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GLASS EFFECT AND THE MICROBIAL PLANKTON-SESTON RELATION IN THE WATER OF LAKES BALATON AND BELSŐ

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According to ZoBell and Anderson (1936), the development of bacteria in water stored in glass containers is initiated by the surface effect of the glass. Later this phenomenon was further supported and it was said that this increase in the number of bacteria as a results of glass effect was produced only in certain species of bacteria easily accommodating to the new environment (Stark et al. 1938; Harvey, 1941; ZoBell, 1943, 1946; ZoBell and Grant, 1943).

The surface-volume relation was not detected in many earlier studies (Lloyd, 1937; Castell and McDermott, 1941) and the results of Taylor and Collins (1949) also prove that the glass effect is more than a simple surface effect. In the water stored in glass container a large number of ecological factors change and the surface effect is only one of the most significant one among these.

It has become known during the first investigations (ZoBell and Anderson, 1936; Heukelekian and Heller, 1940) that the glass effect is significant only in waters with a very low organic content. This is supported by new investigations, too (Hajlov and Finenko, 1968). These results suggest a direct relation between the intensity of the glass effect and the trophic state of the waters.

In the shallow waters without summer stratification this supposed relation has not been investigated at all (Zih, 1929) and therefore we determined the significance of the glass effect in the water of the shallow, highly eutrophic Lake Belső and of the shallow Lake Balaton with a very low organic content. On the basis of the supposed relation in the waters with a low level of organic matter contentration and at the same time with a significant glass effect a large part of the microbial plankton may be attached to the seston. We have not any reliable information about this seston-attachment and so our second purpose was to detect and describe the microbial plankton—seston relation in the above mentioned lakes.

Method

The samples were taken on 6th August, 1969 from the open water of Lake Balaton in front of our Institute and from the centre of Lake Belső with a Francev's sampler (Kuznetsov, 1952) from a depth of 50 cm. The experiments were carried out in a 2 l glass container with row and membrane filtered (pore size: 6 μ) lake water. The samples were incubated at 25 °C in the dark. The quantity of the total microbial plankton was determined by the RASUMOV's method (1932). In addition to the total microbial plankton the intensity of the glass effect was determined by three media with different organic matter content: nutrient, sodium-caseinate and lake water agar (OLÁH and VÁSÁRHELYI, 1970). The slides were sealed with dilution series in five repetition.

To determine the number of the microbial plankton attached to the HCl-soluble inorganic seston fraction 5 ml/l n/20 HCl-solution was added to the samples (after HCl-treatment the samples had a pH value: 6.8—7) membrane filtered (pore size: 6 μ) and compared to the membrane filtered samples without HCl-treatment.

Results

The significance of the glass effect is demonstrated by the ratio (S) between the highest number of bacteria determined on the membrane filter *(Table I)* and on different media *(Tables II, III* and *IV)* during incubation and the initial number of bacteria.

In the filtered lake water the values of S *(Table V)* were always higher than those in the original, unfiltered lake water. The differences between the S values of the filtered and unfiltered lake water were higher in the water of Lake Balaton with low organic matter content than those in the water of the highly eutrophic Lake Belső.

The glass effect measured with the total microbial plankton *(Table I)* comparing with the glass effect determined on various media *(Tables II, III* and *IV)* was small. This indicates that during the incubation the proportion of the different physiological groups changes as a result of glass effect. This selective effect has been also indicated by the fact that the various types of colonies appearing on the agar slides inoculated from the original lake water immediately after sampling have disappeared and only some types of colonies have appeared and become dominant on the agar slides due to the glass effect. The number of the dominant type of the colonies was smaller on media rich in organic matter.

The values of S in the filtered water of the highly eutrophic Lake Belső are smaller than those in filtered water of Lake Balaton *(Table V)*. The difference between the two lakes was the largest on nutrient agar, in the case of the microorganisms requiring high concentration of organic matter. The value of S on the sodium-caseinate agar, in the water of Lake Balaton was higher by two orders of magnitude and on the lake water agar it decreased to one order of magnitude. The value of S determined on membrane filter was nearly the same on both lakes.

On 3th January, 1970 the number of the heterotrophic microorganisms in the ice-covered open water in front of our Institute was $3 \cdot 10^2$ cells/ ml and in the Keszthely-Bay $8.9 \cdot 10^4$ cells/ml. In the unfiltered lake water the number of the heterotrophs from the open water in front of our Institute increased 47 times while in Keszthely—Bay decreased 14 times after a 19 days incubation as a results of the glass effect.

