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Application of polyurethane foam units and calorimetry to microbial monitoring in Lake Donghu

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Abstract

Combined with the national standard biomonitoring method (polyurethane foam units method), calorimetry was applied to study the metabolic activities of PFU microbial communities in fresh water to determine the effects of anthropogenic stresses on the activity of the microbial community. Comparisons were made at four sampling stations with different eutrophic status in Lake Donghu. Water quality variables, species number of protozoa, abundances of microorganisms, biomass, heterotrophy indexes and diversity indexes are reported. The heat rate–time curves of the native and concentrated PFU microbial communities were determined at 28 °C. Growth rate, measured maximum power output and total heat were calculated from the heat rate–time curves. The values of metabolic variables are higher at the more eutrophic stations, which suggests that organic pollution increases the activity of PFU microbial communities. The metabolic variables are in good agreement with chemical and biotic variables. And calorimetry will be useful for biomonitoring of the PFU microbial community.

Keywords: PFU microbial community; Calorimetry; Metabolic variables; Eutrophic status; Lake Donghu; Biomonitoring

1. Introduction

Bacteria, fungi, protozoa, algae and small rotifers exhibit complex interactions and form a specific community termed the microbial community [1]. Polyurethane foam units (PFU) can be used to colonize the microbial communities in water. Water quality is then evaluated by measuring structural and functional variables of the PFU microbial communities. The PFU method was first developed by Cairns et al. [2]. Since 1982, the PFU method has become popular in China and many valuable results have been achieved by this method [3]. It was approved by China Environment Protection Agency and promulgated as the national standard in 1992 as "Water Quality – Microbial Community Biomonitoring – PFU Method" (GB/T 12990-91) [4].

The microbial community plays an important role in nutrient cycling and energy flow in the environment. Study of the activity of the PFU microbial community is of importance to better understand the aquatic ecosystem. Calorimetry is a powerful tool for continuously monitoring the integrated metabolism of a complex system for a prolonged period without disturbance [5]. It has been successfully applied to plant ecology [6], animal ecosystems [7], microbial communities in different soils [8,9], and microplankton in the marine environment [10,11]. In the present study, calorimetry is used in combination with the standard PFU method to investigate the metabolic and structural characteristics of the PFU microbial communities in Lake Donghu in order to determine the effects of anthropogenic stresses on the microbial activities.

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2. Experimental

2.1. Sampling

The study site—Lake Donghu (East Lake), Wuhan, China (30°33′N, 114°23′E) is a subtropical shallow lake with a surface area of 32 km^2 and an average depth of 2.5 m. It is located near the middle reaches of the Yangtz River about 5 km from the river. In the late 1960s, the lake was divided into several parts isolated by artificial dikes: Guozheng Hu, Tanglin Hu, Hou Hu, Miao Hu and Niuchao Hu (Fig. 1) [12]. The Shuiguo Hu (with a surface area of 1.1 km^2), Guozheng Hu (with a surface area of 12.3 km^2) and Hou Hu (with a surface area of 4.9 km^2) were selected as the sites for our study. Four sampling stations, as shown in Fig. 1 were chosen based on their different eutrophic status. Stations 0 and I are hyper-eutrophic, and stations II and IV are eutrophic and meso-eutrophic to eutrophic, respectively.

Samples of PFU microbial communities were collected during September and October 2004. According to the standard PFU method, PFU were suspended 0.3 m below water surface for 15 days, which allowed most microbial organisms to colonize PFU and form equilibrium communities. Then four pieces of PFU at each station were randomly retrieved and transported to the laboratory. The PFU substrates were harvested by squeezing the contents into sterilized beakers.

