the initiation of radical processes of oxidation of the organic compounds dissolved in the water with the accompanying formation of H_2O_2.

Free radicals, chiefly $\dot{O}H$, are the primary products of photochemical conversions of inorganic compounds as a rule.[117–119]

$$NO_3^- \xrightarrow{h\nu,\,H^+} NO_2 + \dot{O}H,$$

$$NO_2^- \xrightarrow{h\nu,\,H^+} NO + \dot{O}H.$$

In addition $O_2^{\cdot-}$ radicals may be formed on photolysis of certain oxides of a semiconductor type.[42,120,121]

$$TiO_2^{2+} \xrightarrow{h\nu} TiO_2^+ + e^- \xrightarrow{O_2} O_2^{\cdot-}$$

Transition metal complexes are subject to photochemical conversion, being decomposed by the action of light with the formation of a metal ion in the reduced form. These processes occur

with the highest efficiency in the case of iron complexes, which absorb light in the near UV region.[122,127]

$$FeOH^{2+} \rightarrow Fe^{2+} + \dot{O}H,$$
$$FeHO_2^{2+} \rightarrow Fe^{2+} + H\dot{O}_2,$$
$$FeC_2O_4^{+} \rightarrow Fe^{2+} + CO_2^{\pm} + CO_2 \text{ etc.}$$

Some copper complexes are reduced in sea water by sunlight.[69]

$$CuX^{+} \rightarrow Cu^{+} + X,$$

where X^- is an anionic ligand.

Manganese[128,129] and chromium[130,131] ions also participate in photochemical conversions.

The role of metal ions in H_2O_2 formation in aquatic media will be discussed in more detail below.

2. The formation of H_2O_2 by redox catalytic processes

Most reactions in natural systems are effected with the participation of dissolved oxygen and variable valency metal ions.

It has been shown that copper ions participate in H_2O_2 formation in sea water.[132] Hydrogen peroxide at a concentration of 10^{-7} M affects the Cu^+/Cu^{2+} ratio and the autoxidation of chloride complexes of Cu^+ and Cu^{2+} leads to the formation of H_2O_2. This process has been studied in detail.[133,134] The mechanism of the process includes the intermediate formation of copper-oxygen complexes.[135]

$$Cu^+ + O_2 \rightleftharpoons (CuO_2)^+ \rightleftharpoons Cu^{2+} + O_2^{\cdot-},$$
$$O_2^{\cdot-} + Cu^+ \xrightarrow{2H^+} Cu^{2+} + H_2O_2,$$
$$CuO_2^+ + Cu^+ \xrightarrow{2H^+} 2Cu^{2+} + H_2O_2.$$

In addition to autoxidation reactions of transition metal ions, oxidation reactions of various readily oxidisable organic compounds, catalysed by metal ions, may contribute to H_2O_2 formation.

Ascorbic acid[136] and dihydroxyfumaric acid[67] are typical examples of reducing compounds which form stoichiometric quantities of H_2O_2 on oxidation by oxygen of the air. These redox ligands (DH^-) react with metal ions, with copper ions in particular, with the formation of the reduced form which participates in the activation of O_2.[138]

$$\left.\begin{aligned} &Cu^{2+} + DH^- \rightleftharpoons CuDH^+ \underset{-H^+}{\dashrightarrow} Cu^+ + D^{\cdot},\\ &CuDH^+ + Cu^{2+} \underset{-H^+}{\longrightarrow} 2Cu^+ + D, \end{aligned}\right\} \text{reduction of metal}$$

$$\left.\begin{aligned} &Cu^+ + O_2 \rightleftharpoons CuO_2 \dashrightarrow Cu^{2+} + O_2^{\cdot-},\\ &CuO_2^+ + DH^- \underset{-H^+}{\longrightarrow} Cu^+ + D + H_2O_2. \end{aligned}\right\} \text{activation of } O_2$$

A radical mechanism forming H_2O_2 is also possible by the catalytic oxidation of reducing agents which are efficient electron donors (H atom).[137,138]

$$O_2^{\cdot-} + DH^- \rightarrow HO_2^- + D^{\cdot-},$$
$$D^{\cdot-} + Cu^{2+} \rightarrow Cu^+ + D.$$

Chain processes are in essence similar reactions, the termination of which occurs by destruction of the chain propagators ($O_2^{\cdot-}$, Cu^+, D^-) on heterogeneous impurities or by recombination with one another.

The catalytic oxidation rate of ascorbic acid and related reducing agents with oxygen is far higher than with hydrogen peroxide, which also leads to the accumulation of H_2O_2 in the medium.

3. The role of microorganisms in the formation of hydrogen peroxide

Van Baalen and Marler[12] and Sinel'nikov[78] were the first to reach the conclusion that the role of algae in the process of forming H_2O_2 is important. Even earlier Mehler[139–141] had demonstrated in classical studies the ability of chloroplasts to produce H_2O_2 under the action of light. It was later shown that, in the process of forming H_2O_2, oxygen may oxidise reduction equivalents in the electron transport chain of photosynthesis.[142–146]

The photochemical formation of H_2O_2 was demonstrated for the first time in 1973 by Patterson and Meyers on irradiating the blue-green alga *Anacystis nidulans* with light of wavelength 620–675 nm.[147]

By adding 3-(3,4-dichorophenyl)-1,1-dimethylurea (DCMU) to block Photosystem II, it was shown that the photosynthetic apparatus was the source of reduction equivalents for the formation of H_2O_2 when reducing O_2. The rate of forming H_2O_2 grew on adding to the medium either inhibitors of the Calvin cycle (PCMB, iodoacetamide) or an inhibitor of phosphorylation (carbonyl cyanide *m*-chlorophenylhydrazone) and also under conditions of CO_2 deficiency. Components of the Calvin cycle suppress H_2O_2 formation.

The conclusion was reached[147] that reduction equivalents, for which O_2 and CO_2 compete, are produced in algae cells by the action of light. It was proposed that the centre of H_2O_2 formation was localised in the final reducing step of Photosystem I.

In addition to *Anacystis nidulans* a large number of fresh water[148] and marine[70] algae were discovered to be capable of producing H_2O_2 in the external medium in the process of photosynthesis. No correlation was noted between the morphological features or the accepted nomenclature differentiation of the algae and their ability to produce H_2O_2. The kinetic characteristics of the process of forming H_2O_2 for the various algae species differed markedly.

It was also discovered[70,148] that certain types of algae are able to produce H_2O_2 not only by the action of light but also in the dark. This shows that the H_2O_2 formation by algae may not be linked solely with photosynthesis.

Palenik and collaborators[149,150] have shown the role of intracellular L-aminoacid oxidases (deaminases) in the formation of H_2O_2 by the reaction:

$$\underset{\text{(L-aminoacid)}}{R{-}CHNH_3COO} + H_2O + O_2 \rightarrow \underset{\text{(α-ketoacid)}}{R{-}CHOCOO^-} + NH_4^+ + H_2O_2.$$

The ammonium ion liberated in the reaction is captured by algae cells (*Pleurochrysis carterae*) and used as a source of nitrogen.

Other surface deaminases are known which participate in the transformation of nitrogen in seawater. These include amino-oxidases, which oxidise primary amines (ethanolamine, putrescine) in the presence of phytoplankton and the polyaminooxidase of the diatom *Thalassiosira weissflogii*, which uses spermine and spermidine as substrates with the formation of H_2O_2 as a side product.[151]

The activity of the surface deaminases is usually large when the growth of phytoplankton is limited by nitrogen. For this reason these processes are usually effected in regions with a low content of inorganic forms of nitrogen (for example in the open ocean).

