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PHYTOPLANKTON AS AN AGENT OF THE SELF-PURIFICATION OF CONTAMINATED WATERS

by

G. G. Vinberg and T. N. Sivko

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FOREIGN TECHNOLOGY DIVISION

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By: G. G. Vinberg and T. N. Sivko

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ABSTRACT: Planktonic algae when grown on a large scale on undiluted city sewage sharply accelerate the process of self-purification; this is expressed in faster initial lowering of the BOR, early termination of the anaerobic phase, the appearance of free oxygen, and in the accelerated onset of nitrification. The accumulation of a large quantity of organic substances synthesized by the algae in the composition of the bodies of the living cells is not reflected in the magnitude of the BOR. With the large-scale development of green algae in self-cleaning sewage the death rate of coliform bacterium is sharply increased. The construction of ponds filled with undiluted sewage is the simplest way to use green organisms as agents of self-purification; it draws attention also as an effective method of purification, especially applicable in regions with warm dry climates. Further study of the conditions favorable to the development of photosynthesizing planktonic organisms will allow setting up problems in the large-scale cultivation of algae as one of methods of utilizing sewage. In particular one should clarify the possibility of using algae grown on sewage to increase of the productivity of piscicultural ponds. English translation; 38 pages.

U. S. BOARD ON GEOGRAPHIC NAMES TRANSLITERATION SYSTEM

Block	Italic	Transliteration	Block	Italic	Transliteration
А а	*А а*	A, a	Р р	*Р р*	R, r
Б б	*Б б*	B, b	С с	*С с*	S, s
В в	*В в*	V, v	Т т	*Т т*	T, t
Г г	*Г г*	G, g	У у	*У у*	U, u
Д д	*Д д*	D, d	Ф ф	*Ф ф*	F, f
Е е	*Е е*	Ye, ye; E, e*	Х х	*Х х*	Kh, kh
Ж ж	*Ж ж*	Zh, zh	Ц ц	*Ц ц*	Ts, ts
З з	*З з*	Z, z	Ч ч	*Ч ч*	Ch, ch
И и	*И и*	I, i	Ш ш	*Ш ш*	Sh, sh
Й й	*Й й*	Y, y	Щ щ	*Щ щ*	Shch, shch
К к	*К к*	K, k	Ъ ъ	*Ъ ъ*	"
Л л	*Л л*	L, l	Ы ы	*Ы ы*	Y, y
М м	*М м*	M, m	Ь ь	*Ь ь*	'
Н н	*Н н*	N, n	Э э	*Э э*	E, e
О о	*О о*	O, o	Ю ю	*Ю ю*	Yu, yu
П п	*П п*	P, p	Я я	*Я я*	Ya, ya

* ye initially, after vowels, and after ъ, ь; e elsewhere. When written as ё in Russian, transliterate as yё or ё. The use of diacritical marks is preferred, but such marks may be omitted when expediency dictates.

PHYTOPLANKTON AS AN AGENT OF THE SELF-PURIFICATION OF CONTAMINATED WATERS

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I

Phytoplankton in many cases obtains decisive values in the process of self-purification of contaminated waters. Especially great is the role of the green organisms of phytoplankton in reservoirs with slow drainage ponds, reservoirs, lakes. Nonetheless, until recently only a few authors have pointed out the dependence of self-purification of the water in open reservoirs on the development of phytoplankton. Thus, for instance, S. N. Stroganov as early as 1914 emphasized that during self-purification of ponds filled by strongly contaminated waters or even by undiluted city sewage the oxygen necessary for life was liberated in the process of the photosynthetic activity of the green organisms of plankton, developing in massive quantities. Working from the experiments of Knaute, conducted at the end of the last century, on measuring the intensity of photosynthesis of plankton in contaminated waters, Cronheim (1904) wrote of the need for a new approach to the appraisal of the value of planktonic algae in the

study of self-purification of rivers. Analogous indications can be found in other authors (Rudolfs and Heukelekian, 1931; Purdy, 1935). However, until recently this question was not the object of systematic investigations. During the study of the oxygen regime of contaminated reservoirs and other medical and biological questions the value of planktonic algae as agents of self-purification has been, for practical purposes disregarded.

In recent years the position has changed radically. First of all it has become widely acknowledged that photosynthetic reaeration is a factor it the self-purification of contaminated waters. It is significant that in a survey of the literature on questions of self-purification of reservoirs after 1951 the factor specially pointed out as on the most significant achievement for the year is the wide acknowledgement of the fact that photosynthesis frequently constitutes the most important factor determining the curve of oxygen dip (Literature Review, 1952). Corresponding presentations and methods have already begun to enter into the practice of designing sources of reaeration and self-purifying capability of individual objects. Thus, for instance, (Nusbaum and Miller 1952), during a study from this point of view of the harbor of the city of San Diego, conducted measurements of the intensity of photosynthesis of plankton by the flask method and on the basis of the obtained magnitudes found that the given reservoir, whose area is equal to 4500 hectares, obtains 81,000 kg of oxygen per day as the result of photosynthetic oxygen evolution by plankton and 59,700 kg of oxygen per day through atmospheric reaeration; this amounts to 1.8 and 1.3 g of oxygen, respectively, per m^2 per day.

In contaminated waters or in waters enriched with biogenic

elements owing to runoff of waters the strong development of phytoplankton organisms and the consequent high value of photosynthetic reaeration is by no means random, but is regular and characteristic for such reservoirs. This phenomenon has already been the object of attention during medical and biological investigations (Sibiryakov, 1951; Drachev and Sosunova, 1953).

We were convinced of the great value of photosynthetic reaeration during self-purification of contaminated waters and of ponds of filled with city sewage, in which massive development of green algae or so-called "green bacterium" is observed, by observations which were partially outlined in preceding works (Vinberg and Sivko, 1952; Vinberg, 1955). The same results were shown by observations conducted by the Byelorussian Medical Institute in the summer of 1952 on the river Svisloch'. According to average data for the summer season of 1952 the seston of a reservoir located above the city of Minsk contained, in all, 5.4 μg/liter of chlorophyll. At the point of maximal contamination of the river the content of chlorophyll was rasied to 16.9 μg/liter (apparently because of patches of oscillatoria) and in the predam part of a second water reservoir located below the city the average content of chlorophyll was equal to 89.7 μg/liter. Moreover, at the first point the relative content of chlorophyll in the seston was close to 0.8% in second to 0.3%, and in third to 2.4% The last figure shows that at this point the seston consisted basically of phytoplankton.

The role of algae in the processes of the cycles of substances and their value as agents for self-purification is certainly by no means exhausted by photosynthetic reaeration. This became especially clear after the practice of purification of liquid waste in the

United States and other foreign countries began to see a new and at the same time most simple method of purification put in use the so-called oxidizing ponds or lagoons, in which photosynthesizing organisms of plankton play the leading role.

In England the possibility of using planktonic algae for self-purification of sewage drew the attention of Abbott. In a number of works (each of which assumes however, a small amount of material) Abbott describes laboratory experiments with sewage inoculated with algae (Abbott, 1948, 1949, 1950, 1952; Stone and Abbott, 1950, 1951). Unfortunately it is not indicated just which algae are discussed, although there is mention of Chlorella and it is said that the algae were very small and freely passed through filter paper. It is ascertained that during incubation in light of sewage in hermetically sealed vessels, after a period of rotting the liquid becomes green and is enriched with oxygen. In spite of the growth of the total content of suspended matter as a result of the accumulation of algae, the biological oxygen requirement [BOR_5] ($БПК_5$) is lowered. In one of Abbott's experiments the quantity of suspended matter increased from 98 to 400 mg/liter in 18 days and the BOR, equal in the beginning to 251, at the end of the experiment constitued 71 mg/liter of oxygen. In this case, in spite of the lowering, the BOR toward the end of the experiment was nevertheless considerable.