TABLE I

Glass effect measured with the total microbial plankton

Sample	Incubation time (in days) and the corresponding quantity of microorganisms (cell/ml $\cdot 10^5$)					
	1. S.D.	2. S.D.	3. S.D.	6. S.D.	7. S.D.	36. S.D.
Lake water (L. Balaton)	2.3±0.1	2.5±0.09	1.4±0.001	1±0.9	0.7±0.01	0.1±0.005
Filtered water (L. Balaton)	1.0±0.07	1.7±0.2	8±1.9	5.1±0.2	0.5±0.07	0.2±0.04
Lake water (L. Belső)	30±1	33±1	78.6±1.5	66±8.3	60.5±6.5	2.4±0.3
Filtered water (L. Belső)	16.6±0.5	— — —	121±3	82.6±15.3	35±3	3.7±0.7

TABLE II

Glass effect measured on sodium-caseinate agar

Sample	Incubation time (in days) and the corresponding quantity of microorganisms (cell/ml $\cdot 10^2$)					
	1. S.D.	2. S.D.	3. S.D.	6. S.D.	7. S.D.	36. S.D.
Lake water (L. Balaton)	9.8±1.2	27±1.0	76.3±1.5	47±1.0	108±1.0	84.6±3.7
Filtered water (L. Balaton)	0.4±0.1	6.5±2.2	626.6±15.3	816.6±40.4	1123±127	803.3±10
Lake water (L. Belső)	453.3±9.5	570±6.3	866.6±1.5	1680±1.9	206.6±9.6	180±10
Filtered water (L. Belső)	74±6.5	370±10	3400±265	2576.6±90	1733±37.9	356.6±6.5

TABLE III

Glass effect measured on nutrient agar

Sample	Incubation time (in days) and the corresponding quantity of microorganisms (cell/ml $\cdot 10^2$)					
	1. S.D.	2. S.D.	3. S.D.	6. S.D.	7. S.D.	36. S.D.
Lake water (L. Balaton)	5.6±0.3	6±0.3	41±2	33.6±2.5	35±1	— — —
Filtered water (L. Balaton)	0.2±0.2	1.7±0.3	220±1.2	626.6±40.2	680±10	86±31
Lake water (L. Belső)	155±1.5	137.6±2.5	165±1.5	362±1.5	160±1	— — —
Filtered water (L. Belső)	44±3.6	186.6±153	1486±100	1190±45.9	1110±5.7	— — —

TABLE IV

Glass effect measured on lake water agar

Sample	Incubation time (in days) and the corresponding quantity of microorganisms (cell/ml $\cdot 10^3$)					
	1. S.D.	2. S.D.	3. S.D.	6. S.D.	7. S.D.	36. S.D.
Lake water (L. Balaton)	0.2±0.003	0.2±0.001	13.9±0.1	2.7±0.1	5.2±0.3	— — —
Filtered water (L. Balaton)	0.01±0.003	0.09±0.01	14±1	22.3±1.5	29.6±1.5	— — —
Lake water (L. Belső)	3.8±0.1	2.4±0.2	4.2±0.1	24.9±0.8	8.1±0.5	— — —
Filtered water (L. Belső)	1.1±0.2	12.3±0.6	132.3±4.9	222.3±5.1	78.3±2	— — —

TABLE V

The proportion between the highest number of bacteria measured during the incubation and the initial number of bacteria (S)

Sample	On sodium-caseinate agar	On nutrient agar	On lake water agar	On membrane filter
Lake water (L. Balaton)	11.02	7.3	69.5	1.08
Filtered water (L. Balaton)	2807.5	3400	2960	8
Lake water (L. Belső)	3.7	2.4	6.5	2.6
Filtered water (L. Belső)	45.9	33.7	202	7.2

In the water of lakes Balaton and Belső the percentage of the microorganisms attached to the seston is different *(Table VI)* The attachment to the seston in the water of Lake Balaton containing smaller amount of nutritive substances is greater. However, when regarding the total microbial plankton this attachment is smaller than that at the heterotrophic microorganisms which at the same time require a higher concentration of the organic matter, too.

TABLE VI

Percentage of the microorganisms attached to the seston

Sample	Determined on sodium caseinate agar	on nutrient agar	on lake water agar	on membrane filter
Lake Balaton	96	96.5	95	56.6
Lake Belső	83.7	71.7	72	44.7

With a treatment of the diluted HCl-solution before the filtration it was possible to separate the organic and inorganic fractions of the seston owing to the large amount of the $CaCO_3$ crystal suspended in the water. The attachment to the inorganic fraction of the seston *(Table VII)* in the water of Lake Belső is small. The same in the water of Lake Balaton was very high, nearly one half of the microorganisms attached to the seston was found in the inorganic HCl-soluble fraction.