2.2. Chemical analysis and PFU monitoring

Chemical analyses of water quality at the four stations were performed according to standard methods [13]. The PFU sample was characterized by measuring protozoan species number, abundances of microorganisms, heterotrophy index and diversity index. The numbers of living bacteria and fungi were determined by colony forming unities (CFU) immediately after sampling. Abundances of protozoa and rotifers as well as species numbers belonging to the different protozoan orders were identified by microscopy within 12 h after PFU were harvested. Generally, three slides from each sample were examined. Ash-free weight was determined according to the standard methods of APHA [14]. Chlorophyll a was extracted and determined by the method in [3]. The optical density of chlorophyll a was measured with an Ultrospec 3000 UV–vis spectrophotometer (Biochrom Ltd., England). Heterotrophy index (HI) is calculated from mean chlorophyll a and biomass by

$$\mathrm{HI} = \mathrm{biomass\,(g\,dm^{-3})/chlorophyll\ a\,(g\,dm^{-3})} \quad (1)$$

where biomass is expressed as the ash-free weight, and diversity index (DI) of protozoa is calculated by Magalef's formula:

$$\mathrm{DI} = (S - 1)/\ln N \quad (2)$$

where S is the protozoan species number and N is the protozoan number in 100 cm^3 PFU samples.

2.3. Calorimetric measurements

The calorimeter is an eight-channel TAM Air isothermal heat conduction calorimeter 3114/3236 (Thermometric AB, Sweden). The calorimetric channels are in a single removable

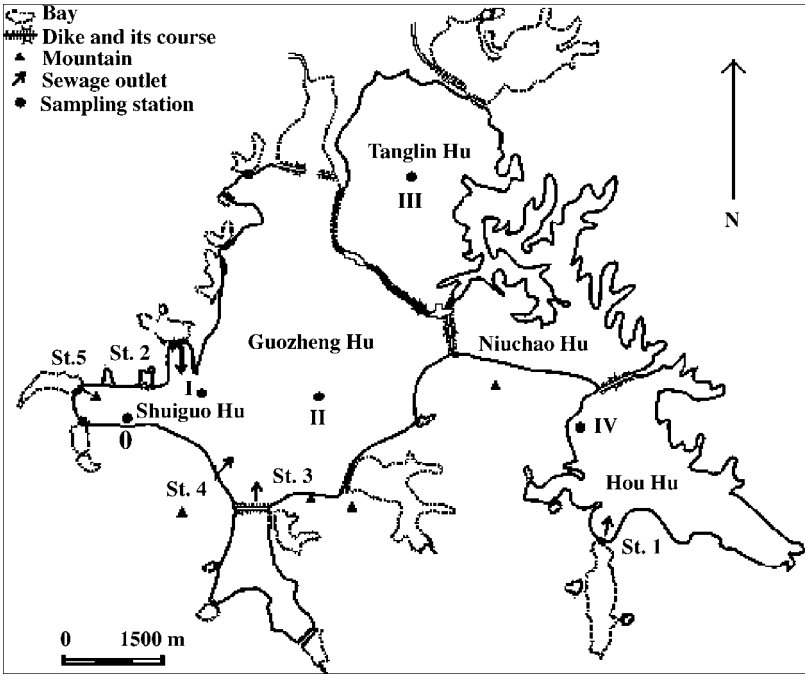

Fig. 1. Map of the sampling stations in Lake Donghu.

Table 1
Chemical characteristics of water at sampling stations (mg dm^{-3})

Station	TN	NH_4^+	TP	COD
0	2.68	0.94	0.28	8.61
I	1.57	0.93	0.21	8.15
II	1.24	0.59	0.17	6.92
IV	0.82	0.29	0.16	6.92

TN: total nitrogen; NH_4^+: ammonium ion; TP: total phosphate; COD: chemical oxygen demand.

block contained in an air thermostat that keeps the temperature within ±0.02 °C. Each channel consists of a sample and a reference vessel. The limit of detection is 2 μW and the baseline deviation over 24 h is ±5 μW. All the calorimetric measurements were performed in hermetically sealed 20 cm^3 glass ampoules at 28 °C.

