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PRODUCTION AND CONSUMPTION OF B GROUP VITAMINS *IN SITU*

A. KURATA

Lake Biwa Research Institute, Vehide-Hama, OTSU, Shiga 520, (JAPAN)

ABSTRACT - The changes in concentration of thiamine, biotin and vitamin B_{12} in water of the soaked bottles for 6 hr *in situ* in the north basin of Lake Biwa were examined successively over 30 hours. The concentrations of the vitamins changed comparatively during 6 hours soaking *in situ*. Generally, the concentrations of thiamine, biotin and vitamin B_{12} were in high levels in the morning and in low levels in the evening both in the soaked bottles and in the surrounding water. The fluctuation ranges in concentration of vitamins in a 5 m deep layer were greater than those in thermocline. From the data obtained with the soaked bottles *in situ*, it was supposed that the decrease of vitamins in concentration during the daytime was attribute to surpass the consumption of vitamins by phytoplankton rather than the production, on the contrary the increase of vitamins in concentration during the night time was attribute to surpass the autochthonous production of vitamins by various kinds of microorganisms than the consumption of vitamins by phytoplankton. The averaged consumption rates of thiamine, biotin and vitamins B_{12} in concentration *in situ* were 4.50, 0.61 and 0.62 mμg/l/h, on the other hand, the averaged production rates of these vitamins were 4.32, 0.43 and 0.95 mμg/l/h, respectively. Bacteria producing vitamins by them was further examined. Some of the bacteria produced two or three kinds of vitamins simultaneously.

Key words: bacteria, freshwater, production of vitamins.

RÉSUMÉ - Les variations des concentrations en thiamine, biotine et vitamine B_{12} dans l'eau des bouteilles immergées *in situ* pendant 6 heures dans le bassin nord du lac Biwa ont été suivies sur 30 heures. Les concentrations en vitamines varient de façon comparable durant les 6 heures d'immersion. Généralement les concentrations en thiamine, biotine et vitamine B_{12} présentent des taux élevés le matin et bas le soir, ceci dans les bouteilles immergées comme dans l'eau de mer environnante. Les concentrations en vitamines varient plus dans une couche de 5 m qu'au niveau de la thermocline. A partir des données obtenues par les bouteilles immergées *in situ*, il est supposé que la diminution diurne de la concentration en vitamines serait due à leur excès de consommation par le phytoplancton. Au contraire, l'augmentation nocturne des concentrations en vitamines serait attribuable à leur production par différents microorganismes. Les taux moyens de consommation de thiamine, biotine et vitamine B_{12} *in situ* sont respectivement de 4.50, 6.61 et 0.62 mμg/l/h; ceux de leur production de 4.32, 0.43 et 0.95 mμg/l/h. Les bactéries produisant des vitamines sont plus particulièrement examinées. Certaines d'entre elles produisent simultanément deux ou trois sortes de vitamines.

Mots clés: bactéries, eau douce, production de vitamines.

INTRODUCTION

We have previously reported a successive diurnal change and a periodical diurnal rhythm in the concentration of B group vitamins having an inverse correlation to the phytoplankton population in the waters of the southern basin of Lake Biwa (Kurata A. *et al.*, 1982). However, it is necessary to clarify the short term continual fluctuation in the concentration of B group vitamins in an *in situ* closed system, in order to more clearly elucidate the interrelationship of the autochthonous vitamin production by microorganisms, and the consumption by phytoplankton requiring these vitamins, without allochthonous vitamin supply and water turbulence. Few reports have been published concerning the successive diurnal changes, and the continual fluctuations in the concentration of B group vitamins

during a short investigative period in the same water body both in the marine and fresh water aquatic environments. However, several reports have been published concerning the distribution of the concentration of B group vitamins. The present paper appears to be the first field study of the continual fluctuations in the concentration of B group vitamins for a successive 30 hour period in an *in situ* closed system.

MATERIALS AND METHODS

Water samples were collected every 6 hours over a 30 hour period from three depths (5 m, thermocline and 30 m) at a station located in the pelagic zone of the main basin of Lake Biwa. 21 subsamples from three layers were enclosed into glass bottles and incubated *in situ* for 6 hours at the same depth of the station. The incubation of the water samples was carried out at every successive sampling time during the investigative period. Immediately after sampling, the water samples collected and the incubated samples were carried back to the laboratory and used for the assay of thiamine, biotine and vitamin B_{12} by the microbiological method discribed in the previous paper (Kurata A. *et al.*, 1976). For the assay of B group vitamins, water samples were concentrated by approximately 50-fold, by evaporation under reduced pressure at 40°C. The concentrations of thiamine, biotin and vitamin B_{12} were determined microbiologically, using *Lactobacillus fermenti* ATCC 9338, *L. arabinosus* ATCC 8014 and *L. leichmannii* ATCC 7830, respectively. Heterotrophic bacteria were isolated from the water and *Egeria densa* using ZoBell 2216 F medium (ZoBell 2216 medium prepared with distilled water) and examined for the production of thiamine, biotin and vitamin B_{12}.

