Effects of Hydrogen Ions on Aquatic Microbial Populations in Korea

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From July 1994 to March 1995, eighteen variables of physico-chemical factors including heavy metals, and of bacteria in the four reservoirs of Kyonggi-Do were investigated to examine the effects of acidic precipitation to bacterial population. The pH range in the study area is from 6.56 to 10.24, which also showed seasonal change extensively compared to other factors. The correlation analysis showed that pH has a significant positive correlation (mean 79%) with the microbial populations in Wangsong reservoir. By multiple regression analysis on all of the seasons and stations, good explanation was obtained for the variation in total direct count of bacteria (71% and 88%, respectively), and the plate count of heterotrophic bacteria (76% and 88%, respectively). In the surface water of Wangsong reservoir, the variation of total count of bacteria was affected by the S/O (soluble sugar/total organic matter ratio) value and the pH, and that of the plate count of heterotrophic bacteria was explained as 63% by pH. However, in other stations they were explained by the NO_2, total organic matter (TOM), soluble sugar (SS), temperature, and dissolved oxygen as 21~91%. On the basis of the results, the bacterial populations on the media at pH 3.0, 4.0, 5.0, 6.0, and 7.0 were compared to determine the effects of acidic proceeding. All the colonies grew the best on the media of pH 7.0, but started to decrease from pH 5.0.

Key words: pH, population, environmental factors, multiple regression

Acidification of freshwater lakes originated from acid rain, which is caused by products of industrial wastes, is one of the most serious environmental problems today (5, 10). The linkage among long-range transport of pollutants, acidic deposition, and status of the biota in freshwaters is slow in coming. Acidic deposition is dominated by two mineral acids, sulfuric and nitric acid (10, 11). Acidic deposition yields a surplus of H^+, which reacts with carbonates and bicarbonates to the extent that they exist and the contact time is permissive. Unreacted H^+ reacts slowly with silicates and displace Ca^{2+}, Mg^{2+}, Na^+, Al^{3+}, and Mn^{2+}. Thus, the Ca^{2+} and Mg^{2+} concentrations in water are probably elevated during the acidification process and water hardness increased (10). Several investigations for the affects of acidification on microbial population have been made at different fields and laboratories, which showed different results. Scheider and Dillon (21) estimated significant differences in the planktonic bacterial populations of acidified and nonacidified Canadian lakes, but Traaen (25) reported no major differences in similar studies in Scandinavian lakes. Schindler et al. (23) found out that the decomposition is not reduced after acidification of experimental lake. Gahnstrom et al. (6) obtained no difference in glucose turnover rate or oxygen consumption between the profound sediments of lakes with pH<5 and pH>6.5. However, Grahn et al. (7), and Hultberg and Grahn (13) reported the accelerated accumulation of organic detritus in acidic Swedish lakes. They concluded that detrital accumulation results from reduced bacterial decomposition which coincides with increased fungal growth on the sedimental surface. Generally, aquatic microbial population size and species diversity are influenced by physico-chemical factors; therefore, survey of the environmental factors are essential for the study of microbial succession (17, 28). Furthermore, to examine how a specific environmental fac-

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tors affect the microbial dynamics and the function *in situ,* the relationships of the specific environmental factor with the other environmental factors or biological factors also have to be considered (2, 3). In spite of the strong influence of the acidic precipitation in the aquatic environment, the studies of acidic deposition in aquatic system have been limited to physico-chemical analysis such as conductivity, area acidic deposition, model of diffusion on the atmosphere acidic pollutant, and model of acidic precipitation (9, 15). The effects of aquatic acidic deposition caused by effluents including acid rain on biological components of the ecosystem have been little known. The relationship of physico-chemical factors and bacteria was investigated to examine the effects of acidic precipitation of atmospheric pollutant from July 1994 to March 1995 in the four reservoirs of Kyonggi-Do. Correlation and multiple regression analysis were used to explain the effects of hydrogen ions and environmental factors on microbial population. On the basis of results, we examined the bacterial population dynamics on the media of various pH values that is at pH 3.0, 4.0, 5.0, 6.0, and 7.0.

Materials and Methods

Study site

From July 1994 to March 1995, samples were collected four times seasonally at 4 reservoirs in Panwol, Wangsong, Wonchun, Schingal (Fig. 1). Collections were made at the deepest point in the reservoirs where the water fluctuation was low.

Sample collection

Samples were collected from the surface to the bottom with Van Dorn water sampler. Water samples for microbiological determinations and chemical analysis were collected with sterile 10 ml cap-tubes and 500 ml bottles, respectively. These samples were kept at 4°C and carried within 24 h to the laboratory for further processing.