The massive development of algae, which in the beginning leads to a sharp reduction in BOR can, starting from a known moment, even lead to an increase in the BOR. However, here it is necessary to consider two points. First, in the opinion of Abbott the BOR always increases to a magnitude smaller than half of the quantity of oxygen which was liberated in the process of photosynthesis. Secondly and

this is especially important in general, in the presence of a sufficiently strongly well-developed phytoplankton the magnitudes of BOR determined by the usual method, i.e., in darkness, lose their meaning. Indeed, when guided by the magnitude of the BOR we should expect absorption of oxygen, whereas in the light for instance during the influx of sewage liquid containing a considerable quantity of green organisms into open reservoirs, conversely will occur not impoverishment but the inverse process of enrichment of the water with oxygen. In order correctly to estimate this peculiarity of the greening of sewage effluent Abbott proposes to produce the determination of the BOR not only in darkness, as usual, but also in daylight.

During the determination of the BOR in light, in those cases when the water is enriched with oxygen the BOR is taken with a negative sign. The difference between the determinations in light and in darkness gives the gross quantity of oxygen liberated in the process of photosynthesis. The ratio of this magnitude to the speed of consumption of oxygen in darkness can, according to Abbott, be used as the index of photosynthetic activity. It is easy to note that the last magnitude depends on the conditions of illumination. In the last work of Abbott he strives to consider this circumstance and tries to standardize illumination with the help of an "actinometer," at the basis of which is assumed the calculation of the rate of accumulation of free iodine in an acidified solution of iodine potassium. However, the conditions of illumination in natural reservoirs are so complicated that determination of the potential photosynthetic activity constitutes a very difficult and as yet unsolved problem.

Along with laboratory observations, certain experiments were conducted on the use of algae for purification of sewage effluent in

the city of Nottingham. The effluent, flowing slowly along special grooves, was contaminated with algae which multiplied and settled on the bottom in the form of dark silt. It was noted that the process of self-purification was sharply accelerated and that no excess reproduction of algae was observed in the reservoirs into which water subjected to such a method of purification was placed (Stone and Abbott, 1950).

On an incomparably greater scale, the purification of liquid waste with the participation of photosynthesizing organisms is applied in the United States and Australia by the method of the above-mentioned oxidizing ponds. The first impetus for application of this method was the observation of self-purification of effluent in ponds formed in 1924 during a breakdown of the purification plant in Santa Rosa in California. Here there is no need to dwell on the already numerous reports about purification of liquid waste in specially erected ponds, initially obtaining propagation in the southern stated of the United States (California, Texas); it is sufficient to introduce the most important conclusions of works of a general character.

In the very first articles about the new method of purification it was emphasized that the photosynthetic activity of green planktonic organisms determines the uniqueness of the conditions which are created in ponds filled with clarified or unclarified sewage. In particular, the extraordinarily sharp twenty-four hour oscillations in the content of dissolved oxygen — from strong oversaturation by day to full absence at night was pointed out. The amplitude of the twenty-four hour variation of active reaction and other factors is correspondingly great (Caldwell, 1946; Espinosa, 1948).

The operational experience with ponds of different dimensions and

character led to the conclusion that for the southern states of the United States the optimum is a depth close to 1 m, with a minimum volume corresponding to a 20-30 day retention time of the effluent in the pond. In certain cases operative ponds have considerable dimensions. For instance, San Antonio, Texas, has a lagoon whose area is equal to 142 hectares; the daily inflow of sewage is 170,000 m^3 (Myers, 1948).

The self-purifying ability of the ponds is characterized by magnitudes of the order of a 50-100 per hectare per day. This means that each 1 hectare of area of the ponds handles sewage from over a thousand persons of the population (Pearse, 1948; Myers, 1948). Similar magnitudes are indicated in the operational experience of lagoons in Australia (Parker et al., 1950). The last author emphasizes the usefulness of preliminary holding of sewage in anaerobic conditions.

Everything which has been said pertains to southern regions, in which open reservoirs do not freeze; this creates the most favorable conditions for the considered method of purification of sewage. Therefore special interest lies in the information about the operational experience of lagoons, finding applications, thanks to their cheapness and simplicity of construction and exploitation, also in the state of North Dakota, bordering on Canada (Heuvelen and Svore, 1954). It reported that in this state, with its severe continental climate, lagoons are used as the method of purification by 13 towns with populations of 500 to 1500 persons. The most interesting fact is that, apparently, even in winter, when the reservoirs are covered with ice and snow, the effectiveness of the process of self-purification is satisfactory, in spite of the anaerobic conditions created during this time. It is important to note that in the conditions prevailing

in the state of North Dakota the ponds designed basically for ground drainage, have a considerably greater period of retention of sewage than those in the southern states, for instance 120 days, and a correspondingly smaller load per unit of area (approximately 250 persons per hectare). It is indicated that the depths as well as the areas of the ponds are very different, but depths of 1-1.5 m are preferable; they ensure sufficient illuminance and, what is no less important, sufficiently favorable conditions for wind and night thermal mixing which is of great significance for correct work of the ponds. In individual cases "oxidizing ponds" can be used also for the purification of certain industrial wastes (Murdock, 1953).

There can be no doubt that also in the conditions prevailing in the USSR, in the first place with respect to small-population towns in the southern parts of the country purification of liquid waste in ponds in many cases can be the most economical and effective method.

This question for the Soviet Union is by no means new; conversely, as was mentioned above, as early as 1913, i.e., long before the foreign experiments in the use of oxidizing ponds, under the leadership of S. N. Stroganov detailed study of the processes of self-purification in ponds filled with undiluted sewage was begun on the Lyublin irrigation field (below Moscow). Later these works were continued on the Lyuberets fields of irrigation (Stroganov, 1914; Bazyakina, Vostokov, and Stroganov, 1919; Zakharov and Konstantinova, 1929). There was also an experiment in growing carp in ponds fed with undiluted waste. The interesting data thus accumulated are outlined in the separately published work of V. A. Meyen (1932), in which the German operational experience of pisciculture ponds fed by waste diluted by 3-5 times is also outlined. As is known, the largest of the many arrangement

of this type was constructed for the purification of the sewage of the city of Munich, whose population in that period was 530,000. The work of the ponds was so successful that in the Iser River, into which the water which has passed through the systems of ponds was discharged. Salmon appeared again besides effective purification of liquid waste, the ponds yielded over a thousand centners [100,000 kg] of fish and and 45 thousand ducks per year (see also Kisskalt and Ilzhofer, 1937).

In spite of the interesting and reassuring results obtained during observations of the ponds on the Lyberets and Lyublin irrigation fields, the investigations were not followed up and the difficulties encountered were not surmounted. Possibly this is at least partially explained by the fact that the data of the investigations were ahead of their time, when the sanitation of big cities had to wait its turn, since the basic attention was directed towards the development of industrial methods of purification. At present, with the incomparably greater technical base the National Economy as a whole and the sharply increased level of sanitary engineering, new possibilities are created for the development of inhabited localities of the most diverse sizes located in various climatic zones of the Soviet Union. In conformity with the huge variety of local conditions, we must apply different methods of purification of liquid waste; we must assume that purification in ponds will not occupy last place among these methods.

Although the basic value of green organisms for self-purification in pond conditions is universally recognized, the complicated mechanism of their action and the conditions ensuring the greatest effectiveness of the process of self-purification one still far from completely clear. In connection with this it was felt to be advisable to conduct

laboratory observations of the course of self-purification of city sewage in the presence of green algae.