RESULTS AND DISCUSSION

Successive variations in the concentration of thiamine, biotin and vitamin B_{12} in waters from 5 m, thermocline and 30 m deep layers were examined over 30 hours at the designated station. Thiamine data are shown as an example in Figure 1. The concentrations of the three kinds of vitamins showed a greater range in the 5 m deep layer than in the 30 m deep layer. Generally, the concentrations of vitamins increased gradually from the

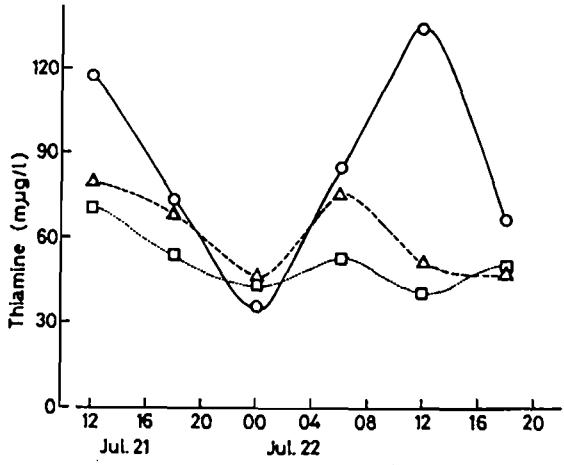

Figure 1 : Fluctuations of the concentration of thiamine for 30 hours in a 5 m deep layer (○——), in a thermocline (△) and in a 30 m deep layer (□...).

evening or midnight, to the morning or noon, by as much as three times in the 5 m layer, and then decreased gradually from the morning or noon to the evening. It is supposed that the decrease in the concentration of vitamins in the photosynthetic layer during the daytime, must be attributed to the consumption of vitamins by phytoplankton requiring vitamins. On the other hand, the increase in the concentration of vitamins must be caused by the surplus of the autochthonous production of vitamins by microorganisms. During the investigative period, stagnation of water was observed during very fine clam weather, without any water turbulence. Therefore, it is considered that the successive variation in the concentration of vitamins showed a tendency to repeate this pattern over 30 hours, because of the elimination of the supply of allochthonous sources of vitamins.

Secondarily, in order to clarify the relationship between the consumption and production of vitamins in water without water movement, and the allochthonous supply of vitamins, the variation in the concentration of vitamins in water of closed systems was examined with an *in situ* incubation of 6 hours. The data on particulate matter, mostly composed of phytoplankton, in the bottles incubated *in situ* for every 6 hours, over a 30 hours experiment, at 5 m, thermocline and 30 m deep layers, are shown in Figure. 2. The values obtained from the bottles after the *in situ* incubation of 6 hours were almost the same as those which were obtained from the surrounding water during the incubation of every layer, except for one example at 5 m.

The variation in the concentration of thiamine in the water of the incubated bottles for every 6 hours, incubating over 30 hours, is shown in Figure 3. After the incubation, the

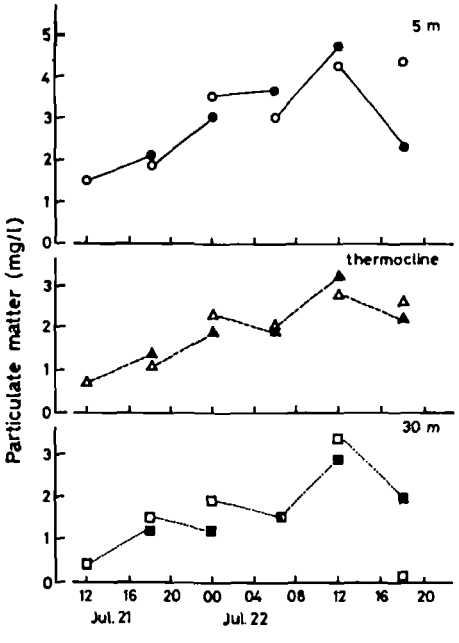

Figure 2 : Variations of particulate matter in the soaked bottles *in situ* for each 6 hours during a 30 hr experiment in a 5 m deep Layer (○ : initial ; ● : after the soaking) and the in a thermocline (△ : initial △ : after the soaking) and in a 30 m deep layer (□ : initial ; ■ : after the soaking).