The quantitative contribution of processes involving deaminases has not been established. The formation of H_2O_2 in abiotic processes occurs at a greater rate than in biotic processes in surface water while at depth the biotic contribution becomes dominant.[15] Palenik and collaborators[149] have established that the

rate of H_2O_2 formation by *Pleurochrysis carterae* reaches $(1-2) \times 10^{-9}$ mole litre^{-1} h^{-1}. Since aminoacid oxidases are widely distributed among microalgae and other deaminase reactions may lead to the formation of H_2O_2, the biological production of H_2O_2 in the dark may be significant in several cases.

It has been shown[152,153] that non-photosynthetic photochemical processes for H_2O_2 formation are also effected efficiently in algae cells by the action of the UV component of sunlight, as a result of the photolysis of water-soluble intracellular components. A portion of the resulting hydrogen peroxide is decomposed by intracellular enzymes (catalase, peroxidase, see below) but a portion of it is eliminated into the external medium. The rate of photoproduction of H_2O_2 and its stationary concentration in the medium depends on both the species of algae and on the age of the culture.[109] The precursor of H_2O_2 in algae cells is the $O_2^{\cdot-}$ radical.

It has been shown[154] that H_2O_2 is formed on autoxidation of ferredoxin. On the other hand the formation of H_2O_2 was detected in membrane preparations from blue-green algae *Anabena* with the ferredoxins washed away.[146]

V. The role of hydrogen peroxide in the formation of a single "cell–medium" redox system

The hydrogen peroxide cycle in natural waters is closely linked with the exchange of oxidation–reduction equivalents between the aquatic organisms and the surrounding medium. It is possible to draw conclusions on the principles of such a redox exchange both within the limits of a cell and between a cell and the external medium which have a bearing on the general principles of the functioning of the living cell.[155]

1. Intracellular redox processes involving H_2O_2

The redox state of the intracellular medium is determined by the interconnected distribution of the oxidised and reduced forms of variable valency metal ions entering the active centres of oxidation–reduction enzymes and labile redox metabolites, such as NADP/NADPH, cytochrome C (Fe^{3+}/Fe^{2+}), ATP/ADP + P (P is phosphate), etc. The oxidation state of cytochrome C determines the respiration rate of a cell and accordingly the rate of phosphorylation processes. Phosphate plays a regulatory role in maintaining the intracellular redox state, its exchange with the external medium is regulated by the cell.

The interaction of intracellular redox systems implies the exchange of oxidation–reduction equivalents between the cell structures. In other words, a system exists for the regulation of the intracellular redox state on which the occurrence of such complex biochemical processes as cell respiration, protein synthesis and the secretion of hormones and neurotransmitters depend.

Hydrogen peroxide together with O_2 play a special role in the redox processes in the cell.

The intracellular content of H_2O_2 in algae cells is determined not only by photochemical processes but also by other oxygen-dependent processes in the cell. Among them a central place is occupied by biological oxidation processes involving O_2, viz. the oxidase (oxygen acts as an electron acceptor), oxygenase (oxygen acts as a donor of an O atom) and the peracid oxidation of lipids occurring with the participation of O_2 and free radicals. The enzymes known to form H_2O_2 in metabolic processes involving O_2 are glycolate oxidase, α-gulonolactone oxidase, aldehyde oxidase, xanthine oxidase, D-aminoacid oxidase, monoamine oxidase, galactose oxidase, prostaglandin synthetase, dihydroorotate dehydrogenase and certain others.

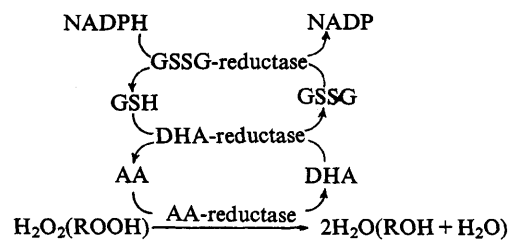
Hydrogen peroxide is formed in the living cell in the mitochondria, microsomes and in peroxysomes.[156–158] The formation of H_2O_2 also occurs on oxidation of NADPH in the endoplasmic reticulum. It was shown[159] that up to 15% of the total oxygen required by a cell for life is converted into H_2O_2.

The H_2O_2 content of different cells under dark conditions varies within wide limits, from 10^{-9} to 10^{-6} M.[159,160] In the case of algae the stationary H_2O_2 concentration reached in the medium on illumination with sunlight varies, depending on the species of algae and their functional state, within the limits 3×10^{-6} to 3×10^{-5} M.[109] It is probable that such high H_2O_2 concentrations are also reached in algae cells. Many algae express H_2O_2 to the external medium which indicates that processes using peroxide oxidation equivalents are weakly developed in them.

The enzymes superoxide dismutase, catalase and peroxidase serve as physiological regulators of the intracellular hydrogen peroxide content.[161,162] In a comparative study of the catalase activity of various species of blue-green and green algae, it was discovered that the blue-green were far less active.[163] In addition the catalase of blue-green algae proved to be more sensitive to the action of solar UV irradiation than did that of green algae.[164]

The deficiency of catalase activity in many algae is compensated by glutathione- and ascorbate-dependent peroxidases.[164] A high glutathione reductase activity and high concentration of glutathione (3×10^{-3} M) were discovered in the blue-green algae *Nostoc muscorum*[165] which is characterised by a high rate of H_2O_2 formation both in the dark and in the light.[148] This alga does not contain glutathione peroxidase,[166] but displays high dehydroascorbate reductase and ascorbate oxidase activity.

An overall scheme has been suggested for regulating H_2O_2 content in the cells of blue-green algae.[166]



where GSH (GSSG) and AA (DHA) are the reduced (oxidised) forms of glutathione and ascorbic acid respectively.

According to such a mechanism relatively large quantities of the H_2O_2, which passes into a cell at a rate up to 30% of the photosynthetic transport of electrons, may be destroyed.

In mammalian cells glutathione has a leading role in maintaining the stationary H_2O_2 concentration. This compound is the main stock of mobile sulphydryl groups and the dependence of the rate of the glutathione peroxidase reaction on hydrogen peroxide concentration complies with an equation of zero order.[167] Up to 70% glutathione peroxidase is found in the cytosol and 30% in the mitochondria. A feature of glutathione peroxidase is the ability to use fatty acid hydroperoxides as oxidising agents. This plays a decisive role in the regulation of peroxide oxidation of lipids. A supply of reduction equivalents in the form of NADPH is necessary for glutathione peroxidase to function.

Various amines and phenols are typical substrates for the peroxidase reaction.[168] The mechanism of action of peroxidase has

been discussed in detail.[134] A peroxidase function is possessed not only by the peroxidases themselves but also by other haem-containing proteins, particularly cytochrome P-450.[169] Chloroperoxidase occupies a special place among the peroxidases as it catalyses the decomposition of H_2O_2 as well as substrate oxidation.[170]

In its turn catalase also possesses peroxidase activity in relation to certain substrates.[171] Thus catalase participates in the peroxidase oxidation of ethanol,[162] causing the development of an unbalance in the redox state of the cell.[172]

The kinetic feature of catalase, in contrast to the peroxidases, is its absence of substrate saturation. When exogenous hydrogen peroxide passes into the cell, on average 40–60% of its metabolism is linked with catalase and the remaining peroxide participates in peroxidase oxidation processes.[173]

In addition to direct H_2O_2 formation in enzymic processes a large portion of the intracellular hydrogen peroxide is formed by the disproportionation of O_2^-.[153] According to one estimate,[160] the stationary concentration of O_2^- in liver mitochondrial membranes is 10^{-11} M. The O_2^- radicals formed in the cell participate both in dismutation reactions, catalysed by superoxide dismutase, and in the initiation of free radical processes.[174,175] In spite of the fact that the O_2^- radical is a natural metabolite of the cell, it is toxic in excess.[174,176]