II

The influence of green algae on self-purificaion of sewage was traced in laboratory conditions. Two large 20-liter bottles were each filled with sewage (liquid) from Minsk. In one of the large bottles we introduced algae (Chlorella pyrenoidosa Chik.) preliminarily grown on sewage in the laboratory. The bottle with liquid inoculated with algae was illuminated by natural light. The temperature during the first series of observations was varied within the limits 23.0-27.4° and during second experience with in the limits 15.6-19.8°. The second bottle, placed in the same conditions, served as the control. Into it we introduced the same quantity of preliminarily heat-killed algae.

Periodically, every few days samples were drawn from each bottle. Proceeding from experience accumulated during analogous observations, we considered it expedient to mix the contents (by a rotary motion) 2 hr before drawing the samples, in order to eliminate oscillations in the results of analysis which might arise because of distribution of algae in the bottles. Samples drawn after the 2-hour standing served for the determination of the content of dissolved oxygen, the active reaction, the BOR_5, oxidizability per Kubel; and contents of ions of ammonium, nitrites, and nitrates. In addition to this we determined bichromate oxidizability per Tyurin (without application of silver salts). Suspended matter was collected by centrifuging the liquid, and with the help of bichromate oxidation the quantity of organic substance in the suspension was determined. In a second test

of suspended matter collected in the same way the quantity of chlorophyll was determined photometrically by an earlier described method (Vinberg and Sivko, 1953). The bichromate oxidizability of the suspension and the content of chlorophyll made it possible to judge the degree of development of the algae.

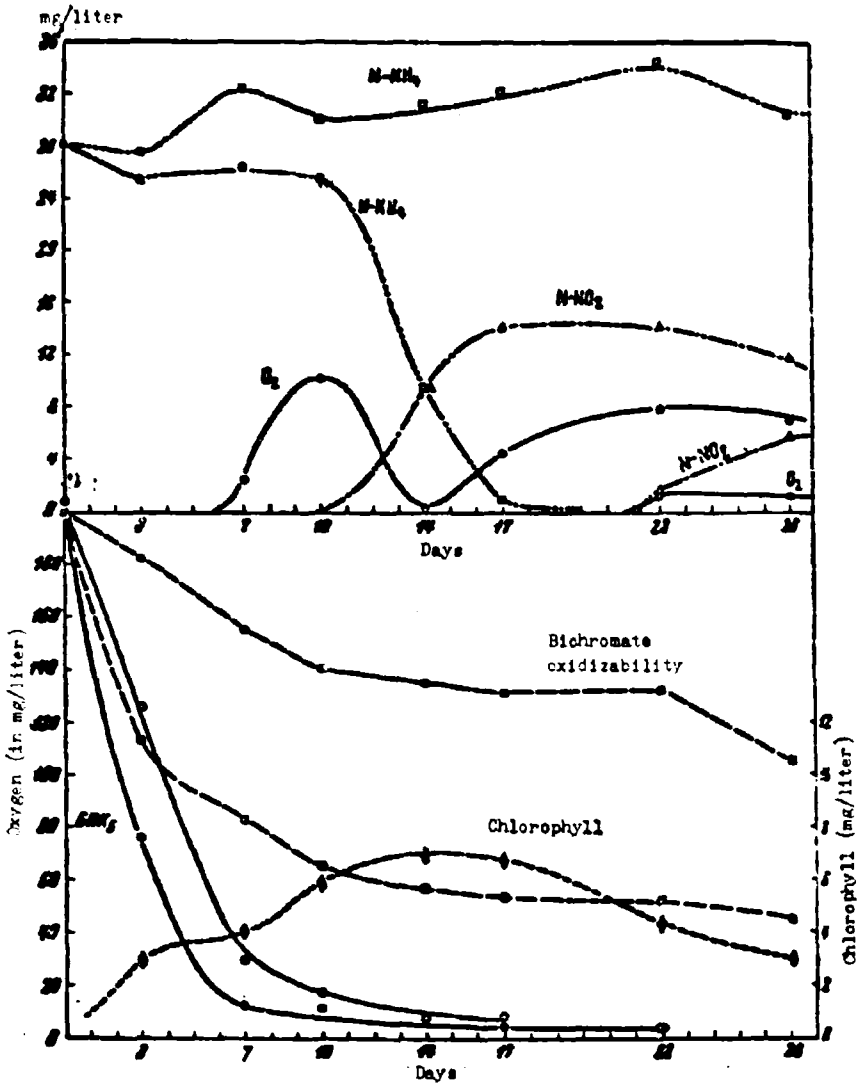

Fig. 1. Influence of large-scale development of algae (Chlorella) on the course of self-purification of sewage. Experiment No. 1. On the horizontal axis — time from beginning of experiment (in day). Data for experimental bottle (self-purification in presence of algae) are shown by blacked signs; data for control by unblacked.

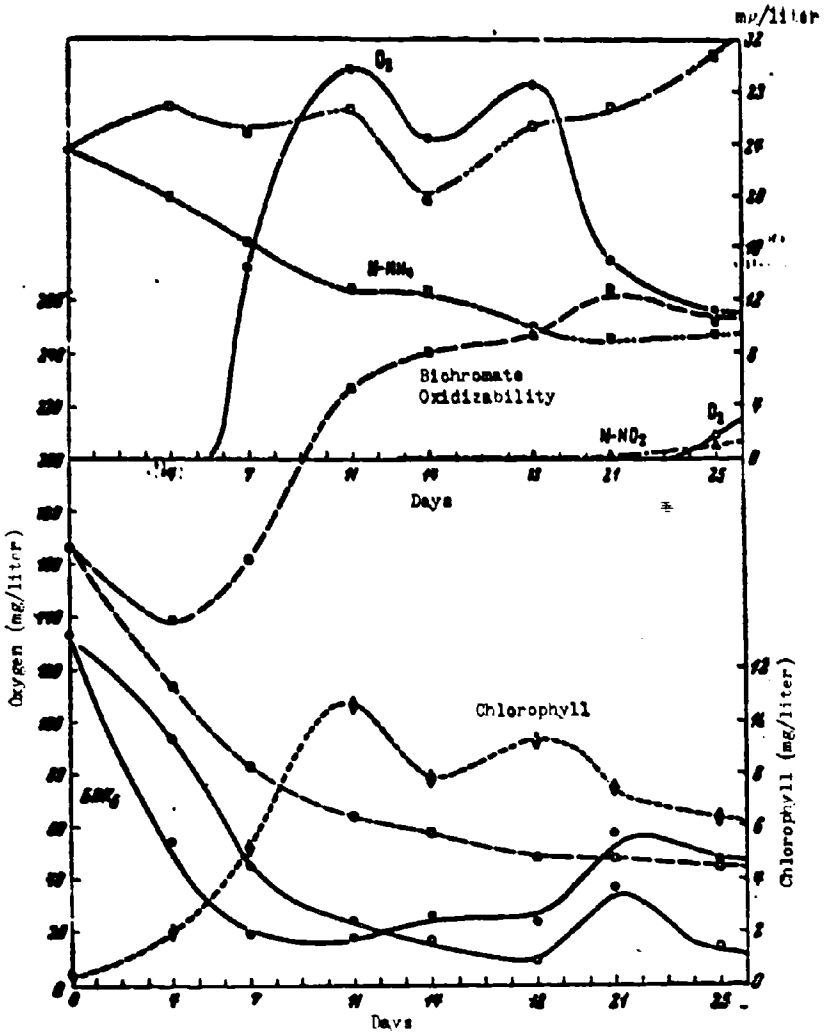

Fig. 2. Influence of massive development of algae (Chlorella) on the course of self-purification of sewage. Experiment No. 2. Data for experimental bottle (self-purification in the presence of algae) are shown by blacked signs; data for controls, by unblacked signs.

