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INSTITUT ZA OCEANOGRAFIJU I RIBARSTVO — SPLIT
FNR JUGOSLAVIJA

Vol. VIII. No. 5.

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SPLIT 1956

MULTIPLICATION OF HETEROTROPHIC SEA BACTERIA IN VARIOUS H-ion CONCENTRATIONS

by

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Under determined conditions sea bacteria decompose about 50% of all the organic matter in the sea, and about 50% of the matter resists to the action of bacteria. About 60—70% of the decomposed matter is oxidated and 30—40% is utilized for the synthesis of bacterial cellular matter (Waksman and Carey 1935, ZoBell and Grant, 1943). The multiplication of bacteria in sea water is therefore conditioned chiefly to the quantity of disponible organic matter. Investigations further more proved that the bacterial decomposition of organic matter, respectively the multiplication of sea bacteria, is conditioned to a lot of factors. So were examined the influence of various kinds of organic matter, the influence of temperature, the quantity of oxygen, the volume of water and some other factors (Whipple 1901, Waksman, Carey and Reuszer 1933, Waksman and Renn 1936, ZoBell and Anderson 1938, ZoBell 1940 and 1943, and others).

Up to the present day it has been ascertained that the H-ion concentration has a remarkable effect on the productivity and activity of the enzymal system of bacteria, on the structure of the products from bacterial assimilations and dissimilations of some carbohydrates (Watt and Werkman 1951, Osborn et al. 1937 cit. Werkman and Wilson 1951) on the multiplication of various bacterial species (Stephenson 1949) and cultures (Knaysi 1951). The influence of this factor on the multiplication of sea bacteria and respectively on the intensity of decomposition of organic matter in sea water, is unknown up to day. This is why we undertook to investigate the effect of H-ion concentrations on the multiplication of heterotrophic sea bacteria under laboratorial conditions, since their number permit to form conclusions about the intensity in the process of decomposition of organic matter in water (Kriss 1954).

I am glad to be able to express my gratitude to my collaborators, the laboratory-technicians Ojdana Marović and Peter Bilić, for

the assistance they lent to me in carrying out the experiments as well as in the technical work in the compilation of the manuscript.

Method of work

In five flasks provided with wide necks, which were previously sterilized, we poured in each 9 lit. of sea water, filtered through thick filter-paper, in order to remove from the water all organisms, except bacteria. The flasks were marked with the letters A, B, C, D and K. The flask »K« was the so-called control flask, i.e. in this flask the H-ion concentration was left unchanged. In the other flasks we altered the H-ion concentration by adding 2% HCl. In order to prevent the compensation of the consumed oxygen in the water during the experiments we put into each flask on the surface of the water a layer about 3 cm. thick of raw engine oil twice sterilized. Through the cork stopper were let down till the bottom, some thin glass tubes through which every 24 hours were taken samples of water for analysis. The flasks, during the time while the experiment lasted, were kept in the dark at a temperature of the air varying from 20 to 24° C. and the average temperature of the water was of 22,4° C.

The number of heterotrophic bacteria, namely of sea-water bacteria which under laboratorial conditions can multiply on the nutrient broth of organic compositions, was determined by the method of bacterial counting on dishes with the use of the usual agar-bases. (Cviić 1953). As we expected to find large quantities of bacterial cells in water, we proceeded to the dilution of the original water from the experiments with sterile sea water in a proportion of 1:100 till 1:100.000. We inoculated 10 cm^3 of nutrient broth with 1 cm^3 of diluted water sample, the cultures were incubated for 7—8 days at a temperature of 24° C. and for the counting of the colonies we used a magnifying glass and a Helige-apparatus. Each sample of water was parallely inoculated on 3 Petri-dishes and the arithmetical mean was taken as the number of bacteria per 1 cm^3 of water in the experiment.

To determine the H-ion concentration we used the electric pH-meter (Radiometer-pH-meter 22, Copenhagen). The quantity of oxygen in the water was determined by a somewhat modified Winkler's method. The samples of water for analysis from the flasks in the experiment, were taken every 24 hours. All the experiments were repeated thrice under the same conditions, and as the variation in the results were insignificant, all further repetitions were deemed useless.

Experiments and Results

For the sake of a basic orientation, before the very examination of the growth of heterotrophic bacteria in the various H-ion concentrations, it was necessary to examine the behaviour of pH medium in the various speeds of development and multiplication of heterotrophic sea bacteria, since about them, up to now, we have no data yet.