Hydrogen peroxide may display a toxic effect at concentrations significantly exceeding the physiological.[177,178] At high concentrations hydrogen peroxide inactivates the transformation of DNA[179] and also induces chromosomal aberrations in ascites tumour cells.[180] Other physiological changes by the action of H_2O_2 are known.[178]

On the other hand the physiological concentration of H_2O_2 is not only non-toxic but is also necessary for normal cell activity.[155] It has been shown[172,181–183] that hydrogen peroxide participates in the regulation of cellular respiration. It was also shown that by itself H_2O_2 does not induce the peroxide oxidation of lipids,[184] it does not destroy mitochondria[185] but it is used by plant cells to induce intracellular reduction processes.[186]

It is known that a normal (stationary) level of peroxide oxidation of lipids is inherent for the cell membrane.[169] This is necessary for the regulation of the various cell functions and for the whole organism such as the setting of the metabolic state of the respiratory chain, changes of membrane permeability, etc. It is also known that lipoperoxides are obligatory intermediates in hormone biosynthesis (prostaglandin, progesterone), and participate in the hydroxylation of the cholesterol group of steroids.[175]

Under hyperbaric conditions a simultaneous increase occurs in the concentration of H_2O_2 and of free radicals in the cell which leads to a reduction in the NADPH/$NADP^+$ ratio. In its turn this causes a reduction in the content of reduced glutathione in the cell and correspondingly to the activation of peroxide oxidation of lipids. The reaction of O_2^- with H_2O_2, catalysed by metal complexes, may participate in the initiation of peroxide oxidation of lipids.[187,188] It was shown[184] that this reaction is also responsible for the inactivation of algae cells under the action of solar UV radiation.

This same reaction seemingly serves as a basis for the phenomenon of "photooxidising death", the sudden cessation of the "bloom" of blue-green algae in a deficiency of CO_2.[189,190] Under these conditions both hydrogen peroxide and its precursor, the O_2^- radical, are formed in the cell. The cells become sensitive to the action of light not only in the absence of CO_2 but also by the action of DCMM, the inhibitor of Photosystem II.[190] "Photooxidative death" is accompanied by decolourisation of the photosynthetic pigments, but this process is not linked with cell death.

The source of CO_2 in the "bloom" process of blue-green algae is not so much atmospheric carbon dioxide gas as the activity of bacteria. The more extended and stable bloom of eutrophated reservoirs in particular is linked with this circumstance.[191] The high sensitivity of blue-green algae to photooxidative inactivation explains why algae of this type are better able to function under conditions of oxygen deficiency, in a quasi-reducing medium and under weak illumination.

2. Exchange of oxidation–reduction equivalents between a cell and the external medium

Isolation of a cell from the extracellular medium is essential to preserve the continuity of the intracellular redox state. The presence of exchange with an extracellular redox system increases the possibility of a cell changing the direction of the intracellular redox processes but the danger of an adverse action of the external medium on the internal redox state of the cell is contained.

Many intracellular metabolic processes are linked with the transmembrane transfer of oxidation–reduction equivalents from the cell to the external medium and *vice versa*.

Hydrogen peroxide, together with O_2, serves as a carrier of oxidation equivalents in nature. Consequently the problem of H_2O_2 transport within the limits of the cell and through the cell membrane is important. It has been shown[155] that the mitochondrial membrane is weakly permeable to H_2O_2 while other biological membranes are fairly readily permeable. So from hyperoxic conditions, where large quantities of H_2O_2 are formed,[192] 10–40% hydrogen peroxide penetrates the cytosol and may then pass from the cytosol to the external medium[157] The high permeability of cell membranes for H_2O_2 also follows from the data on the kinetics of the formation and destruction of hydrogen peroxide in cultures of algae.[84,153]

Many intracellular metabolic processes are linked with the transmembrane transfer of reduction equivalents from the cell into the external medium. The obvious role of such exchange is displayed in the vital activity of microorganisms. Thus the initial stage of the multiplication of many bacteria is linked with the formation of a reducing medium.[193] If a substance having a reducing nature is introduced into the culture medium then the induction period in the multiplication of bacterial cells disappears but on introducing an oxidising agent it is increased.[193] In the growth process of bacterial cultures the redox potential of the medium is reduced and anaerobic strains are characterised by large shifts of potential in comparison with aerobic.

It has been shown[194] that ferricyanide is reduced in yeast cultures. Addition of citrate, a component of the Krebs cycle, accelerates this process, although citrate itself does not react with ferricyanide. This experiment shows that reduction equivalents formed in the Krebs cycle are partially eliminated into the external medium. The flux of reduction equivalents is determined by the intracellular redox system and is increased on inhibiting the processes transferring them to O_2.

Transfer of reduction equivalents to the external medium serves as one of the main processes securing the optimum redox state of the cell. An essential role in transmembrane transfer of reduction equivalents is assigned to the ascorbate/dehydroascorbate redox system.[155]

The available data show that a close interconnection exists between the metabolic activity of microorganisms and the redox state of the surrounding aqueous medium. The existence of a single

redox system for the intra- and extracellular media is in agreement with the ideas of Skadovskii,[195] who considered a reservoir to be a biological individual, and also with the hypothesis of Academician Vernadskii that natural water is a bioinert body.[196] In fact a natural aquatic medium like a monoculture of microalgae, must be considered as a specific organism possessing an insignificant integration of cells. In this "organism" extrametabolites of hydrobionts may play the role of "media hormones" regulating the composition of cenoses.[197] A decisive role in setting the qualitative state of an aquatic medium under natural conditions belongs to the smallest forms of hydrobionts, since, as emphasised,[195] the margin between physicochemical and biological processes is eroded as the dispersity of the hydroecosystem is increased.

Possible mechanisms for the participation of microorganisms in the redox systems within reservoirs include the following five main types of reaction:[151]

1) the excretion of a compound of a reducing nature into the external medium,

2) transmembrane electron transfer to an extracellular electron acceptor,

3) catalysis of extracellular redox reactions by an enzyme localised on the external surface of the cell,

4) catalysis of redox reactions by an extracellular enzyme bound to the cell surface with the participation of a reducing agent excreted by the cell,

5) catalysis by the cell surface of non-enzymic redox reactions with the participation of extracellular redox factors.

The expression of soluble organic compounds into the external medium is a characteristic of growing cells. According to one estimate[198,199] 5–10% of the total carbon of phytoplankton is expressed into the medium and serves as a source of reduction equivalents for carrying out chemical and biological reactions. Many of the excretions participate in complex-forming reactions with metals[200–202] and thereby affect their redox catalytic activity. Thus, certain aquatic microorganisms are capable of producing siderophores binding iron ions.[203] Other bacteria using sulphate as a source of electrons eliminate sulphides which participate in the reduction of metals.[204]

Electrons formed in the cell in photoreactions (or in respiratory processes) may be transferred to the outside of the cell surface and used in the reduction of various cellular substrates. These electron-transfer processes are effected by trans-plasma membrane reductase, which transports electrons from the intracellular donor (NADH, NADPH) to an extracellular electron acceptor. Such membrane-impermeable oxidising agents as ferricyanide, cytochrome C and copper complexes are reduced directly on the cell surface.

Microorganisms may also catalyse extracellular redox reactions (and hydrolysis reactions) through ectoenzymes immobilised on their outer surfaces. A whole class of surface enzymes exists, such as deaminases, typical of phytoplankton which catalyse the elimination of NH_4^+ on oxidation of nitrogen-containing organic compounds[151] with simultaneous reduction of O_2 to H_2O_2 (see Section IV).