The activity of a PFU microbial community was observed by adding 10 cm^3 of a PFU sample into an ampoule. The reference ampoule was filled with 10 cm^3 sterile distilled water. The concentrated PFU microbial community was obtained by concentrating 25 cm^3 mixed native PFU sample from one station onto a CA-CN millipore membrane with diameter of 50 mm and pore size of 0.22 μm. The activity of the concentrated PFU microbial community was measured by placing the wet membrane with concentrated cells into a calorimetric glass ampoule with 10 cm^3 filtered lake water from the same station. The reference ampoule was filled with 10 cm^3 sterile distilled water and a piece of sterile membrane. The heat rate was recorded every minute by use of the Picolog software supplied with TAM Air. The heat rate values of each sample were obtained from triplicate, independent curves. During the calorimetric measurements, parallel ampoules were cultured at the same condition, and the numbers of heterobacteria, fungi, protozoa and rotifers in the parallel ampoules were observed at the first, second, and last day by microscope.

2.4. Statistical analyses

The data in Tables 3–5 are given as the arithmetic mean ± S.D. for three repetitions. The ANOVA statistical method was used to assess the significance of differences in measured variables among the sampling stations at $P \leq 0.05$. Correlations among variables were also analyzed at $P \leq 0.05$.

3. Results

3.1. Chemical analysis of water quality

Chemical characteristics of the four stations are shown in Table 1. The concentration of total nitrogen decreases gradually from stations 0 to IV. A slight decrease of the concentration of total phosphate is also found with the decrease of trophic level. The concentrations of NH_4^+ at stations 0 and I are approximately the same, but higher than the other stations. Stations 0 and I show an increase in the concentration of COD as compared with stations II and IV.

3.2. Biotic characteristics of the PFU microbial community

Given in Table 2 are the species numbers belonging to the different protozoan orders in the native PFU samples. The greatest species numbers of phytomastigophora, ciliate and protozoa are observed at station IV, which presents the highest species diversity. Station 0 has the fewest species of phytomastigophora, but the most of zoomastigophora and sarcodina. More species numbers of protozoa are observed at stations 0 and IV than at stations I and II. Species number of phytomastigophora, which reflects water quality, shows no difference between stations I and II. However, some species

Table 2
Species composition and number of protozoa

Station	Species number				Total species number
	Phytomastigophora	Zoomastigophora	Sarcodina	Ciliates	
0	13	7	6	12	38
I	16	4	4	5	29
II	16	1	2	4	23
IV	17	5	5	21	48

Table 3
Abundances of the particular microorganisms in the PFU communities

Station	Number of heterobacteria ($\times 10^4$ cm^{-3})	Number of fungi ($\times 10^4$ cm^{-3})	Number of protozoa (individual/cm^3)	Number of rotifer (individual/cm^3)
0	1.48 ± 0.46	0.73 ± 0.32	3320	530
I	0.98 ± 0.25	0.70 ± 0.42	3380	100
II	0.75 ± 0.00	0.75 ± 0.00	3250	30
IV	0.09 ± 0.02	0.01 ± 0.002	2680	30

Values are given as mean ± S.D. ($n = 3$).

Table 4
Diversity index and heterotopy index

Station	Biomass (g dm^{-3})	Chlorophyll a (mg dm^{-3})	Heterotophy index	Diversity index
0	2.23 ± 0.23	5.1 ± 1.9	438	2.99
I	0.96 ± 0.01	2.3 ± 0.5	421	2.28
II	0.78 ± 0.02	2.1 ± 0.0	372	1.81
IV	0.44 ± 0.004	1.5 ± 0.2	295	4.58

Values are given as mean ± S.D. (n = 3).

like *Stentor* that can endure serious pollution are found in a greater number at stations 0 and I.

The main composition of the PFU microbial community is given in Table 3. Abundance of fungi is the lowest at station IV, but approximately the same among the other stations. The highest abundance of rotifers is found at station 0. Overall, the abundance of microorganisms is higher at the more eutrophic stations.

Heterotrophy index (HI) denotes the percentage of heterotrophes in the microbial community. The higher the diversity index (DI), the better the water quality. From Table 4, there are increases in biomass, chlorophyll a and HI with increasing eutrophic level. DI is the highest at station IV, but the lowest at station II because of its least protozoan species. Although the values of HI at four stations are relatively high, implying their poor water quality, station IV has the lowest HI and highest DI. Therefore, the water quality of Lake Houhu is the best among all the studied areas of Lake Donghu.