We availed ourselves of the experience obtained by ZoBell (1941 and 1943) who has ascertained that in flasks having a larger surface of attachment richer bacterial populations are developed. In three flasks containing 10 l. we augmented the surface of attachment in different ways, i.e. in one flask (A) we left the normal surface, in the second (B) the surface of attachment was augmented by putting in the bottom of the flask a layer 3 cm. thick of well washed sea sand, and in the third flask (C) besides sand were also put three upright small bundles of glass tubes. The flasks were sterilized in autoclave and then into each of them was poured 9 lit. of original sea water. The experiment lasted 16 days and confirmed our expectations. In the flask »A« the development was the weakest, i.e. the maximal development was attained on the fourth day and amounted to 17×10^3 bacteria per cm^3. In the flask »B« the maximal bacterial development was attained on the third day (175×10^3 bacteria per cm^3.) and in the flask »C« the maximum was on the third day and amounted to 358×10^3 bacteria per cm^3. and consequently it was the highest. After having attained the maximal development, the number of bacteria suddenly dropped off in all the three flasks and after 6—7 days it attained the average of 5×10^3 bacteria per cm^3.

The H-ion concentration in the flasks was measured every day and the results are shown in Fig. 1. — It appeared that during the development of the bacterial cultures pH rather fluctuated, but that in that fluctuation there were mainly two stages. From the beginning of the

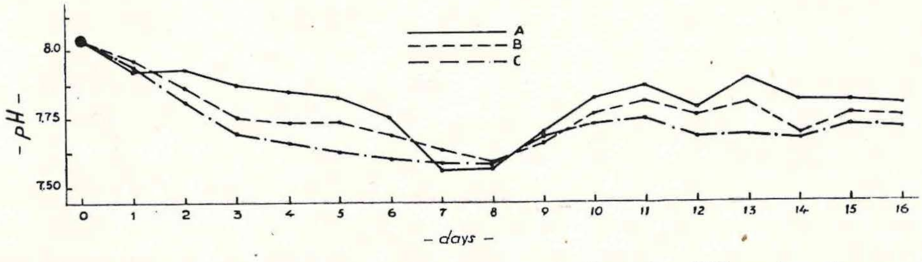

Fig. 1. Behaviour of sea water H-ion concentration in different cultures. A: poorer. B: middle and C: richest bacterial culture.

experiment (ph — 8,02) till the seventh day in all the three flasks pH suddenly dropped off till 7,60 and then, after a certain standstill it again began to rise and on the sixteenth day amounted to 7,72 till 7,79. We might therefore conclude that during the first seven days of development in the bacterial populations predominated the processes of biogenetic transformation of the organic material, the products of which acidified the medium and caused therefore the production of CO_2, of organic acids, assimilation of NH_3, creation of NO_2 and NO_3 and others. In the second part of the experiment (from 7 to 16 days) predominated the processes the final products of which had alkalinic character, namely consumption of CO_2 some salts of organic acids, creation of NH_3 reduction of NO_2 and NO_3 and others. On the other hand the results of these experiments show that pH fluctuation is connected with the speed of development and abundance of bacterial populations. This was evinced in the first stage of the cultures development (from 0—7 days) when the dropping of pH was the swiftest and the strongest in the flask »C«, namely in the flask having the most abundant bacterial population. Slower and slighter was the dropping of pH in the flask »B« and the slowest and slightest in the flask »A«. The same serial order appeared also in the second stage of the bacterial populations' development which appeared to be dropping and equalizing in all the flasks (from 7—16 days) where the renewed rise of pH was the least in flask »C«, bigger in flask »B« and the biggest in flask »A«.

It is obvious, then, that sea bacteria with their biochemical activity in decomposing organic matter act totally on the H-ion concentration of the medium in the sense of a rise of the latter, i.e. of acidification and that in general this process has a tendency to neutralize the medium. On the other hand the intensity of this process depends of the speed of the bacterial populations' development, i.e. on the intensity of the biochemical processes.

Taking in consideration the results obtained in the afore mentioned preliminary experiment, we examined the multiplication of heterotrophic sea bacteria in the various H-ion concentrations. The method adopted in performing the experiment has been already described in the »M e t h o d o f W o r k« and the results obtained are to be found in F i g. 2 and 3.

As said before in the flasks K, A, B, C and D which were filled with 9 lit. of filtered sea-water each, we altered PH by adding determined quantities of 2% HCl. In such a way we obtained a H-ion concentration varying from 8,15 to 6,81. From the beginning of the experiment till the second day in all the flasks PH dropped (Fig. 2). After the second day in the control flask (»K«) the dropping of pH continued till the end of the experiment. In the flasks »A« and »B« after the second day pH rose and after the third till the fourth day almost up to the end of the experiment it dropped again. In the flasks »C« and »D« after the second day pH rose more abruptly and then up to the end of the experiment it rose gradually. From the thirteenth day in all the four flasks (A, B, C and D) pH was rising and the final pH in all the five flasks varied from 7,36 to 7,42. Consequently in all the flasks there was a tendency to a manifestation of an intensive development of the bacterial populations