In contrast to the intracellular enzymes functioning in strictly controlled conditions (ionic strength, pH, content of Ca^{2+}, Mg^{2+}, etc.) the ectoenzymes are closely dependent on the state of the external medium. In particular metal ions contained in the aqueous medium may lead to inactivation of ectoenzymes. For example, alkaline phosphatase in a phytoplankton culture or in full-scale samples are inhibited by copper(II) ions even at very low concentrations in the medium.[205] The high sensitivity of the ectoenzymes to the presence of metals may determine the ecotoxic properties of the latter, differing from their toxicity towards metabolic processes such as photosynthesis or respiration, which are integrated with a large number of biochemical reactions.

The reduction of extracellular substrates may also occur directly on the reduced parts of the cell surface without the participation of enzymes. Such reactions may proceed both in the dark and in the light. The reactions are non-enzymic and require regeneration of the reducing agent. The reduction of ions of copper and iron by microalgae cells may serve as an example of such reactions.[151]

Many forms of microalgae, including diatoms,[150] pyrophytes[206] and blue-green algae[207] reduce intracellular electron acceptors. Using *Thalassiosira weissflogii* as an example it was shown[208,209] that three types of reducing agents participate in these processes:

1) reducing agents eliminated by cells and not regenerated in the absence of microalgae,

2) reduced parts of the cell surface which may be inactivated by pretreatment of the algae with oxidising agents,

3) surface reductases, the activity of which does not depend on illumination.

There are compounds dissolved in the culture medium of *T. weissflogii* after filtration of the algae which reduce complexes of copper(II)[208] and iron(III).[209] The chemical nature of the reducing compounds is unknown. It has been suggested that they may be polysaccharides forming part of the cell wall (polygalacturonic acid) or a sulphydryl-containing protein or amino acid.[208]

Two types of reduction centre are encountered on the surface of cell membranes. In the alga *T. weissflogii* such centres are not regenerated, but are oxidised on preliminary treatment with copper(II) ions and such treatment does not affect the ability of cells to reduce metal complexes by the enzymic route. The surface redox reactions of cells are catalysed by reactive groups in the case of *Dunaliella tertiolecta*.[208]

The reduction of copper(II) occurs only in light (>425 nm) and the copper(I) ions formed are oxidised by molecular oxygen. Regeneration of the reduced groups on the cell surface is effected by extracellular photochemical processes. Intact cells of *T. weissflogii* reduce membrane-permeable copper(II) and iron(III) complexes with a potential in the range from −10 to +100 mV both in the light and in darkness by enzymic processes involving transmembrane reductases.[151] Plasma membrane redox activity is linked with the consumption of nitrate by the algae,[210] such that the rate of formation of reduction equivalents by the cell reaches 10% of the rate of reduction of nitrate.[15]

Transmembrane reductases are also present in other microalgae and macrophytes and probably in water bacteria. The evidence for this is the oxidation and reduction by cells of various membrane-permeable compounds. Plasma membrane redox systems also participate in the reduction of variable valency metal ions in cells of higher plants and mammals.[211]

Plant cells are able to reduce iron(III) chelates, including Fe EDTA.[212] This occurs in two ways. The first is by the action of ectoenzymes bound to the cell surface, forming NADH under the action of malate dehydrogenase and using the NADH in the reduction of iron(III) chelates by ferri-NADH oxidoreductase.[213] The second route is the formation of a superoxide radical in the plasma membrane redox system, this radical then participates in the reduction of Fe(III).[214] The extracellular reduction of iron is closely linked with its consumption by phytoplankton cells.[215–217] The subsequent oxidation by hydrogen peroxide of metal ions reduced by ectoenzyme action serves as an important link in the cycling of variable valency metal ions in aquatic media.

A numerical assessment has been made of the rate of formation of Cu(I) in coastal sea waters by the action of cellular water-soluble reducing agents.[69] This rate was 2 nmole litre^{-1} d^{-1} which proved to be 10 times greater than the rate expected for plasma membrane reductase of algal cells at a population of 10^7 per litre water. The conclusion was drawn that it was necessary to allow for the contribution of bacterioplankton and also of abiotic processes for a complete estimate of the reduction rate of copper.[151]

Confirmation has been obtained in the case of manganese of the participation of ectoenzymes both in its oxidation and in reduction. The manganese cycle in the illuminated zone of natural waters includes a redox transition between dissolved Mn(II) and particulate Mn(IV).[218–223] The oxidation of Mn(II) is effected by the photosynthetic activity of fresh water algae.[219] The resulting Mn(IV) forms part of the composition of extracellular carbonic anhydrase which catalyses the conversion of bicarbonate to CO_2. In oxygenated[220] and anaerobic[219] sea water the oxidation of Mn(II) by aquatic microorganisms is effected by a different mechanism. The measured rate of Mn(II) oxidation is 100 times greater than the rate of uptake of manganese by phytoplankton[220] and far greater than the rate of abiotic oxidation of Mn(II) in a sterile medium.

Two processes make the main contribution towards maintaining manganese in the reduced state in water close to the surface. These are the photoreduction of manganic oxide[222] and the reductive dissolution of MnO_x by hydrogen peroxide[224] produced particularly by phytoplankton aminooxidase.

Hydrogen peroxide is also implicated in the intra-algal iron cycle, participating in both the oxidation of Fe(II)[225,226] and in the reduction of Fe(III) in the light. Biota may promote these reactions through the activity of their own extracellular enzymes.

VI. Processes decomposing hydrogen peroxide in natural waters

The formation of H_2O_2 in natural waters by photochemical and catalytic processes is accompanied by processes decomposing it. Judging by the diurnal dynamics of the H_2O_2 content of aquatic media the processes forming and decomposing H_2O_2 are balanced and one may speak of the stationary (or quasi-stationary) concentration of hydrogen peroxide. This concentration depends not only on the rate of H_2O_2 formation but also on the kinetic characteristics of the decomposition process.

The decomposition of H_2O_2 in natural waters has been discussed many times.[31,80,82,152,227–230] The half-conversion time of H_2O_2 in eutrophic waters is about 1 h.[31] It has been shown[229] that in oligotrophic waters in the dark the half-conversion time for decomposition of hydrogen peroxide is 130 h and 10 h in fresh water. These data show that, as in the process of formation, abiotic and biotic processes may participate in the decomposition of H_2O_2.

1. Abiotic processes for the decomposition of H_2O_2 in natural aquatic media

The whole data accumulated on the catalysis of H_2O_2 decomposition by transition metal ions may be used to describe the abiotic processes of H_2O_2 breakdown in natural media.[134,231] Natural waters are characterised by a low metal ion concentration, neutral pH value, indefinite composition and low ligand concentration. Several monographs and reviews have been devoted to the study of the forms in which metal ions exist in natural waters.[200,201,232,233]

It may be concluded on the basis of the data available that of the metal ions, which are potential catalysts of H_2O_2 decomposition, only copper ions and chelate complexes of iron are found in a homogeneous state in natural aquatic media.[234]

The relative contribution of the various metal ions to H_2O_2 decomposition has been studied.[235] It was shown that the catalytic breakdown of hydrogen peroxide in neutral aqueous solution is effected mainly by microcolloidal iron hydroxide and by copper ions. In the first case the process is effected by a nonradical mechanism and in the second through the formation of free radicals.[132,236]

$$CuOH^+ + HO_2^- \rightarrow Cu^+ + O_2^{\cdot-}.$$

The reaction of Cu^+ and H_2O_2 has been studied in detail.[133] The primary product of this reaction is the CuO^+ species which may be considered as a hydrolysed Cu^{3+} ion.[1] In the absence of extraneous reactive substrates this species oxidises water (and is decomposed) with the formation of an OH radical.[133,237]

$$Cu^+ + H_2O_2 \xrightarrow[-H_2O]{} \begin{array}{c}(OH^-)CuO^+\\ \uparrow\downarrow -H^+\\ CuO^+\\ \uparrow\downarrow H^+\\ CuOH^{2+}\end{array} \xrightarrow[H_2O]{} CuOH^+ + \dot{O}H$$

In a similar manner the OH radical appears in the reaction of Fe^{2+} and H_2O_2 in neutral medium as a result of the conversion of a first-formed particle of the type of $FeH_2O_2^{2+}$ or FeO^{2+}.[1]

In the presence of an H donor species such as CuO^+ and FeO^{2+} react with the donor by a two-electron mechanism.[237]

$$CuO^+ + DH_2 \xrightarrow[-H_2O]{} Cu^+ + D.$$

In this case hydrogen peroxide is consumed in the catalytic oxidation of the donor without the formation of intermediate free radicals.