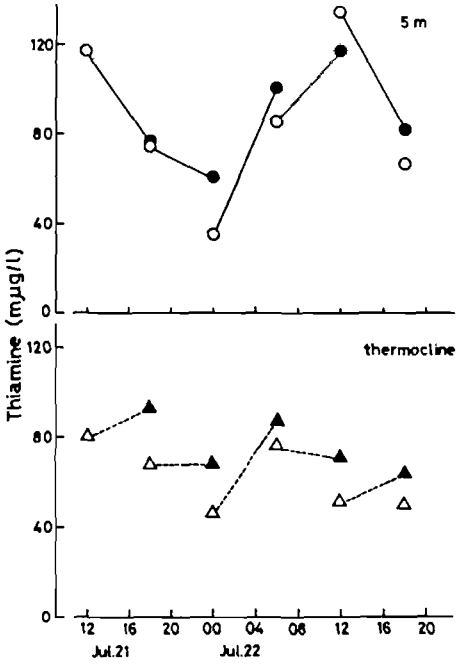

Figure 3 : Variations of the concentration of thiamine in the soaked bottles *in situ* for each 6 hr during a 30 hours experiment in a 5 m deep layer (● : initial ; ○ : after the soaking) and in a thermocline (△ : initial ; △ : after the soaking).

concentrations of vitamins in the water of the incubated bottles varied following the same pattern has the surrounding water, for every vitamin studied. The decrease in the concentration of vitamins in the water from the bottles during the daytime incubation undoubtedly signifies that the consumption of these vitamins, by phytoplankton requiring the vitamins, exceeds the availability of vitamins supplied by various kinds of producers. On the contrary, the increase in concentration of vitamins during the night incubation obviously signifies that the production of these vitamins surpasses the consumption by phytoplankton. The consumption rate of thiamine in the water of incubated bottles ranged from 0.33 to 8.65 mμg/l/h, and averaged 4.50 mμg/l/h in a 5 m deep layer and the thermocline for each *in situ* incubation. On the other hand, the production rate of thiamine in those bottles ranged from 0.20 to 11.07 mμg/l/h, and averaged 4.32 mμ g/l/h. The consumption of biotin ranged from 0.05 to 1.70 mμg/l/h and averaged 0.61 mμg/l/h, while the biotin production ranged from 0.28 to 0.60 mμg/l/hr and averaged 0.43 mμg/l/h. In the case of vitamin B_{12}, the consumption ranged from 0.09 to 1.63 mμg/l/h, while the production ranged from 0.59 to 1.17 mμ/l/h and averaged 0.95 mμg/l/h. Generally, for every vitamin, both the consumption rate and the production rate were higher in the 5 m deep layer than in the thermocline. The diurnal changes and the successive fluctuations in the concentration of B group vitamins in the southern basin of Lake Biwa have been previously reported (Kurata A. *et al.*, 1982). The data obtained in this study strongly supports this earlier work. It has also been that the primary production rate by an unicellular algal population, especially an ultra-phytoplankton population, showed a seasonal inverse relationship of the concentrations of B group vitamins in the waters of Lake Mergozzo, northern Italy (Kurta A. *et al.*., 1976). Recently, Pommel (Pommel B., 1975) reported the existence of an inverse relationship in the seasonal changes of the concentration of vitamin B_{12} and the population of *Oscillatoria rubescens* in lake Leman. The diurnal and successive variations of any kind of vitamins in the aquatic environments have hardly been reported. However, it appears that the concentrations of vitamins in aquatic environments generally decrease during the day and increase during the night, and the phenomenon will be repeated periodically if the water mass is calm and stagnant. The decrease of vitamin concentrations in the waters of the euphotic zone must be attributed to photosynthetic activity or primary production by phytoplankters.

To ascertain the production of vitamins by bacteria in water, heterotrophic bacteria were isolated from lake water and macrophytes, and their vitamin productivity was examined. The results are shown in Figure 4. Many bacterial strains isolated from the lake water

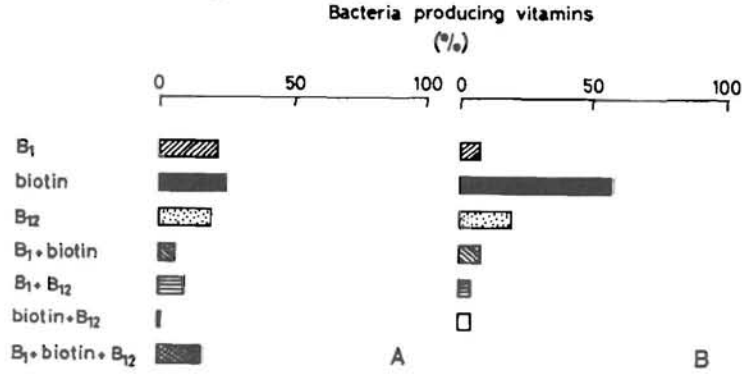

Figure 4 : Production of each combination of vitamins by bacteria isolated from *Egeria densa* (A) and water (B).

actively produced biotin and particularly most of the epiphytic bacteria isolated from *E.densa* which produced three kinds of vitamins. Accordingly, it is thought that the production of vitamins by these bacteria must be one of the most important autochthonous sources of vitamins in the pelagic zone of the lake.

That is to say, that role of B group vitamins must be very important to the increase of the algal community and the primary productivity on the aquatic environment, and that far more attention must be paid to the production and circulation of these vitamins in the hydrosphere.

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