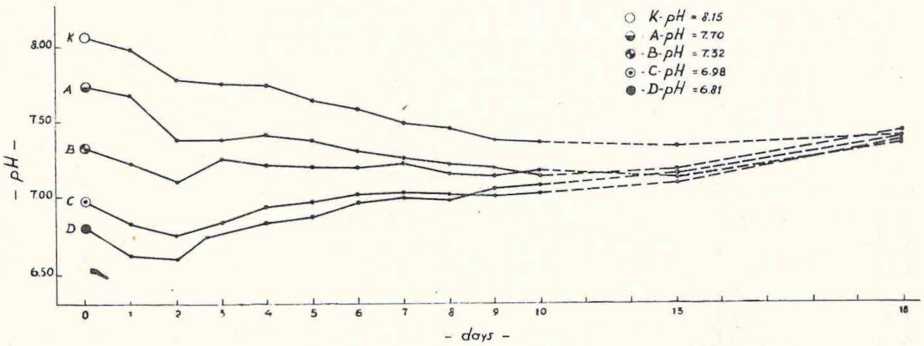

Fig. 2. Behaviour of H-ion concentration in sea bacterial cultures with different initial pH of sea water.

dropping of pH on the first and second days. By all means this was a manifestation of an intensive development of the bacterial populations in all the flasks (Fig. 3), respectively of copious biochemical processes decomposing organic matter, the accompanying and final products of which, at that time,acidified the medium. After the second day the number of bacteria in the cultures suddenly dropped and the H-ion concentration in all the flasks till the end of the experiment had a tendency to move towards a neutral reaction.

Taking in consideration the development of bacteria up to the third day, namely till the day when the initial pH had not yet been sensibly corrected, we can affirm that there existed a direct connection between H-ion concentration and the development of bacteria populations (Fig. 3). The most luxuriant bacterial multiplication was found in the flask having the lowest initial pH (flask »D«, pH—6,8) then in the flask »C« with a somewhat richer initial pH (6,98). In the flasks »K, A and B« in which the initial H-ion concentration was in the region from alkalic towards neutral (8,15 to 7,32) the development of the bacterial populations was weaker. From the third day to the end of the experiment the number of bacteria per cm³. remained in general the same in all the flasks.

If we compare the fluctuation of pH in the preliminary (Fig. 1) and in the main experiment (Fig. 2) it strikes us that the fluctuation of pH in the preliminary experiment was much stronger. Also the fluctuation in the number of bacteria per cm³. in this experiment was stronger than in the main one. This may be ascribed to the fact that in the preliminary experiment we did not filter the sea water and consequently did not remove from it the living and the broken down plankton organisms. Therefore the multiplication of bacteria, respectively of the single

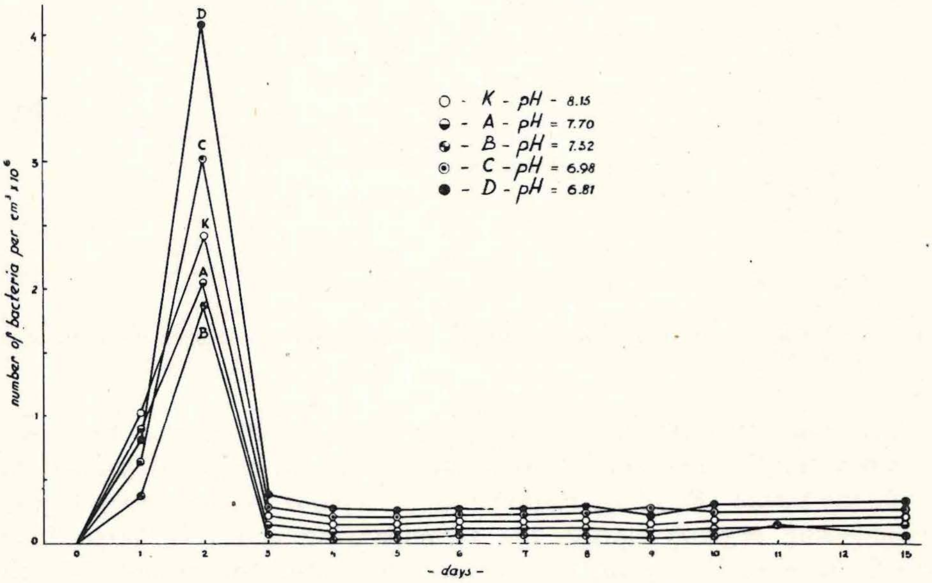

Fig. 3. Development of bacterial cultures at different initial H-ion concentrations of sea-water.

bacterial groups, was irregular. Waksman, Carey and Reuszer (1933) had namely asserted that some of the zooplankton constituents are liable to decomposition in the first stages, i.e. at the begining of the experiment (e.g. the proteins) and later follows the decomposition of other constituents (e.g. hitine and fat substances).