The H donors themselves (redox ligands) may participate in the formation of free radicals[239] by virtue of the specific property of partial charge-transfer of the complexes.[1,238]

$$Cu^{2+} + DH^- \rightarrow CuDH^+,$$
$$CuDH^+ + H_2O_2 \xrightarrow[-H_2O]{} Cu^{2+} + D^{\cdot} + \dot{O}H.$$

It must be emphasised that the metal ion in this reaction remains in the oxidised state. The introduction of redox ligands into an aqueous medium containing H_2O_2 and copper ions may initiate the formation of OH radicals in it, which play an important role in processes of self-purification of natural waters.[240] On the other hand the introduction of H_2O_2 into a medium containing the H donor DH_2 may lead to the oxidation of this reducing agent without the formation of free radicals.

$$Cu^+DH_2 + H_2O_2 \xrightarrow[-2H_2O]{} Cu^+ + D.$$

Strictly speaking these two extreme cases also illustrate the difference in principle between the two redox situations which may occur in natural waters, viz. an oxidative and a quasi-reductive state of the aquatic medium (see below). In the first case reducing agents initiate the breakdown of H_2O_2 into free radicals, in the second hydrogen peroxide is consumed in the oxidation of a reducing agent introduced into the medium.

In addition to catalytic breakdown, the decomposition of H_2O_2 into free radicals by solar radiation is important under natural conditions.[96,241,242]

2. The role of microalgae in H_2O_2 decomposition

The separation of the biotic and abiotic contributions to the kinetics of H_2O_2 decomposition[230] observed in natural waters is extremely arbitrary since natural water is in principle a catalytic oxidation–reduction system of an open type. As a result of the activity of microorganisms there is a constant exchange in it of oxidation–reduction equivalents between the biota and the aquatic medium. It is possible to interrupt this flux by filtration of water samples through a fairly fine porous filter (usually 0.22 μm). However, stopping the flux changes the observed kinetic picture in an appreciable manner. Consequently more adequate information may be obtained by combining full-scale investigations on H_2O_2 decomposition in natural waters[80,82] with the investigation of model systems in cultures of algae and bacteria.

A detailed study of the kinetics of H_2O_2 decomposition by microalgae has been carried out.[152,153] The main conclusion of the investigations was the existence of two basic types of biotic processes of H_2O_2 decomposition which differed in the dependence of reaction rate on the initial hydrogen peroxide concentration.

For young cultures of algae the reaction is first order with respect to H_2O_2 concentration and its rate (w_1) is proportional to the biomass of the microorganisms (chlorophyll concentration).[152] In the case of natural algocenoses and old cultures there is in addition a contribution from a reaction of zero order with respect to H_2O_2 concentration,[84,153] the rate of which (w_0) is also proportional to the biomass of algae. The relative contributions of the first and zero order reactions depends on the initial hydrogen peroxide concentration (the greater its concentration, the greater the contribution of the first order reaction) and also on the state of algocenosis or of the alga culture.

Investigations carried out with whole and powdered algae and adding inhibitors of various reactions showed[84] that the first order reaction is caused by the action of catalase, while peroxidase processes make the main contribution to the zero order reaction. The catalase reactions are localised in the cell membrane fractions and the peroxidase in the water soluble fraction (not separated on centrifugation for 5 min at 10000 rpm).

Kinetic saturation of the peroxidase reaction at relatively low H_2O_2 concentrations is linked with the limited rate of intake (w_r) of peroxidase substrates (H donors). Experiments with an added dehydrogenase inhibitor (*m*-cresol) showed[84] that substrates of the peroxidase reaction pass into the medium as a result of the action of dehydrogenases.

It was shown[82] that reducing agents, H donors, react efficiently with H_2O_2 and are not only formed in algae cells but are also eliminated from them to the external medium. The rate of formation of reducing compounds (w_r) is proportional to the algal biomass and depends on the ecological situation in algocenosis. Blue-green algae are the most efficient producers of reducing agents.

The rate of decomposition of hydrogen peroxide in natural waters (w_d) is determined by the rate of formation of reducing compounds and the rate of their catalytic decomposition (w_1).

$$w_d = w_0 + w_1 = w_r + \varkappa_1 [H_2O_2],$$

where $\varkappa_1$ is the observed first order rate constant.

In a quasi-stationary state the rate of decomposition of H_2O_2 coincides with its rate of formation (w_f).

$$w_d = w_f = w_r + \varkappa_1 [H_2O_2].$$

From which it is obvious that

$$[H_2O_2] \simeq (w_f - w_r)/\varkappa_1.$$

In other words, the content of hydrogen peroxide in a natural aquatic medium is determined by the difference in the rates of flux within the reservoir of H_2O_2 and reducing agents which react with O_2 far less efficiently than with H_2O_2. The two redox states of the aquatic medium, which differ in principle, are distinguished by the ratio of these rates (see above).

As was indicated, a change in the redox state of the medium accompanies the bloom of blue-green algae and is caused by their ability to produce reducing compounds in the external medium which react efficiently with H_2O_2.

VII. Radical processes for self-purification linked with the hydrogen peroxide cycle

As is seen from the data given in Sections IV and VI the processes of forming and breaking down H_2O_2 in natural waters are accompanied by the formation of free radicals in the medium. Of the other processes leading to free radicals mention must be made of the possible role of radiochemical processes, collapse phenomena (effects of cavitation of gaseous microbubbles in deep tanks[243,244]), acoustic phenomena,[245,246] uptake of reactive atmospheric gases[94,247–249] and biological processes.[250]

Radiochemical processes are only effected after radiation contamination of a locality,[249] since the natural radiation background is small. The contribution of collapse and acoustic phenomena to the formation of free radicals has not been studied quantitatively. The uptake by water of atmospheric ozone may make a significant contribution.[249,251]

In the absence of contamination, O_3 dissolved in water breaks down by a radical chain mechanism with the participation of $\dot{O}H$, and $O_2^{\cdot-}$ radicals in chain propagation reactions.[248,252–254] Ozone reacts with reducing agents (H donors), when present in the water, with subsequent breakdown into radicals.[248]

$$O_3 + DH^- \rightarrow HO_3 + D^{\cdot},$$
$$HO_3 \rightarrow \dot{O}H + O_2.$$

Quantitative assessment of the contribution of the various routes to the formation of free radicals is difficult.[96] For practical purposes it is important to have an estimate of the stationary concentration of free radicals. It is possible to obtain this assessment both by direct measurements and by calculation, determining independently the rate of formation of radicals and the observed rate constant of their destruction in the medium in question.