The results of two experiments are shown in Figs. 1 and 2. For the control bottles in both experiments was a fast lowering of the magnitudes of BOR in the first days of incubation characteristic. Basic lowering occurred in the first 7-10 days from the beginning of observations. Is lowered also the magnitude of bichromate oxidizability is also reduced, but at a slower rate than the BOR; as a result

the magnitude of bichromate oxidizability, which in fresh sewage is very close to the BOR, in subsequent periods of the observations considerably exceeds the BOR. All the described changes set in anaerobic conditions, since small quantities of free oxygen appear only 20-24 days from the beginning of the experiment. During the entire period of observations (months) the content of ions of ammonium remains at a high level (nitrogen, 27.5-34.5 mg/liter) in the first and 20-30.6 mg/liter in the second experiment).

The experimental large bottles were characterized by fast reproduction of the algae, which was manifested in only 24 hours by a sharp strengthening of the green color of their contents. As Figs. 1 and 2 show, in the experimental bottles the maximum content of chlorophyll was observed after 11-14 days. The intense reproduction of Chlorella and the accumulation of chlorophyll proceeded also in the first twenty-four hours, when strictly anaerobic conditions still ruled. Apparently the oxygen which the algae produced during this time was immediately expended on the oxidation of unstable organic substances.

At the time of maximum development of the algae the liquid becomes intensely green and the content of chlorophyll attains 7-10 mg/liter, which exceeds even the maximum quantities of chlorophyll which we observed in ponds of sewage on the filtration fields of Minsk. Such quantities of chlorophyll correspond to several tens of millions of Chlorella cells per ml.

The reproduction of the algae is accompanied by a growth in the bichromate oxidizability of the suspension, which in the second experiment attained a very great magnitude 186 mg/liter of oxygen. We remember that with the applied method approximately 90% of the

available organic substance is oxidized. As has been shown (Vinberg and Platova, 1951), the magnitudes of bichromate oxidizability found by this method are numerically close to the magnitudes of dry weight. Therefore, without going into detail, for tentative comparisons it is fully possible to assume that bichromate oxidizability expressed in milligrams of oxygen is numerically equal to dry weight. This enables us to get an idea of the relative content of chlorophyll in the suspension, which according to the experiment turns out to be within the limits 4-6.5% of the dry weight.

This is a very high content of chlorophyll, which, as is known from the literature is observed in algae only under favorable conditions of development in weak light. In our case the obtained magnitude can serve as yet more proof that the growth of the bichromate oxidizability of the suspension was basically caused by accumulation of algae.

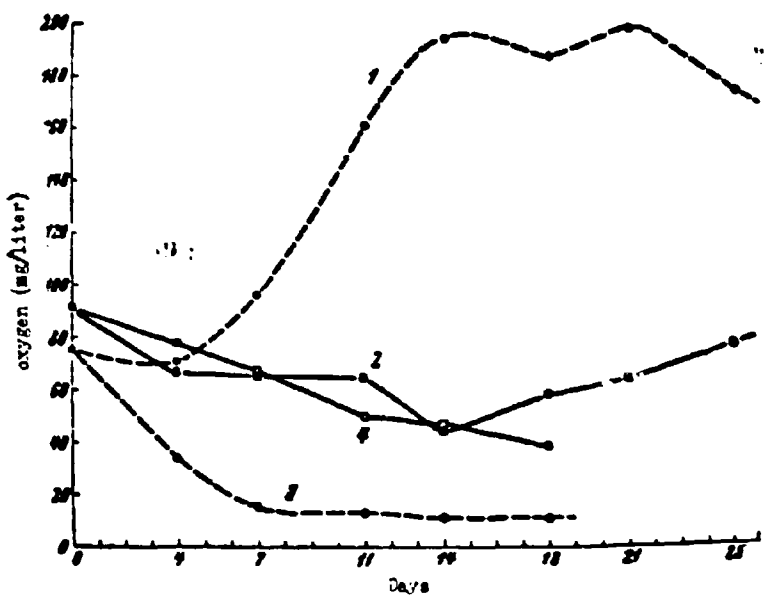

Fig. 3. Bichromate oxidizability of suspended substances (1 and 3) and dissolved organic substances (1 and 4), according to experiment No. 2. 1 and 2 — experiment; 2 and 3 — control.

A very important point is the fact that with such a strong increase in the bichromate oxidizability of the liquid in the experimental bottle when filtered through a diaphragm filter was lowered at approximately the same rate as that in the control (Fig. 3). This very important circumstance shows that in the conditions of the given experiment the accumulation of a huge quantity of algae cells did not lead to an increase in the content of dissolved organic compounds.

In the light of these data the results of the determinations of BOR in the experimental and control bottles become intelligible. In the experimental bottle, despite the increase in the oxidizability of the suspension not only was a smaller rate of descent of the BOR not observed, but conversely, in the first, anaerobic, period of the presence of the algae the reduction in the BOR the occurred at a considerable higher rate than the control. Thus, for instance, three days after the beginning of the first experiment the BOR_5 the experimental bottle was reduced by 138 mg per liter of oxygen and that in the control by 78. In the second experiment, in four days the reduction of BOR_5 in the experiment was equal to 78 mg per liter of oxygen and that in the control, to 40. After the first period of rapid lowering when the BOR is established at a low and more or less stable level, it is impossible to ascertain any regular distinctions between the experimental and control bottles. All this persuasively attests to the fact that the presence of even maximum quantities of cells of living algae does not lead to an increase in BOR.

As one would be led to expect, the massive development of algae strongly shortens the anaerobic phase of self-purification. After as little as 5-6 days from the beginning of the experiment free oxygen appears in the liquid, whereas in the control the appearance of free

oxygen is noted only after 20-24 days. Besides this, in distinction from the control, after the first appearance of free oxygen its content starts to increase rapidly and attains very large magnitudes. In the second experiment as early as 7 days to 14.37 mg/liter and after 11 days it was 29.47 mg, despite the fact that the experiment was conducted in bottles not filled to the top; their contents, moreover, were mixed before the taking of samples so that a certain exchange with the atmosphere was possible. The creation of aerobic conditions involves the possibility of nitrification. Indeed in this relation we observed a sharp difference between the experimental and control bottles. In the control nitrites were ascertained for the first time in the first experiment only at 25 days from the beginning of observations, and in the second no nitrites were detected up to the very end of observation. In the experimental bottles in the first experiment nitrites were detected at 7 days and in the second at 11 days hours from the beginning of the experiment. In the first experiment the quantity of nitrites quickly attained considerable magnitudes (14.0 mg/liter of nitrogen; after 11 days nitrates were ascertained, whose content remained at a low level (not over 0.5 mg/liter of nitrogen) to the end of the observations. In the second experiment nitrification proceeded very weakly and only toward the end of observations did the content of nitrites approach 1 mg/liter of nitrogen, while no nitrates were detected at all.

According to this the first and second experiments characteristically differ one from another with respect to the content of ions of ammonium. In both cases, in distinction from the control, where the content of ions of ammonium remained at approximately the same level during the entire period of observations, in the experimental bottles

the content of ions of ammonium was strongly lowered. In the first experiment, with intensely proceeding processes of nitrification in last period of observations, ammonium existed only in the form of traces, whereas in the second experiment, with weakly expressed nitrification, a considerable content of ions of ammonium was retained up to the end of observations (9-10 mg/liter of nitrogen, which is 38-42% of it initial quantity).