3.3. Metabolic characteristics of the PFU microbial communities

Fig. 2 shows the heat rate–time curves from the native PFU microbial communities. The heat rate–time curves show a decreasing trend. The observation results revealed that abundance of microorganisms decreased gradually and only a few microorganisms were alive at the end of calorimetric measurements. The maximum heat rates at stations 0, I, II and IV are 0.12 ± 0.009, 0.07 ± 0.006, 0.06 ± 0.005 and 0.03 ± 0.007 mW, respectively, consistent with the level of eutrophication.

Typical heat rate–time curves of the concentrated PFU microbial community are depicted in Fig. 3. The parallel observation showed that numbers of bacteria and fungi increased at the first day in ampoules while numbers of protozoan and rotifers declined. At the second day, abundances

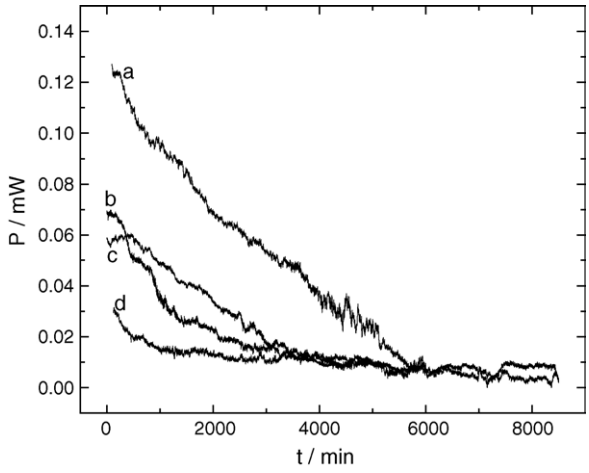

Fig. 2. The heat rate–time curves of the native PFU microbial communities at station 0 (a), station I (b), station II (c) and station IV (d).

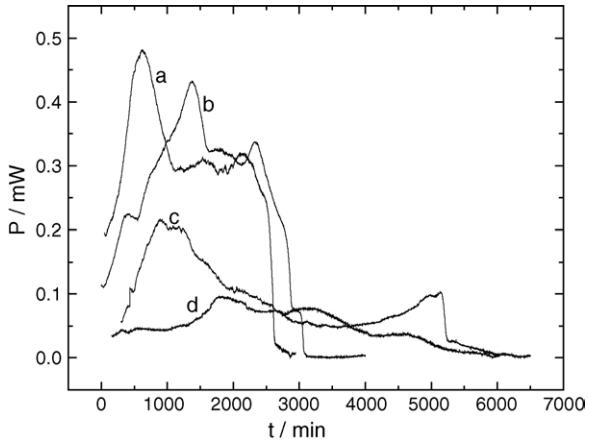

Fig. 3. The heat rate–time curves of the concentrated PFU microbial communities at station 0 (a), station I (b), station II (c) and station IV (d).

Table 5
Metabolic properties of the concentrated microbial communities

Station	P_{max} (mW)	r (pW s^{-1})	Q_T (J)	Q_{Log} (J)	P_m (mW)
0	0.68 ± 0.09	55 ± 5.00	46.03 ± 2.39	20.21 ± 7.81	0.56 ± 0.09
I	0.65 ± 0.14	25 ± 3.33	51.97 ± 0.84	22.94 ± 0.73	0.44 ± 0.01
II	0.38 ± 0.10	30 ± 5.00	31.76 ± 7.87	6.38 ± 2.15	0.22 ± 0.03
IV	0.16 ± 0.04	15 ± 1.67	21.74 ± 4.27	4.35 ± 1.13	0.10 ± 0.01

P_{max} is the potential maximum heat rate, r the growth rate, Q_T the total heat, Q_{Log} the total heat in the increasing period, and P_m is the measured maximum heat rate. Values are given as mean ± S.D. (n = 3).

of bacteria and fungi decreased. At the end of the calorimetric measurements, the microorganisms almost died. The shapes of the curves are similar, particularly for stations 0 and I. The kinetic process in the increasing period of the curves follows the classical logistic model:

$$P_t = P_{\max}/(1 + \exp(a - rt)) \quad (3)$$

where P_t is the heat rate at time t, r the growth rate and $P_{\max}$ is the potential maximum heat rate.