1. Free radicals in natural waters

The methods of detecting free radicals in natural waters are based on the use of various types of radical "trapping". Depending on concentration, the "trap" may intercept either all the radicals formed in the medium or only a small proportion of them. In the first case the rate of formation of radicals in the system is determined from the rate of supply of the trap or the rate of accumulation of its reaction product with the radical. In the second case, knowing the

rate constant for the reaction of the trap with a given radical, it is possible to assess the stationary concentration in the medium.

Mill and collaborators[93] applied the trap method to the assessment of the free radical content of natural waters for the first time. They used toluene to intercept $\dot{O}H$ radicals and pyridine to intercept RO_2 radicals.

The dye *p*-nitrosodimethylaniline (PNDMA), which is widely used for the same purpose in radiation chemistry, was used as a trap.[81] This dye is gradually decolourised in samples of natural waters, chemical–biological processes making a decisive contribution. The rate is sharply reduced in filtered samples.

The trapping method has been significantly improved in recent years. The use of stable nitroxyl radicals for trapping gives the best results.[255–262] The stable product formed with this or with any radical may be identified chromatographically and the nature of the initial radical is thereby clearly established. Using such traps data have been obtained on the stationary concentrations and formation rates of free radicals and of other reactive intermediates in various natural waters (Tables 2 and 3).

Table 2. Typical values for the concentrations of reactive intermediates in natural surface waters.[107,261,262]

Reactive species	Rate of formation, mole litre^{-1} sec^{-1}	Decay rate constant, sec^{-1}	Concentration in surface layer, M (depth 1 m)	Note
*S	$(3–300)\cdot10^{-9}$	$5\cdot10^{5}$	$(1–5)\cdot10^{-13}$	
$^1O_2$	$(3–300)\cdot10^{-9}$	$2.5\cdot10^{5}$	$10^{-14}–10^{-13}$	sea environment
	–	–	$10^{-14}–10^{-12}$	fresh waters
	–	–	$8\cdot10^{-14}(5\cdot10^{-14})$	fresh waters [263]
ROO	$10^{-11}–10^{-10}$	0.1–1(?)	$10^{-11}–10^{-10}$	
OH	–	10^{7}	$2\cdot10^{-19}$	sea environment
	$10^{-11}–10^{-10}$	$(0.2–2)\cdot10^{5}$	$(2–6)\cdot10^{-16}$	fresh waters
	–	–	$2\cdot10^{-18}(4\cdot10^{-17})$	fresh waters [263]
e_{aq}^-	$(5–10)\cdot10^{-11}$	$(0.5–1.5)\cdot10^{7}$	$(1–2)\cdot10^{-17}$	
	–	–	$1.2\cdot10^{-17}(5.2\cdot10^{-18})$	[263]
$O_2^{\cdot-}$	$10^{-11}–10^{-7}$(?)	10^{-3}(?)	$10^{-9}–10^{-8}$(?)	
	0.14–0.79 * nM kg^{-1} min^{-1}	–	–	sea environment [262]

Note. Numerals in square brackets denote references.

measured by the rate of decolourisation of PNDMA in distilled water. The proportion of radicals participating in the reaction with inhibitors of radicals contained in a water test sample is determined by the rate of decolourisation of PNDMA in natural water. Since the values of k_i for the overwhelming majority of traps lie in the range 10^8 to 10^{10} M^{-1} sec^{-1},[264] it may be assumed that $k_i \sim 10^9$ M^{-1} sec^{-1} and, depending on the size of the $\Sigma k_i[S_i]$ parameters, the observed concentration in aqueous medium of the trap for $\dot{O}H$ radicals (S_e) is assessed from:

$$S_e = \Sigma[S_i] \approx 10^{-9}.(\Sigma k_i[S_i]).$$

Table 3. Measured and estimated stationary concentrations and production rates (w) of $\dot{O}H$ radicals under the action of sunlight in samples of sea and fresh water.[96]

Sample	[OH], 10^{18} M	$w_i\times10^{12}$ mole litre^{-1} sec^{-1} (nM h^{-1})	Contribution from various radical sources, % (source concentration, μM)			
			NO_3^-	NO_2^-	H_2O_2	DOC*
Open ocean surface water (Sargasso Sea)	1.1±0.1	2.8±0.2 (10.1)	1 (0.05)	–	4 (0.05)	95 (200)
Gulf stream surface water	1.2	3.1 (11.2)	–	–	–	–
Deep water samples (Sargasso Sea, 700 m)	6.3±0.3	15.9±0.7 (57.2)	19 (10)	1 (0.01)	3 (0.1)	77 (70)
Deep Gulf Stream water samples (700 m)	5.8	14.7 (52.9)	–	–	–	–
Subtropical coastal waters (Biscayne Bay, Florida, high tide)	9.7±1.2	24.4±3.0 (87.8)	2 (2.0)	–	2 (0.2)	96 (300)
Ditto (low tide)	13.7±1.7	34.5±4.3 (124.2)	–	–	–	–
Coastal waters of temperate latitudes (Vineyard Sound, Massachusetts)	10.6	26.5 (95.4)	–	–	–	–
Equatorial upwelled water	7.4	18.6 (67.0)	3 (5)	25 (0.2)	3 (0.1)	65 (200)
Coastal upwelled water	26.3	66.1 (238)	7 (15)	35 (1)	6 (0.1)	52 (300)
Fresh water enriched with DOC	840	420±58 ($1.5\cdot10^3$)	–	–	–	–

*DOC, dissolved organic compounds.

2. Kinetic methods of assessing the stationary concentration of radicals and their role in the self-purification of natural aquatic media

As has been indicated it is necessary to know the rate of formation and the observed rate constant for disappearance in order to assess the stationary concentration of free radicals.

The last of these parameters is determined fairly simply for $\dot{O}H$ radicals using PNDMA. A method has been developed[81,240] for measuring the "inhibiting ability" parameter in an aqueous medium, viz. $\Sigma k_i[S_i]$ sec^{-1} where S_i is the trap for $\dot{O}H$ radicals contained in the water, k_i the rate constant for the reaction of $\dot{O}H$ with the trap S_i. The essence of the method is that $\dot{O}H$ are formed by the photolytic decomposition of H_2O_2 and the rate of formation is

If H_2O_2 is added to natural waters and the change in S_e is followed as H_2O_2 is decomposed, then it is possible to estimate the fraction of H_2O_2 decomposing into radicals.

$$\alpha = \Delta S_e/\Delta[H_2O_2].$$

According to one estimate[265] this fraction varies for different natural waters from 0.1 to 0.5 and is 0.3 on average. Thus about 30% hydrogen peroxide is decomposed into $\dot{O}H$ radicals in natural waters.

The process of forming H_2O_2 also proceeds through the intermediate formation of radicals. As was shown in Section IV, $O_2^{\cdot-}$

radicals serve as precursors of H_2O_2. The disproportionation of two $O_2^{\cdot-}$ radicals in neutral medium occurs with low efficiency,[266] and at low formation rates the catalytic processes involving copper ions are more probable.

$$O_2^{\cdot-} + Cu^{2+} \rightleftharpoons CuO_2^{+} \rightleftharpoons Cu^{+} + O_2,$$
$$O_2^{\cdot-} + Cu^{+} \rightleftharpoons CuO_2^{+} \xrightleftharpoons{2H^+} Cu^{2+} + H_2O_2.$$

The magnitudes of the rate constants for the reactions of $O_2^{\cdot-}$ with Cu^{2+} and Cu^{+} are close[267] so that the ratio of the rates of these reactions is determined by the redox ratio $[Cu^{2+}]/[Cu^{+}]$. In an oxidising medium the reaction of Cu^{+} with H_2O_2 leads to a reduction in the H_2O_2 yield due to the reaction of $O_2^{\cdot-}$ with Cu^{+}. This is reflected in the observation that in the presence of the enzyme superoxide dismutase (SOD) the rate of H_2O_2 formation in natural waters is usually increased.[98,99] If the rate of H_2O_2 formation is half the formation rate of $O_2^{\cdot-}$ radicals in the presence of SOD then in its absence a portion of the $O_2^{\cdot-}$ radicals participate in the reduction of H_2O_2 to OH radicals.

$$O_2^{-} + H_2O_2 \xrightarrow[H^+]{Cu^{2+}/Cu^{+}} O_2 + \dot{O}H$$

Correspondingly, it is possible to assess the extent of transformation of initially formed $O_2^{\cdot-}$ radicals into OH from the difference in the rates of the photochemical formation of H_2O_2 in the presence and absence of SOD. This fraction varies depending on the chemical composition of the aquatic medium, but is usually about 50%.