It is very probable that the weak development of nitrification in the second experiment was connected with the fact that in this experiment (apparently due to the especially strong reproduction of algae) a sharply alkali reaction was created. After as little as 7 days the pH equalled 8.25. In subsequent periods of observations the values of pH were not exactly established, but exceeded 10. In the first experiment, during the entire period of all time of observations the value of pH did not go above 8.7. In the considered conditions the active reaction of the medium depends on a complicated combination of different processes. Basic roles will be played by on the one hand, alkalinization advancing due to the consumption by the algae of bicarbonate carbonic acid [?], and on the other hand by acidification as a result of consumption of ions of ammonium, which occurs by means of exchange on the hydrogen ion. Therefore, depending upon conditions the growth of algae can lead both to acidification and to alkalinization of the medium. The different courses of the active reaction in the experiments indicates that there is a certain optimum quantity of algae at which they manifest a positive action on the process of self-purification and do not give rise to such complications as excessive alkalinization of the medium.

The observation convincingly showed that when the process of self-purification of sewage proceeds in light in the presence of green algae

the initial lowering of BOR is accelerated, the anaerobic period is sharply shortened, and the stage of nitrification sets in correspondingly earlier. In the presence of algae the lowering of the total content of organic substances is delayed, and the content may even grow; however, accumulation of organic substances in the form of living algae does not lead to an increase in the BOR and in general renders a completely different (frequently directly opposite) influence on the oxygen regime and cycle of matter in a reservoir than do the unstable, easily oxidized organic substances of fresh contaminations.

III

From the medical hygiene point of view, any method of purification should be judged not only by such indices of the degree of total contamination or the attained stage of purification as the BOR, the content of dissolved oxygen, nitrites, and so forth, but also by direct microbiological data first of all, by the magnitude of the colititer, characterizing the conditions of survival of intestinal flora. Articles dedicated to the purification of sewage in ponds contain some — however, quite scant — data indicating a lowering of the number of cells of coliform bacteria in these conditions. For instance, according to the data of Parker et al., (1950) the initial colititer, equal to 0.0000012 ml, after passage through an anaerobic lagoon was changed very weakly, increasing only to 0.000004 ml, and in an aerobic lagoon was raised to 0.003 ml. Pearse (1948) gives data of various authors, according to which in Texas in lagoons with a 15-day stay of liquid the colititer increases from 0.00001 ml to 0.02—0.2 ml, which in his opinion is similar to the effect of chlorination.

Close magnitudes (0.01-0.1 ml) were observed also in ponds of sewage on the filtration fields of Minsk with the mass development of "green bacteria" (Vinberg and Sivko, 1952). According to the given data, during self-purification of sewage in ponds conditions are created for rapid dying off of intestinal flora, although the colititer remains lower than that in uncontaminated waters.

Conditions in ponds are very complicated and variable. Therefore the basic regularities of the interaction between algae and bacteria can be clarified by only means of laboratory experiments. It is obvious that an alga can render an influence on the periods of survival of bacteria either directly, by means of specific metabolic products of the antibiotics type, or indirectly, due to the creation of conditions unfavorable for the survival of bacteria.

In a work of the Japanese author Kurokawa (1941), known to us only by an abstract, it is affirmed that the filamentous alga Spirogyra has shown a lethal action on the bacteria of typhus, cholera, and dysentery. Kurokawa explains this effect by the fact that the bacteria, attracted by the oxygen which is produced by the algae, accumulate near their cells, where as a result of photosynthesis there is created a strongly alkali reaction of the water, lethal to the bacteria.

In view of the fact what interrelation between algae and bacteria are virtually unexamined our observations made during the above-described experiments are of some interest. Along with chemical analysis during every observation we determined also the total number of bacteria by means of the standard method of counting colonies on MIA (meat-infusion agar) and the determination of the colititer.*

*All microbiological data given in this work were obtained by A. I. Rest, chief of the microbiology lab of the Byelorussian Sanitation Institute.

With respect to the total number of saprophytic bacteria, the obtained data do not enable us to note any sort of definite influence of the algae. Both in the experiment and in the control, during the entire period of observations in the total number of bacteria remained, during the considerable irregular oscillation, at a level of several tens or hundreds of thousands per ml.

In distinction from this, there was a clearcut difference between the experiment and control jars with repsect to the rate of dying off of coliform bacteria. As Fig. 4 shows, in both experiments, starting from the 10th-12th day the colititer increased considerably faster in the experimental bottles than in the controls, so that after 19-22 days a titer of 10 ml was attained. In the control bottles during this time the colititer was equal to 0.004 ml. Subsequently in the experimental bottles the colititer continued to increase and toward the end of observations in the second experiment (after 25 days) was over 333 ml, i.e., it already approached the standard established for potable water.

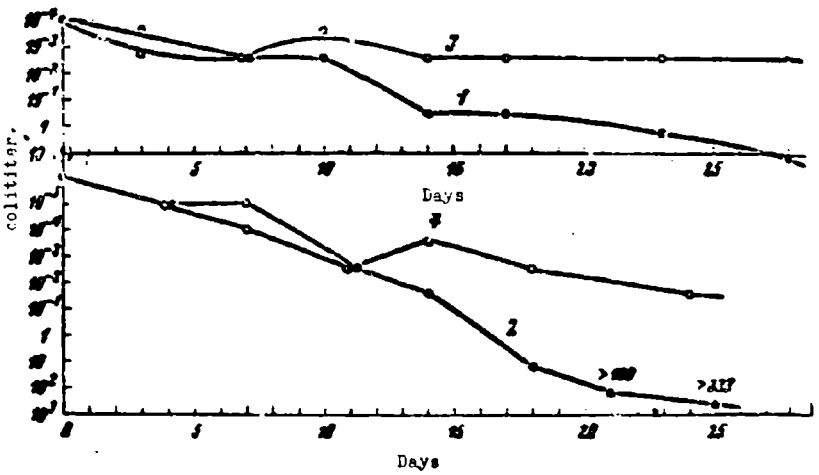

Fig. 4 Influence of the massive development of Chlorella on the dying off of coliform bacteria in sewage according to experiments No. 1 and No. 2. On the horizontal axis – time from beginning of experiments days. 1 – experiment No. 1; 2 – experiment No. 2; 3 – control in experiment No. 1; 4 – control in experiment No. 2.

Thus the experiments convincingly showed that the massive

development of algae in self-purifying sewage creates sharply unfavorable conditions for the survival of coliform bacteria.

Our data are insufficient for a judgement about the precise nature of these unfavorable conditions. Nonetheless, it is impossible not to turn our attention to the fact that in the initial period of the experiments, for a quite prolonged period (10-12 days), the rate of dying off of coliform bacteria was identical in the experiment and in the control, despite the fact that this is the period of the most intensive multiplication of algae in the experimental bottles and their accumulation up to a maximum. Thus the young, intensively multiplying chlorella in our experiment did not render a direct antibiotic action on coliform bacteria, if we judge this by the periods of their survival. There are no bases to consider that the increased death rate of the bacteria in the experimental bottles is wholly caused by the active reaction, since in the first place the alkaline active reaction was created considerably earlier than the appearance of the difference between the experiment and the control with respect to the periods of survival of coliform bacteria and secondly and most convincing, in the first experiment, in which the increase in the death rate of the bacteria was expressed fully distinctly, there was, in general, no strong alkalinization of the medium.