The values of r and other metabolic variables are given in Table 5. $P_{\max}$ and Q_T show significant differences among the four stations except between stations 0 and I. No significant differences are observed in Q_{Log} between stations 0 and I as between stations II and IV. The values of r show significant differences among the stations except between stations I and II. Significant differences in P_m are observed among the four stations.

4. Discussion

Chemical variables especially the essential biological nutrients in lake water directly affect the nutrient cycling and productivity, which make them the direct indexes in assessing the eutrophic level of lake. High concentrations of chemical variables at four stations suggest the seriously organic pollution in Lake Donghu. However, chemical variables provide only the concentrations of pollutants, but not their ecological effects. The PFU microbial analyses for biomass, chlorophyll a, abundances of the main microorganisms and heterotrophy index at four sampling stations, indicate the eutrophic gradient changes along the lake. However, variations in the protozoan species richness and diversity index at four stations are not consistent with the changes of water quality. It is also found that there are less protozoan species at stations I and II than stations 0 and IV. This can be explained by the differences in the habitat. Stations 0 and IV are close to the lakeshore, while stations I and II are in open water. Generally, protozoa species are more abundant at the shoreline than in open water [15]. Furthermore, in a previous study [16], the pattern of biotic variables indicated that, between unpolluted and seriously polluted water, there was a transition zone where correlations of diversity index and species number with chemical pollution index changed from positive to negative and HI from non-significant-positive to significant-positive correlation. Therefore, it is difficult to make an objective estimation of water quality from biotic variables obtained by PFU biomonitoring, although their changes can be used to monitor water quality.

Calorimetry detects the heat dissipation of the whole living system. The native PFU samples are associated with low signal levels, high signal levels are obtained from the concentrated PFU samples with the membrane filter technique, similar to heat production of microplankton in seawater obtained by Lopukhin and Kamenir [10]. However, the curves of the native PFU samples do not form an apex like microplankton and instead show only a decreasing trend, which was caused by the decreasing of the abundance of microorganisms during the metabolic measurements. Calorimetric ampoules are in darkness so algae are not able to photosynthesize but do respire. And with consumption of oxygen and nutrients, the metabolic rate of microorganisms will decline, which lead to the decrease of their abundance. The heat production curves of the concentrated PFU microbial communities resemble those for the multiplication of bacteria and fungi in soils [17–19]. The PFU samples have many small clay particles that adhere to the membrane filters. Furthermore, results from the microscope revealed that bacteria and fungi were growing fast in the increasing phase of heat rate–time curves, and that the population of protozoan, algae and rotifers were declining in all the phases. In an equilibrium community, ciliates and flagellates of protozoan graze aquatic bacteria so that the growth of bacteria is always in the log phase [20]. Consequently, the fast growth of bacteria in the increasing period is mainly due to the decrease of protozoa. From the comparisons of microbial communities at different eutrophic stations, the values of their metabolic variables are significantly higher in the more eutrophic ecosystem, which is consistent with the metabolic results of marine bacterioplankton [11]. Metabolic data at stations I and II are in reasonable ranges with regard to their eutrophic levels, and are not influenced by their differences in habitat. So, thermal activity monitoring of the microbial community by calorimetry can measure the biological effects at the community level caused by eutrophication.