In the quasi-stationary approach the rates of formation and breakdown of H_2O_2 are therefore equal to one another and in these processes the rate of OH radical formation is an appreciable fraction of the hydrogen peroxide formation or decomposition. For a rough assessment of the rate of formation of OH it is possible to take the rate of the photochemical formation of H_2O_2 in the aquatic medium:

$$w_i \approx w_f.$$

Knowing w_f and the parameter $\Sigma k_i[S_i]$, it is possible to assess the stationary OH concentration and the apparent rate constant for the radical oxidation of any contaminating substance $P(k_p)$:

$$[OH] = w_i/\Sigma k_i[S_i],$$
$$k_p = k_{(OH+P)}[OH] = k_{(OH+P)}w_i/\Sigma k_i[S_i],$$

where $k_{(OH+P)}$ is the bimolecular rate constant for the reaction of OH with P.

Various peroxidase self-purification processes are efficiently brought about in the presence of H_2O_2 in aquatic media in addition to the radical reactions.[152] Thus 3,4-dichloroaniline undergoes practically no conversion in a state of natural algocenosis in the dark in the absence of added H_2O_2 and is rapidly consumed if hydrogen peroxide is added to the algae.[265] In a similar manner various amines, which are peroxidase substrates, are oxidised on storing with algal cultures.[152]

The rate of the H_2O_2 cycle within reservoirs thereby reflects the efficiency of radical (and peroxidase) processes of self-purification of aquatic media. This enables assessment of the potential for chemico-biological self-purification of aquatic media, which is essential for assessing the acceptable loading of organic contaminants in natural reservoirs both in combination with and in relation to individual compounds.[71] The quantity of hydrogen peroxide formed (and decomposed) in natural waters gives an idea of the quantity of dissolved organic compounds which may be oxidised in extracellular chemico-biological processes (the chemico-biological self-purification potential). Under actual conditions a portion of the H_2O_2 is formed without the formation of reactive intermediates and the breakdown of H_2O_2 may be effected by a catalase mechanism, also without the formation of intermediates.

VIII. The hydrogen peroxide cycle within reservoirs and the biological wealth of aquatic media

On a global scale the fluxes of oxidation and reduction equivalents in the biosphere are balanced in the processes of photosynthesis and respiration. In addition to this global redox system there also exists in natural waters quasi-stationary redox subsystems comprising hydrogen peroxide and reduction equivalents of biotic origin. In view of the diversity and complexity of the mechanisms of H_2O_2 reactions with indigenous reducing agents under natural conditions, useful information for describing them adequately may be obtained from a study of model catalytic redox systems.

1. Modelling redox processes involving hydrogen peroxide within reservoirs

Ascorbic acid, hydroquinone and glutathione stand out among the most widely distributed reducing agents in nature as peroxidase substrates.[155] The redox catalytic reactions involving ascorbate ion have been studied in most detail.[268] As was indicated above, ascorbic acid is oxidised more efficiently by oxygen than by hydrogen peroxide. Another feature of ascorbic acid is the initiation of coupled processes of radical oxidation of various compounds difficult to oxidise.[239] A third feature is that ascorbic acid is an efficient H donor, for example in relation to organic radicals,[137] preventing the oxidation of organic substances. At the same time ascorbic acid radicals, stabilised by a system of double bonds, are kinetically extremely inert.[138]

Hydroquinone is a precursor of aquatic humus. In spite of its wide practical use in various investigations the mechanism of the redox catalytic reactions of hydroquinone are still unclear. A detailed kinetic investigation has been carried out of the catalytic oxidation of hydroquinone by hydrogen peroxide in the presence of copper ions.[269] It was found that in addition to non-radical processes hydroquinone participates in coupled radical oxidations of compounds difficult to oxidise. Hydroquinone thereby serves as the most suitable reducing agent to put into the medium in the presence of H_2O_2 to model the radical self-purification process induced by reducing compounds of biogenic origin.

Thiol compounds form part of proteins, humic compounds and metabolic products of water organisms, particularly micro-algae.[270,272] However the mechanism of the catalytic redox reactions involving thiols, and glutathione in particular, has been insufficiently studied. This gap has been partially closed.[269] It was shown that glutathione possesses extremely important features.

First of all glutathione readily reduces copper ions and forms stable (covalent) mono and less stable bis complexes with Cu^{+}.

$$2Cu^{2+} + 2GSH \xrightarrow[-2H^+]{} 2Cu^{+} + GSSG,$$
$$Cu^{+} + GSH \rightleftharpoons CuGSH^{+},$$
$$CuGSH^{+} + GSH \rightleftharpoons Cu(GSH)_2^{+}.$$

The complexes $CuGSH^+$ and $Cu(GSH)_2^+$ reversibly add O_2 (see Ref. 273) but are not decomposed to electron transfer products. The reduction of O_2 is effected directly to water by reaction of the oxygenated complex with a second glutathione copper complex, avoiding the formation of intermediate hydrogen peroxide.

$$Cu(GSH)_2^+ + O_2 \rightleftharpoons (GSH)_2CuO_2^+,$$

$$(GSH)_2CuO_2^+ + Cu(GSH)_2^+ \xrightarrow[-2H_2O]{} 2Cu^+ + 2GSSG.$$

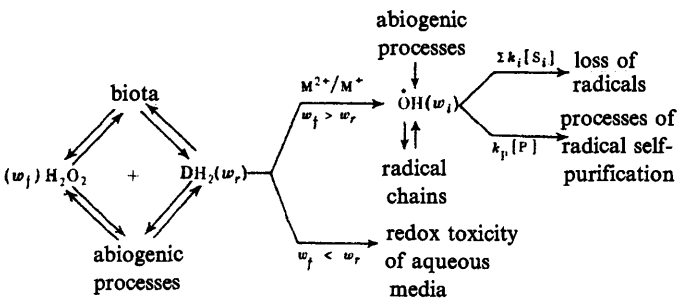
Evidently the oxidation of glutathione will proceed extremely slowly at low natural concentrations of copper.

The glutathione Cu^+ complexes react far more efficiently with hydrogen peroxide.

$$Cu(GSH)_2^+ + H_2O_2 \xrightarrow[-2H_2O]{} Cu^+ + GSSG.$$

Glutathione and seemingly other thiol compounds bind copper ions in the form of unreactive, biologically inaccessible complexes. Glutathione may thereby work as a reducing agent, modelling the onset of toxicity and the inhibition of self-purification processes of natural aquatic media by reducing toxicants.

Generalised results of full-scale and laboratory investigations[274] have enabled us to propose a dynamic chemico-biological redox model for natural aquatic media.