The beginning of the increased rate of dying off fell in a period later than the time of appearance of free oxygen and coincided with the period of maximum oxygen content and the beginning of the appearance nitrites. This creates the impression that the increased death rate of the coliform bacteria is connected with the achievement of a certain threshold of oxidizing-reducing conditions in the medium. On the other hand, this is the same time at which the maximum content of algae occurs, so that the possibility is not excluded that at that moment

when a reduction begins in the number of cells of the algae, i.e., their dying off, an antibiotic action, absent earlier, will appear.

In addition to the described observations, we conducted experiments with tap water into which we introduced B. coli communae washed from agar cultures and centrifuged and washed Chlorella. The experiment was set up in three variants. With identical initial densities of coliform bacteria (colititer 0.000004 ml) and Chlorella (1.08 mg/liter chlorophyll) we intoduced into first and third phials chlorous ammonium to a final concentration of 3 μg/liter nitrogen. All phials were incubated at a temperature varying from 22 to 27°. The first phial, serving as the control, was in darkness; the second and third were in natural illumination by diffuse light. Observations were made at 3, 6, 9, and 12 hours then at 1, 2, 3, 4, 5, and 6 days from the beginning of the experiment. In the control phial, as one would expect, the content of chlorophyll remained on at one level to the end of the observations. The index of the concentration of hydrogen ion (pH), initially equals to 8.19, also remained constant and only after 5 days shifted somewhat in the direction of acidification.

It is very interesting that in darkness, in spite of the presence of a considerable quantity of living Chlorella, the colititer remained constant for the first three days and was increased by only one place through 5 days. Toward the end of the observations it was equal to 0.00004 ml. Thus, in the absence of photosynthesis the algae did not render an influence on the period of survival of coliform bacteria.

In the second and third phials reproduction of the algae occured and as early as twenty-four hours from the beginning of the experiment the content of chlorophyll in the second phial increased 30%; that in

third phial grew by 37%. The higher rate reproduction of the algae in third phial, to which chlorous ammonium was added, was clearly manifested 2 days from the beginning of the experiment, when the content of chlorophyll in the second phial was equal to 1.68 and that in the third to 2.98 mg/liter (275% of the initial). Subsequently the content of chlorophyll did not increase more. In both of the phials standing in light there was alkalinization of the medium as a result of photosynthesis; it was stronger in the second phial, where at 12 hr from the beginnning of the experiment the value of pH was raised above 9.2, while at the same point pH in the third phial was 8.9. This was in spite of the more intensive development of the algae, which is explained by consumption of the ammonium ions existing here,

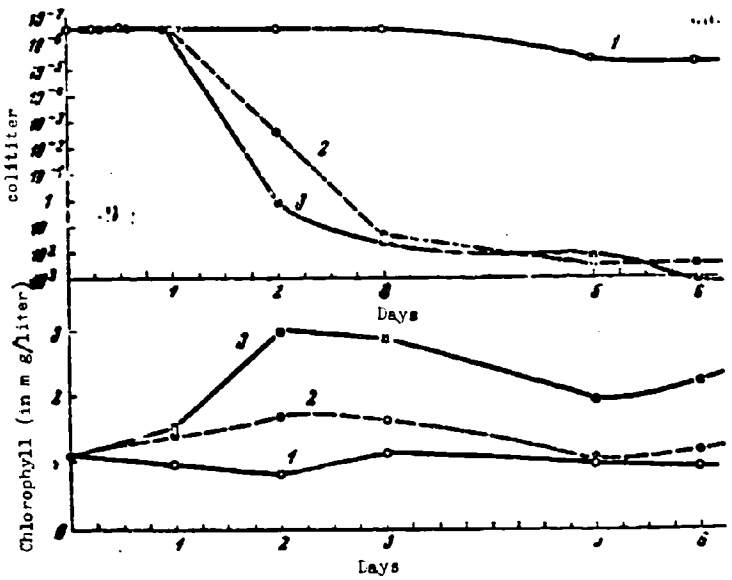

Fig. 5. Dying off of coliform bacteria in tap water in the presence of Chlorella in light and in darkness. Phial No. 1 — in darkness; phials No. 2 and No. 3 in light. In phials No. 1 and No. 3 tap water with addition of chlorous ammonium to a final concentration 3 of 3 mg/liter. On the horizontal axis time from beginning of experiments, in days. 1 — phial No. 1; 2 — phial No. 2; 3 — phial No. 3.

with concomitant acidification of the medium. Upon the expiration of the first twenty-four hours the active reaction was sharply alkaline in both the second and third phials. In spite of this the colititer still remained at the initial level at this time. However, by the following day, i.e., 48 hours from the beginning of the experiment, the colititer in both of the phials in the light turned out to be sharply raised, and in second phial was equal to 0.004 ml. In the third phial, in which at this time there was a maximum quantity of algae, the colititer turned out to be very high over 1.1 ml.

Thus in these conditions in only one way the colititer increased from 0.000004 to 1.1 ml (more exactly, to over 1.1 ml). This means that the number of coliform bacteria was lowered by 300,000 times in 24 hours. On the following day the distinction between the second and third phials was smoothed and the colititers were 43 and 56 ml, respectively. After 6 days the colititer was over 500 ml in both phials. Remember that at this time the colititer in the control phial was equal to 0.00004 ml, i.e., the number of coliform bacteria per unit of volume was 12.5 million times greater.

Thus in the conditions of the given experiment it was again observed that the photosynthetic activity and the growth of green algae (Chlorella) lead to a sharp reduction in the life expectancy of coliform bacteria.

IV

When our observation of the role of green organisms in the process of self-purification were already conducted, publication began of the work of Ludwig, Oswald, and other colleagues of the Institute of Engineering Research of the University of California

concerned with experimental study of this question (Ludwig et al., 1951; Oswald et al., 1953a, 1953b; Ludwig and Oswald, 1952).

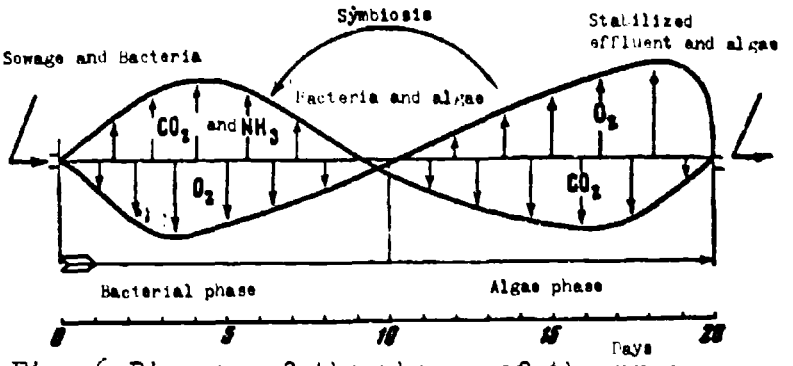

Fig. 6 Diagram of the phases of the process of self-purification in ponds of sewage (per Oswald et al., 1953).