TABLE VII

Percentage of the microorganisms attached to the inorganic HCl-soluble seston

Sample	Determined on sodium caseinate agar	on nutrient agar	on lake water agaz	on membrane filter
Lake Balaton	47	53	42	57
Lake Belső	9	11	7	23

Discussion

My investigations have supported the selective feature of the glass effect. The explanation of this phenomenon may be attributed partly to the selective adsorption of organic matter on the solid surface (ZVJAGINCEV and VELIKANOV, 1968) and to the different adsorptive affinity of bacteria to the solid surface (ZVJAGINCEV, 1959). According to the present results the measured selective effect increases with the increasing organic matter content in the media used for the detection of the glass effect. This directly proves that the glass effect stimulates first of all the proliferation of the microorganisms requiring higher concentration of the nutritive substances. This further supports that the values of S indicating the significance of the glass effect are the highest in nutrient agar containing the largest amount of organic matter and the smallest in the total microbial plankton measured on the membrane filter *(Table V)*.

According to KRISS's idea (1963) the dilute organic matter besides concentration on the solid surface changes its quality and the stabile organic matter characterizing the majority of the oligotrophic waters becomes more available for the water microorganisms. On the basis of this explanation the quantity of this stabile organic matter has been measured in marine waters (KRISS, 1968). KRISS's explanation is based on the fact that during storage the number of heterotrophic microorganisms being very sensitive to changes in the easily available organic matter content increases and the quantity of the total microbial plankton remains nearly at the same level. This fact was supported by the results obtained by MELCHIORRI-SANTOLINI (1966). The glass effect measured with the changes of the total microbial plankton quantity was small in our experiments, too.

The values of S in the water of Lake Balaton sampling from section "A" with a very low level of organic matter were higher by several orders of magnitude than those in the water of the highly eutrophic Lake Belső. This suggests a direct relation between the significance of the glass effect and the trophic level in waters. This has been also proved by the "negative glass effect" observed under ice in the water of the Keszthely-Bay with a very high biomass of heterotrophic microorganisms.

The large percentage of the microorganisms attached to the seston and especially the attachment to the inorganic seston fraction prove that in the water of Lake Balaton having a significant glass effect due to the low organic matter content the surface effect has a very important role in the turnover of the in vivo organic matter, too. From this it follows that in the development of the intensive self-purifying ability of Lake Balaton (OLÁH, 1969) the $CaCO_3$ crystals with a large surface area suspended in the water exhibit a very important role besides the high summer water temperature even at the mud-water interface and the high oxygen saturation.

Summary

1. In the water of lakes Balaton and Belső with different trophic level the significance of the glass effect has been indicated by the vaule of S, the proportion between the highest number of bacterial measured during the incubation and the initial number of bacteria.

2. In the filtered water the values of S were always larger than those in the unfiltered water and increased parallel with the increasing organic matter content in the media employed in detecting of the glass effect. In the filtered water of Lake Balaton the values of S was higher by two orders of magnitude, than those in the water of the highly eutrophic Lake Belső. This suggests a direct relation between the significance of the glass effect and the trophic level.

3. In Lake Balaton the 56 per cent of the total microbial plankton was attached to the seston in the investigated period. At the heterotrophic microorganisms this percentage increased to 96. In the water of Lake Belső the seston-attachment was smaller (45 and 84 per cent). The differences between the two lakes were even higher in the case of the attachment to the inorganic seston.

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AZ ÜVEGHATÁS ÉS A MIKROBIÁLIS PLANKTON—SZESZTON KAPCSOLAT A BALATONBAN ÉS A BELSŐ-TÓBAN

Oláh János

Összefoglalás

1. A különböző tápanyagellátottságú Balaton és Belső-tó vízében vizsgált üveghatás nagyságát az inkubálás folyamán mért legmagasabb baktériumszám és az eredeti baktériumszám aránya (*S*) mutatja.

2. Az *S* értékek a szűrt vízben mindig nagyobbak mint a szűretlen vizben és az üveghatás nagyságának felmérésére használt táptalajok szervesanyag tartalmának növekedésével párhuzamosan nőnek. A Balaton szűrt vizében az *S* két nagyságrenddel nagyobb volt mint az erősen eutróf Belső-tó szűrt vizében. Ez arra utal, hogy összefüggés van az üveghatás nagysága és a tó trofikus állapota között.

3. A Balaton teljes mikrobiális planktonjának 58 százaléka szesztonhoz rögzült a vizsgálat időpontjában. A heterotrófoknál ez az arány 96 százalékig emelkedett. A Belső-tó vizében a szesztonhoz rögzülés kisebb. A teljes mikrobiális planktonnál 45 százalék, a heterotrófoknál 84 százalék. A szeszton szervetlen részéhez való rögzülésben a két tó között a különbségek még nagyobbak. A Balaton vizében tehát a felülethatás fontos szerepet játszik a tó szervesanyag forgalmában.