The gist of the model is as follows. In a natural aquatic medium biotic and abiotic processes are in constant operation forming oxidation and reduction equivalents, which react with one another. The results of these interactions are determined by the ratio of the fluxes within reservoirs of reactive oxidation equivalents, hydrogen peroxide (w_f) and the reducing compounds (DH_2) reacting efficiently with them (w_r). If the flux of oxidation equivalents predominates ($w_f > w_r$), an oxidative state is set up in the medium. Reducing agents entering the medium are either oxidised by hydrogen peroxide or initiate conjugated radical processes of self-purification of the aquatic medium from the contaminating compounds (P). If the flux of reducing agents exceeds the H_2O_2 flux ($w_r > w_f$) then a quasi-reductive redox state is set up in the aquatic medium (on increasing the flux the reducing agents will be gradually oxidised by oxygen dissolved in the water). Under these conditions various toxic effects arise in the water.[82]

2. Hydrogen peroxide and the biological wealth of an aquatic medium

The participation of H_2O_2 in the self-purification of an aquatic medium is closely linked with maintaining its biological wealth. However there is another aspect to biological wealth connected with the hydrogen peroxide cycle within reservoirs. One speaks of the redox-toxicity factor of a medium which arises when quasi-reductive conditions become established in an aquatic medium.[80,82]

It has been shown[275] that the presence of H_2O_2 is necessary in an aquatic medium for the development of fish in the early stages. This is linked with the possible participation of peroxy oxidation equivalents in bringing about metabolic redox processes. In fact a certain optimum H_2O_2 content exists in a natural aquatic medium, at which the chemico-biological redox processes within the reservoir are balanced. Unbalance of these processes may lead to two specific situations.

As was indicated in Section VII, in an aquatic medium the H_2O_2 cycle is closely linked with the free radical cycle. At the same time (see Section V) the H_2O_2 cycle is closely linked with the microelement cycle (particularly manganese ions). On increasing the free radical processes the oxidation of Mn(II) may be accompanied by the formation of metastable microcolloidal particles with mixed valency Mn(III,IV)[276,277] possessing strongly expressed oxidative properties. This is linked with the fact that the Mn(IV) ion, although having a high oxidation potential, is extremely inert in reactions of ligand substitution by virtue of the particular features of its electronic structure (d^3). Correspondingly the reduction of Mn(IV) by reducing compounds is brought about comparatively slowly. The Mn(III) ion (d^4), on the other hand, has a labile coordination sphere and is an efficient oxidising agent, possessing a high oxidation potential. The Mn(III) ion in microcolloidal particles of mixed valency is reduced to the Mn(II) ion which readily disproportionates with a neighbouring Mn(IV) ion with the formation of two more Mn(III) ions, i.e. particles with even more marked oxidative properties. The formation of such particles is particularly characteristic of aquatic media subjected to radiation contamination.

The hyperoxidising state of the medium, when metastable microcolloidal particles of bound or adsorbed Mn(III,IV) are present in the water, proves to be toxic for fish, a disease of the gills is observed. The factors controlling the emergence of the hyperoxidising state of the medium have not yet been clarified. Another, more completely studied phenomenon linked with the unbalancing of oxidation-reduction processes is the formation of a quasi-reducing state of an aquatic medium (see Section VI). Under these conditions a whole range of negative factors arise.

There is seemingly a reduction in the ability of the medium towards self-purification which leads to the accumulation of a whole series of toxic agents in the water.

Since the emergence of quasi-reducing conditions is usually accompanied by vigorous development of blue-green algae in the water, the majority of negative consequences are linked with precisely this factor. Blue-green algae occupy a special position in the ecosystem of modern reservoirs. In summer months they frequently become the dominant species in the phytoplankton.

The combination of an ability to photosynthesise, to consume organic compounds from the external medium[278] and to fix atmospheric nitrogen[279] causes the high adaptability of these microorganisms. In comparison with green algae and diatoms, blue-green algae effect photosynthesis at far lower light intensities and in the dark respiration consumes far less energy. The inhibitory effects of elimination and breakdown products of blue-green algae have great importance for other representatives of the algoflora.[280] Blue-green algae are most suited to living in reducing conditions. In the bottom layers of water they may vigorously assimilate sulphur in its sulphydryl form.[281]

The massive development of blue-green algae leads to many negative consequences. At bloom in a reservoir the pH of the medium increases, sometimes to values of 10–12. This creates

pathogenic microflora and of stimulants of intestinal illnesses, including cholera bacillus.[281] The situation is very dangerous when, in their life cycle, many forms of blue-green algae eliminate into the aquatic medium compounds which possess a strong toxic action.[280,282–285] Cases of mass destruction of animals and diving birds linked with the bloom of blue-green algae have been recorded in many regions of the Earth since the middle of this century.[286]

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The combination of literature data and our investigations indicates the important role of hydrogen peroxide in the aquatic component of the environment. Seemingly the evolution of aerobic life in the ocean also proceeds with the participation of H_2O_2 as the basic form of activated oxygen.[287] The oxidising character of the medium is also a condition for the rich functioning of the water ecosystem.

Under the influence of anthropogenic factors the content of H_2O_2 in natural aquatic media may be increased or decreased. An increased concentration of H_2O_2 into the overshoot zone is recorded on dropping unpurified domestic effluent into a reservoir. A sudden reduction of hydrogen peroxide concentration is possible on dropping biologically purified effluent into the reservoir due to reaction with reducing compounds introduced with the effluent.

The content of H_2O_2 in the air and in atmospheric moisture may be reduced (with SO_2 overshoot) or increased (with overshoot of organic substances) in a similar way. When the air is contaminated with sulphur oxides, sulphuric acid and incompletely oxidised sulphite will enter the soil ecosystem in place of oxidation equivalents such as H_2O_2. Such a substitution has an unfavourable action on the functioning of the soil ecosystem.[288]

In addition to its effect on the oxidation-reduction state of aquatic media, hydrogen peroxide makes an appreciable contribution to the formation of free radicals and participates in the self-purification of waters from difficultly oxidisable organic compounds. Free radicals may also have a significant effect on the biological richness of aquatic media, participating in the oxidation of compounds involved in biochemical cycles, in the initiation of peroxide oxidation of lipids, etc. Seemingly in natural waters, as in the cell, there is a physiological optimum both in H_2O_2 content and in the stationary concentrations of active intermediates.

The effect of anthropogenic factors on the H_2O_2 content of natural waters may be compensated by artificial addition of oxidation equivalents (H_2O_2, soluble peroxy compounds) to water or to the soil ecosystem (in irrigated farming). By varying the H_2O_2 content it is possible to regulate the biological richness of aquatic media, to suppress the development of undesirable microorganisms, blue-green algae in particular, and to intensify self-purification processes. The practical application of such measures to large bodies of water and areas requires large quantities of H_2O_2. Consequently a promising direction for investigation is the design of heterogeneous systems (with combined photo-sensitisers) generating H_2O_2 efficiently under the action of sunlight. The manufacture of small scale electrochemical generators of H_2O_2 based on the cathodic reduction of O_2 would also be promising.

An absolute requirement is the putting into practice of prophylactic measures to prevent the introduction of reducing compounds into reservoirs together with effluent which has passed biological purification. Water-submersible sources of UV irradiation may be used for this purpose. Under the action of hard UV radiation H_2O_2 is formed in waste water, radical chain oxidation processes are initiated, pathogenic microflora perish, aromatic compounds are hydroxylated, organochlorine compounds are dechlorinated and the overall content of dissolved organic compounds is reduced.

Regrettably the solution of many ecological problems encounters a shortage of H_2O_2. In practice H_2O_2 is not used in the purification of waste water, in spite of the fact that there is a multitude of patents to that effect and hydrogen peroxide is applied widely worldwide in the purification of domestic and industrial waste water.[289]

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