Physico-chemical enviromental analysis

Temperature, pH, salinity, dissolved oxygen, conductivity, and turbidity were measured at central area of the lake on a boat with a water quality analyser (Horiba. U-10, Horiba, Japan). Water samples were filtered through membrane filters (Gelman, 47 mm, pore size, 0.45 μm), and ammonia, nitirite, and phosphate were measured according to the method of APHA (4). Soluble sugar was measured with the Anthrone method (19). Total organic matter was measured with the Walkley method (27).
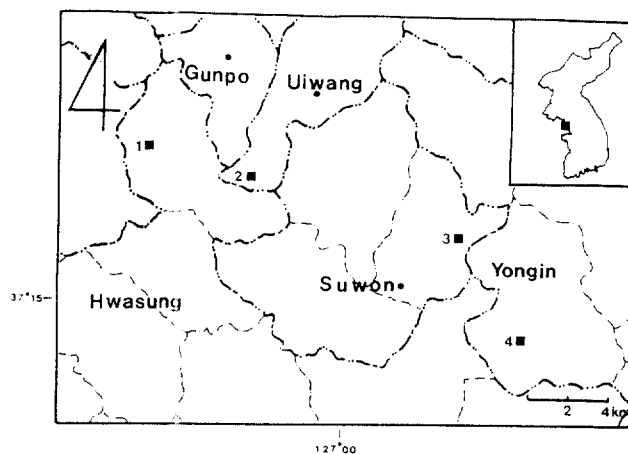

Fig. 1. Locations of four surveyed reservoirs in Kyonggi-Do, Korea. St. 1, Panwol Reservoir; St. 2, Wangsong Reservoir; St. 3, Wonchun Reservoir; St. 4, Schingal Reservoir.

Heavy metal analysis

The amounts of metals were measured with inductively coupled plasma (ICP) after treating as APHA (4).

Microbiological analysis

Total counts of bacteria : Water samples were fixed with buffered formalin (final concentration 4%). Direct counts were made with the method of Hobbie *et al.* (12). Nuclepore polycarbonate filters (Nuclepore Co., pore size 0.2 μm, φ 25 mm) were prestained with Sudan Black B. The fixed water sample (1 ml) was mixed with 1.5 ml of acridine orange solution (1 : 10,000 in 6.6 mM phosphate buffer, pH 6.7). After 5 min, the sample was filtered through the prestained filter. The mounted filter was examined and counted with a Epifluorescence microscopy (Axioplan, Zeiss, Germany). At least 20 fields were examined per filter.

Plate counts of heterotrophic bacteria : Nutrient agar (Difco) and Zobell 2216e medium (1) were used for plating viable heterotrophic bacteria. After incubation at 25°C for 7 days and 30°C for 3 days, the colonies were counted.

pH adaptation

To determine whether any of the bacteria had adapted to low pH, the water isolates from each lake were inoculated on nutrient agar and Zobell 2216e agar plate that had been adjusted to pH 3.0, 4.0, 5.0, 6.0, and 7.0 with the 1 N H_2SO_4 (5). The colonies were incubated at 25°C for 7 days and 30°C for 3 days to determine whether growth would occur at the various pH values.

Analysis of the data

All the data were analized using SAS stat. Version 5 (19).

Results

Physico-chemical environmental parameter

The variation in the parameters investigated is summarized in Table 1. The annual means of each environment factors were as following: 17.2℃ in temperature, 8.1 in pH, 11.36 mg/l in dissolved oxygen, and 20 NTU in turbidity. The concentrations of total organic matter and soluble sugar were 250 mg/l and 4.22 mg/l, respectively. NH_4^+-N, NO_2^--N, and PO_4^3-P were 704 μg/l, 28.6 μg/l, and 74 μg/l, respectively. Through the seasons, the pH was changed from 6.56 to 10.24, which was higher in the summer (pH 9.25~10.24) than in the winter (pH 6.93~8.72). At station 2 in Wangsong reservoir, the pH values showed conspicuous difference between the summer (pH 10.24) and the winter (pH 7.33), especially. In the summer, the increase of pH values was probably related to blooming of cyanobacteria, *Anabaena*. The increased pH in the water induced by the *Anabaena* bloom may have additionally favoured cyanobacteria over other phytoplankton groups (24). The concentration of nitrogen and phosphate in the water is often regarded as a limiting factor, which also causes the eutrophication and water-blooming. Seasonal change of the concentration of NH_4^+-N, NO_2-N, and PO_4^3-P ranged from 0.1 to 3573 μg/l, from 0.4 to 303.5 μg/l, and from 0.1 to 451.0 μg/l, respectively, in which the mean values were as high as those in eutrophication. The ranges of total organic matter and soluble sugar concentration were 1.0~8.8 mg/l and 177~490 mg/l. The S/O value (soluble sugar/total organic matter) was 0.00~0.45, which was the highest at station 2 (0.34). The numbers of total bacteria and heterotrophic bacteria ranged from 1.4 to 63.4×10⁶ cells/ml and 0.1 to 48.1×10⁴ cells/ml at all the stations, respectively. The total bacteria and heterotrophic bacteria were the highest at station 2 (25.7~26.3×10⁵ cells/ml and 2.6~24.5×10⁴ cells/ml, respectively), which showed positive correlation with organic material.