As a control for the bacterial decomposition of organic matter we availed ourselves of the oxygen consumption which we also determined every day in all the flasks. The oxygen density in water at the beginning of the experiment was in all the flasks 6,59 mgr/1. Two days later the biggest consumation was found in the flask »D« (3,23 mgr/1) then in flask »C« (2,47 mgr/1) and further on in the flask »K« (1,82 mgr/1), »A« (1,65 mgr/1) and »B« (1,63 mgr/1). Consequently also the data about the consumption of oxygen for the first two days of the experiment confirm that there was, at that time, the most intensive decomposition of organic matter in water with the highest H-ion concentration. The consumption of oxygen during the first five days of the experiment was quicker and then till the end of the experiment it was gradual and generally uniform in all the flasks. On the eighteenth day, namely at the end of the experiment, the quantity of oxygen in the water varied from 0,09 to 0,27 mgr/1.

Discussion about the results

It appears from the results of our experiments that the changes of H-ion concentrations provoke a strenghtened activity of the enzymic system which is responsible for the growth and the division of the bacterial cells. Since the multiplication of the cells was the most intensive with the value pH in the acid region, one should presume that in such a region the optimum is pH for the development of the aforesaid enzymes, or, again this pH provokes in another way a brisker activity of the enzymic system. The same connection of the pH medium and the bacterial populations was found by Emery and Rittenberg (1952) in the sediments, where with the lowest pH came the most developed bacterial population. On the other hand our experiments show that also in the cultures of sea bacteria, by means of deamination, respectively of decarboxylation (Gale 1946) is effected a turnover of pH media towards the acid, respectively towards the alkalic. In other words also in the cultures of sea bacteria H-ion concentration affects their life activity and the multiplication of bacterial cells and the consequence thereof is that in the acid media there is a strenghtened activity of the enzymic system, respectively a faster decomposition of the disponible organic matter in sea water. — It is not to be excluded that these results may be connected with the finding of Buljan (1953) who in a basin filled with sea water, enriched with P-PO_4 and corrected pH (with the aid of H_2SO_4) to 7,26 — 8,35 remarked a fifteen times bigger growth of clorophyll than in a similar basin without correction of pH, which was 8,36 — 9,00. This growth of clorophyll may also be, partly, a consequence of the strenghtened bacterial activity which occurs because of the acidification of the medium, which led to a strenghtened mineralization of the organic matter, putting so at the disposal of the autotrophic planktonic organisms bigger quantities of available salt.

SUMMARY

We examined the behaviour of H-ion concentration in heterotrophic sea bacteria cultures and the multiplication of heterotrophic sea bacteria in various H-ion concentrations under laboratorial conditions.

1) Sea heterotrophic bacteria increase the H-ion concentration of natural sea water, respectively dislocate pH from alkalic towards acid reaction. This process is more intensive in rich bacterial cultures.

2) In acid medium (initial pH 6,81 and 6,97) the multiplication of heterotrophic sea bacteria at the beginning of the experiment is more intensive than in alkalic medium (initial pH 7,32 and 7,72). In bacterial cultures with different initial H-ion concentration pH shifts from acid towards alkalic and neutral reaction.

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Received for publication December 30, 1955.

RAZMNOŽAVANJE HETEROTROFNIH MORSKIH BAKTERIJA PRI RAZNIM KONCENTRACIJAMA H-iona

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Kratak sadržaj

Ispitivano je ponašanje koncentracije H-iona u kulturi heterotrofnih morskih bakterija i razmnožavanje heterotrofnih morskih bakterija pri raznim koncentracijama H-iona, pod laboratorijskim uslovima.

1. Morske heterotrofne bakterije povisuju koncentraciju H-iona prirodne morske vode, odnosno pomiču pH od alkalne prema kiseloj reakciji. Ovaj proces je intenzivniji u bogatijim bakterijskim kulturama.

2. Razmnožavanje heterotrofnih morskih bakterija je u početku eksperimenta intenzivnije u kiselijim sredinama (inicijalni pH 6,81 i 6,97), nego u alkalnim sredinama (inicijalni pH 7,32 i 7,72). U bakterijskim kulturama, s različitom inicijalnom koncentracijom H-iona, mijenja se pH pomicanjem iz kiselog i alkalnog prema neutralnoj reakciji.

Tiskanje završeno 30. VII. 1956.

Tisak: Novinsko-izdavačko poduzeće »Slobodna Dalmacija« — Split