Proceeding from the experience in the application of lagoons, which in their opinion turned out to be the simplest and most economical method of purification of liquid waste in conditions of a sunny and dry climate, these researchers set as their goal the study of the "symbiosis" between bacteria and algae, in well-controlled laboratory conditions. "Symbiosis" here is understood in the sense that during self-purification of sewage in light the vital activity of saprophytic bacteria and photosynthesizing organisms changes the medium in different, and in many respects directly opposite, directions; as a result of this, these organisms are mutually capable of developing with one another. It is possible to consider that self-purification of sewage in ponds passes through two phases; bacterial and alga. During the first phase as a result of bacterial decomposition, oxidation, and mineralization of unstable organic substances the water is impoverished of dissolved oxygen, the reserves of which are supplemented from the atmosphere. In the opposite direction, i.e., from the water to the atmosphere, pass excess quantities of carbon dioxide and ammonia In the second phase, as a result of the photosynthesis of algae, the

water is oversaturated with oxygen, the surplus of which departs into the atmosphere, while carbon dioxide enters the water from the atmosphere (Fig. 6). In reality these phases are not separated in space nor in time; conversely, during the construction of lagoons measures one taken for the best possible mixing (admission of liquid into the middle part of the reservoir, devices for recirculation of part of the drainage, removal of obstacles impairing mixing by the wind, and so forth). Nonetheless, mixing in ponds is very imperfect, which sharply lowers their effectiveness as purifying constructions. In ponds far from all the possibilities which with appropriate conditions one inherent in the use of algae for processing and utilization of liquid waste, are realized. In order to increase the effectiveness of the use of algae, it is first necessary to clarify the dependence of their vital activity on the conditions of the medium.

During the study of this question by the California collective of researchers wide use was made of modern laboratory technology. Various installations were tried for observations of the development of algae on sewage and clarification of their value as agents of self-purification. It turned out that a closed system with a constant leak of fresh sewage liquid is of little use, since in these conditions it is very difficult to attain the according to the terminology of the authors equilibrium state at which conditions in the culture are kept constant indefinely. This requirement is easily carried out in the so-called open system, with a continuous current of air through the medium in which the algae grow.

When an installation of the open type all of the most important results published by the mentioned authors are obtained. In the construction which they used the growth of algae occurred in glass cylinders with 1.2 liter capacity, along the axis of which they passed

a gas-discharge lamp, illuminating the culture from within. The installation was filled preliminarily with prepared sterilized sewage, which has been contaminated with several strains of bacteria separated from sewage, and with a bacteriologically pure culture of a certain species of algae. Aseptic conditions were observed. Daily a certain part of the contents of the cylinders was replaced by a new medium, which made it possible to watch the development and results of the vital activity of the algae at various periods of the stay of the sewage in the experimental cylinders. For instance with daily replacement of $\frac{1}{4}$ of the culture the period of stay is equal to 4 days; with replacement of $\frac{1}{10}$ of the culture, to 10 days, etc.* Cultures with various periods of stay were maintained for a prolonged time. In each of the cultures the equilibrium state corresponding to the given retention time set in rapidly; this state could be maintained indefinitely. They compared the data obtained in such equilibrium cultures. Temperature and illumination were automatically supported at a certain level (usually 25° and 1200 foot-candles); the quantity of passed air (500 ml/min) and the influence of other conditions were calculated.

The growth and state of the algae were recorded by means of counting the number of cells in a unit of volume and the determination of their dimensions, volume, dry weight, chemical composition (in particular the content of chlorophyll), external appearance, and so forth. Detailed chemical analysis of the medium allowed a close watch to be kept on the degree of utilization of separate biogenous elements

*Here and below we observe the terminology accepted in the literature on purification of sewage: the [English] term "retention time" we translate as "period of stay." In application to cultures of algae this term is close in meaning, although not identical to, the term "turnover of culture," after N. S. Gayevskaya (1953).

changes in the active reaction, in BOR, etc. In this way was studied the value of various retention times, the concentration of sewage, the intensity and periodicity of light, temperature, and certain other factors were studied for two different species of algae: Euglena gracilis and Chlorella pyrenoidosa.

With small retention time, i.e., large loads of sewage, the cells multiplied at maximum rate; this means that all cells are "young". With very small retention times even the maximum division rate turns out to be insufficient. Therefore with a decrease of load the number of cells in a unit of volume increases and at certain retention time, different for different species, it attains a maximum. For Euglena the maximum density of cells is attained with a duration retention time of 7 days and for Chlorella, at three days, when 1 ml contains 35 million cells. During this time the dimensions of the cells are minimal. For instance, with a 7 day retention time the dry weight of 1 million cells of Euglena equals 0.56 mg, while at 20 days it is 1.22 mg. However, the maximum increase of cells is attained during a smaller retention time, when the density of cells is considerably less than the maximum.

The greatest daily increase of Euglena composed 0.08 g/liter [sic] with a total weight of available cells of 0.16 g/liter. Culture of Chlorella gave a maximum increase of 0.11 g/liter dry weight, in twenty-four hours. By transmission of air containing 1-2% carbon dioxide the maximum increase can be increased. Euglena in these conditions gave an increase of up to 0.26 mg/liter.

We should add that authors who developed methods of mass cultivation of algae in synthetic media obtained densities of cultures a few times larger and correspondingly larger yield of algae

(Gayevskaya, 1953; Algal culture, 1953).

Summarizing in their last paper the most important of their results, Oswald and the other participants of the works (Oswald et al., 1953a) wrote: "Under optimum conditions the cells of the algae remain young, i.e., are maintained in the logarithmic phase of growth. Such cells have a relatively high content of chlorophyll, carbohydrates, and fats. Like the increase in the algae, the production of oxygen is also great, and the production of oxygen exceeds the requirements of all the living organisms of the system.... Under favorable physical conditions, the factor limiting increase it usually the source of carbon. When the load is lowered and the source of carbon becomes still more organic, the cells either cannot divide or divide at a strongly reduced rate. Many of the cells become "old," i.e., they store carbohydrates and fats at the expense of the limited sources of carbon. The cells contain little chlorophyll, their increase is reduced and the production of oxygen can be less than the total requirement for it...."

The optimum luminous intensity for Euglena depends on the concentration of sewage and the retention time. For sewage with a BOR of 90 mg/liter the optimum luminous intensity lies within the limits of 400-1200 foot candles (4300-12,900 lux). When luminous intensity passes the optimum the cells become "old"; this is to a certain degree similar to that which occurs with a lowering of load, i.e., during a shortage of food as the result of prolonged retention time. Further, recalling the action of hyperoptimal luminous intensity on the construction and decomposition of chlorophyll, the authors arrive at the conclusion that periods of intense illumination alternated with periods of darkness are favorable or even necessary to increase the total final rate of photosynthesis. On the whole,

as Oswald emphasizes, the tempo of cell division, the rate of increase and production of oxygen, and of synthesis and destruction of chlorophyll should be considered as interconnected variables, the character of the dependence among which is determined by the conditions of the medium — primarily by the conditions of feeding, luminous intensity, and temperature.

The considered investigations showed what with respect to the studied processes distinct and sufficiently clearly expressed specific distinctions appear in the experimental species of algae. For instance, in comparing Euglena and Chlorella as agents of self-purification authors indicate a preference for Euglena, in spite of their smaller rate of reproduction, since in cultures of Euglena the BOR of the centrifugate, i.e., the content of dissolved organic substances, turned out to be somewhat lower than in the cultures of Chlorellas.

Besides this, there are certain data according to which Chlorella, indistinction from Euglena, suppress the reproduction of bacteria. However, this important conclusion has an insufficiently convincing foundation Oswald and colleagues (Oswald et al., 1953a) limited themselves to reporting, without indicating the conditions under which the given magnitudes were obtained, that in sewage without algae the total number of bacteria per ml was equal to $1 \cdot 10^8$, in the presence of Euglena it was $5 \cdot 10^7$, and in the presence of Chlorella, $1 \cdot 10^6$, i.e., in the presence of Chlorella it was in 100 times less than without algae. Hence they conclude that Chlorella, by suppressing the development of bacteria, can retard the mineralization of organic substance of contaminations. Apparently these authors do not consider the fact that many algae (in particular Chlorella) themselves are able to assimilate dissolved organic substances. For instance, it is

known that in media for the cultivation of Chlorellas urea can serve as a good source of nitrogen.