Information on the structure and function of an ecosystem is essential to study the effects of eutrophication on the ecosystem. In our study, biotic variables obtained by PFU biomonitoring provide structural information of microbial community. Metabolic variables obtained by calorimetry reveal the whole characteristics of nutrient cycling and energy flow in microbial community. Therefore, they represent the functional information of microbial community. Then, can metabolic variables reveal biotic information? And how do chemical factors in the water influence the activity of microbial community? To answer these two questions, correlation analyses were done for the metabolic, chemical and biotic variables. The results in Table 6 show firstly that metabolic

Table 6
Correlation between metabolic properties and biotic variables

	$P_{\max}$	r	P_m	P'_m
Phytomastigophora species number	−0.75	−0.99*	−0.85	−0.99*
Biomass	0.78	0.98*	0.88	0.99*
Chlorophyll a	0.73	0.98*	0.85	0.98*
HI	0.99*	0.79	0.96*	0.86
Abundance of heterobacteria	0.94	0.92	0.96*	0.96*

$P_{\max}$ is the potential maximum heat rate, r the growth rate, Q_T the total heat, P_m and P'_m are the measured maximum heat rates of the concentrated and native PFU microbial communities, respectively.
* $P < 0.05$ ($n = 4$).

Table 7
Correlation between metabolic properties and chemical variables

	P_{max}	r	Q_T	P_m	P'_m
TN	0.85	0.97*	0.71	0.93	1.00*
NH_4	1.00*	0.72	0.98*	0.96*	0.81
TP	0.84	0.91	0.72	0.94	0.97*
COD	0.92	0.76	0.88	0.97*	0.87

P_{max} is the potential maximum heat rate, r the growth rate, Q_T the total heat, P_m and P'_m are the measured maximum heat rates of the concentrated and native PFU microbial communities, respectively.
* $P < 0.05$ ($n = 4$).

variables including r and P'_m have negative relationships with species number of phytomastigophora, but positive correlations with biomass and chlorophyll a, which is similar to the metabolic results of microbial community in soils [21,22]. P_{max} and P_m have positive correlations with HI that represents the percentage of heterotrophes in microbial community. This shows that the heat dissipation of the concentrated microbial community is mainly by heterotrophes. The maximum heat rates of the native and concentrated microbial communities (P_m and P'_m) have a positive correlation with abundance of heterobacteria. Although bacteria are very small, they can grow fast and appear at a high abundance. And they can use not only dissolved organic matter (DOM) but also particulate organic matter (POM). They provide food to protozoa, algae and rotifer. In aquatic systems the productivity of bacteria is above the 25% of what is produced by photosynthesis [1]. No thermodynamic variables have significant correlations with the species number of zoomastigophora, sarcodina and ciliate and diversity indexes of protozoa, which show that species diversity cannot influence the metabolic characteristics of whole microbial community. Many significant correlations above between metabolic and biotic variables suggest that functional information obtained by calorimetry is consistent with structural information of microbial community obtained by PFU biomonitoring. In addition to that, in PFU biomonitoring, the main functional variables are obtained by the identification of protozoan species that is difficult and takes more time. However, calorimetry is simple, automatic, fast and sensitive. Therefore, metabolic variables obtained by calorimetry can be applied to biomonitoring of the PFU microbial community.

The results in Table 7 show a positive relationship between metabolic and chemical variables, suggesting that the enhanced microbial activity at the more eutrophic stations might be attributed to organic pollution. The correlations between metabolic and biotic variables show that heterotrophes especially heterobacteria make the greatest contribution to the heat production of microbial community. In ecosystem heterotrophes mainly use organic substances as carbon sources. And the abundances of heterotrophes especially heterobacteria are confined by organic substances. Then organic substances can indirectly influence the heat production of microbial community. So the metabolic variables can be used to support the chemical variables in biomonitoring.

5. Conclusion

Calorimetry has been demonstrated to be a suitable tool to study the metabolism of the native and concentrated PFU microbial community in fresh water. The estimation of metabolic variables of natural microbial communities by calorimetry quantifies the dynamic characteristics of the whole ecosystem, which is not achievable with other methods. They show a positive response with pollution level, which can be used to support chemical and biotic variables. Therefore, calorimetry will be useful for biomonitoring of the PFU microbial community.

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