Thanks to experimental works, already at present many important points have been clarified which promote understanding of the role of algae as agents of self-purification; however, in view of the complexity of the considered phenomena still more questions, set in turn for study, await solution. Nonetheless the practice of the application of ponds as a method of purification and the corresponding experimental works have shown with full clarity that the use of algae during purification of liquid waste is of great interest in many respects. Certainly, we are not limited to the most primitive and therefore least effective form of using photosynthesizing organisms, in ponds, although this method of purification can and should be widely applied where it is needed. In the future, obviously, special installations will be developed which will allow more effective use of the potential capabilities of photosynthesizing microorganisms.

Algae can be used not only as agents of the process of self-purification, but also as one of interesting and promising possibilities for the utilization of sewage for the production of useful products. Besides this, when the problem of methods of large-scale cultivation, collection, and utilization of algae is solved, it will mean that methods of removal of biogenous elements from sewage and runoff waters will be found. The detection of such methods has in recent years been the object of a number of investigations, since in many cases, especially in thickly populated regions, the dumping into open reservoirs of effluent which has undergone fully sufficient purification but is strongly loaded with biogenous elements has led to undersirable phenomena – for instance, to extremely intense "coloring" of water by

blue-green algae.

It is possible to outline various directions of possible utilization of algae grown on sewage; for instance, their use as cattle feed or as green fertilizer to increase of the food base of natural reservoirs and artificial piscicultural ponds, and finally as a biochemical raw material. The most — discussed is the prospect of their use as cattle feed. In connection with this, in special works and popular articles of foreign authors there is frequent repetition of the winged expression: "From sewage to beefsteak." In principle this is realizable, but it still is necessary to surmount many difficulties, including those of an economic order, before we can seriously speak of the practical realization of this possibility.

It seems to us that a considerably closer prospect is that of using algae grown on sewage to increase the productivity of fish-farm ponds. As is known, in the Soviet Union successes have been scored in the development of methods of mass cultivation of planktonic algae for use as a means of increasing the productivity of pond farming (Gayevskaya, 1952, 1953). Sewage is a cheap medium, readily available in huge quantities, which is fully useful for this purpose. By using this medium a huge quantity of Protococcales algae, needed for pond farming can be obtained. It is important that this use of liquid waste in the interests of pond agriculture is not connected with a need to construct the ponds near inhabited localities, as in the use of liquid waste in piscicultural ponds by the German method. The grown algae, after on their concentration (which is simple to do) can be transported alive over great distances. Many of them can easily withstand a prolonged stay under anaerobic conditions.

In order to realize in practice the possibilities which are now only more or less close prospects, it is necessary still to surmount

various difficulties (for instance, to develop profitable methods of collection and storage of algae, etc.). Some of these questions are still unsolved but at present they are no longer the subject of scientific fantasies or theoretical considerations, but one under intense study, in the laboratories of many countries. The difficulties and unsolved questions encountered during the cultivation of algae on sewage exist in equal measure with any other means of large-scale cultivation of algae, not connected with sewerage. The large-scale cultivation of algae has been allotted much attention and in this rapidly developing field a great deal of experience has already been accumulated (Algal culture, 1953). It is sufficient to say what at the biggest experimental installation of this nature, in Cambridge (United States), containing 4500 liters of culture with an area of insolation of 56 m^2, they have already obtained, in a short period, 45 kg of dry substance of Chlorella, where the maximum average daily "harvest" reached 11 g/m^2. Just how great this magnitude is will stand out clearly if we consider that it corresponds to a yield of 110 kg of dry substance from 1 hectare in one day (not in one year!). Although this is the maximum magnitude and on the average, over prolonged periods of time, the yield of algae obtained in practice was less, nevertheless these figures show just how serious are the already-obtained results of large-scale cultivation of algae, despite the fact that we speak of first attempts of this nature, in which many difficulties still have not been surmounted.

Much has been done also with respect to the development of possible methods of collecting algae by means of gravitational sedimentation with or without application of coagulators, centrifuging, or combined methods and also of methods of drying and storage of

harvested algae. As a result of thorough laboratory study of light, of the regime most favorable for effective photosynthesis, the most rational ways of utilizing the energy of solar radiation are clear in principle. It is obvious that all these data must be considered during the development of methods of the application of algae for the purification and utilization of sewage.

From what we know about the light regime most favorable for the growth of algae and from experience with large-scale cultures, we can make the following conclusion; in order to intensify and standardize as far as possible the process of self-purification flowing with the participation of algae, special installations will be needed to ensure carbon supply and intense mixing of thick suspensions of algae. During intense mixing of the poorly transparent suspensions each of the cells will be in conditions of intermittent illumination, at which the greatest utilization of light is ensured. Just how these arrangements will be designed and built will be shown by future studies which should be organized in scientific research establishments developing questions of medical-hygiene and sanitation engineering.

The success of works of this kind will be determined not only by whether they are organized on the required scale and are sufficiently equipped in terms of contemporary laboratory technology, but also by the approach used in the combined development of the medical-hygiene, microbiological, chemical, and hydrobiological sides of the question. It is already clear just how important are the specific peculiarities of various algae, which must be known and used; also clear is the necessity of drawing together the available physiological data. In brief, the solution of the considered medical-hygiene and sanitation engineering problems requires the work of qualified

biologists. Medical hydrobiology encounters a complicated problem. It is not a question of a new variant of a system of model organisms, but a problem lying in a completely different plane. The question of algae as agents of self-purification constitutes one of the special aspects of the general problem of the functional value of living organisms in a reservoir. The solution of problems of this kind is possible only with quantitative appraisal of phenomena and requires the development of unique methods, responsive to the stated goals, which pass for beyond the framework of traditional methods of "medical appraisal of reservoirs on the basis of biological investigations" (Kononov, 1953) which, unfortunately, at present sometimes tend to limit the horizon of medical hydrobiologists.

We cannot fail to note that the matter concerns not only special questions which should occup branch institutes. Questions connected with large-scale cultivation of algae are in the closest way interlaced with questions arising during the study of large-scale development of planktonic algae in natural reservoirs or fish ponds, etc., and constitute particular aspects of the overall problem of the primary productivity of reservoirs. The consequences of large-scale development of algae for sanitation and hygiene and the fish industry have a common basis - the objective natural regularities of development of autotrophic organism creating primary production is most closely connected with the regularities of the biotic cycle of matter in reservoirs. Hence the need is clear for study of this circle of phenomena also from the more general hydrobiological or limnological point of view. From this example it is possible to see the unrealistic nature of naive attempts to negate the need for the existence of general hydrobiology, occupied with questions whose solution is

necessary in equal measure for each of the specialized hydrobiological disciplines, serving separate branches of the national economy.

Conclusions

1. Planktonic algae when grown on a large scale on undiluted city sewage sharply accelerate the process of self-purification; this is expressed in faster initial lowering of the BOR, early termination of the anaerobic phase, the appearance of free oxygen, and in the accelerated onset of nitrification. The accumulation of a large quantity of organic substances synthesized by the algae in the composition of the bodies of the living cells is not reflected in the magnitude of the BOR.

2. With the large-scale development of green algae in self-cleaning sewage the death rate of coliform bacterium is sharply increased.

3. The construction of ponds filled with undiluted sewage is the simplest way to use green organisms as agents of self-purification; it draws attention also as an effective method of purification, especially applicable in regions with warm dry climates.

4. Further study of the conditions favorable to the development of photosynthesizing planktonic organisms will allow setting up problems in the large-scale cultivation of algae as one of methods of utilizing sewage. In particular one should clarify the possibility of using algae grown on sewage to increase of the productivity of piscicultural ponds.

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