Concentrations of heavy metals

Hg, Cd, Fe, Cu, Zn, Mn, and Pb concentration has been shown to be lower in the studied area than those of environmental standard made by the Ministry of Environment (16). The concentration of heavy metal ions showed no seasonal difference (Table 1). Schindler and

Table 1. Ranges and means of the parameters at reservoirs of Kyonggi-Do from July 1994 to March 1995.

Parameter	Range	Mean							
		1S	1B	2S	2B	3S	3B	4S	4B
Environment									
Temperature (℃)	4.3~33.8	17.2	17.2	17.5	17.5	17.0	17.0	17.1	17.1
pH	6.6~10.2	7.9	7.9	8.9	8.9	7.8	7.8	7.9	7.9
Conductivity (mS/cm)	0.18~0.39	0.19	0.19	0.34	0.34	0.21	0.21	0.34	0.34
DO (mg/l)	4.5~19.3	11.2	11.2	13.5	13.5	11.1	11.1	9.7	9.7
Turbidity (NTU)	8~133	29.5	29.5	57.8	57.8	16.0	16.0	31.8	31.8
Inorganic matter									
NH_4^+-N (μg/l)	0.1~3573.0	79.0	158.0	1442.0	2209.0	219.0	233.0	669.0	623.0
NO_2-N (μg/l)	0.4~303.5	5.9	8.1	52.3	29.1	84.1	9.4	24.3	15.4
PO_4^3-P (μg/l)	0.1~451.0	46.0	141.0	95.0	238.0	25.0	18.0	15.0	14.0
SS (mg/l)	1.0~8.8	3.1	3.8	6.4	5.8	3.7	3.7	3.9	3.4
TOM (mg/l)	177~490	197.3	326.5	197.3	241.5	282.5	244.8	323.5	258.5
S/O value	0.00~0.45	0.02	0.01	0.03	0.02	0.02	0.01	0.01	0.01
Heavy Metal									
Cd (μg/l)	1.0~37.3	0.8	0.6	0.7	0.7	7.7	0.8	0.7	10.4
Al (μg/l)	182.0~9680.0	1531.0	1292.0	389.0	708.0	435.0	3740.0	1040.0	2089.0
Fe (μg/l)	53.0~512.0	199.0	116.0	158.0	168.0	205.0	232.0	99.0	199.0
Mn (μg/l)	1.7~25.4	4.3	5.0	9.4	8.1	3.7	3.7	5.1	8.8
Cu (μg/l)	2.3~59.2	5.2	4.5	6.6	7.2	6.1	6.0	18.2	5.8
Zn (μg/l)	11.0~261.0	90.0	113.0	49.0	72.0	76.0	49.0	118.0	93.000
Pb (μg/l)	0.1~189.0	87.6	81.1	69.5	83.3	82.9	76.7	76.8	126.9
Microorganism									
Total bacteria (10⁵ cells/ml)	1.4~63.4	6.1	9.8	25.7	26.3	8.8	16.6	7.2	10.9
Heterotrophic bacteria									
NA (10⁴ CFU/ml)	0.1~34.3	1.4	1.7	2.6	7.8	1.7	1.2	1.0	8.9
Z-25 (10⁴ CFU/ml)	0.1~48.1	2.2	2.0	10.6	24.5	1.2	0.7	8.3	9.6

S: Surface layer of station, B: Bottom layer of station, SS: Soluble sugar, TOM: Total organic matter, S/O value: SS/TOM ratio.

Table 2. Significant independent variables in the regression models for the parameters of bacterial populations based on data from seasonal change.

Season	Dependent variable	R^2(%)	Independent variables (P<0.2)
JUL	Total bacteria	65	COND (63); NH$_4$ (2)
	Heterotrophs		
	NA	67	NO$_2$ (59); S/O (7); DOC (1)
	Z-25	97	NO$_2$ (91); TEMP (6)
SEP	Total bacteria	64	COND (41); SS (13); DO (10)
	Heterotrophs		
	NA	72	S/O (38); COND (27); DO (7)
	Z-25	77	COND (51); PO4 (26)
DEC	Total bacteria	66	DO (64); COND (2)
	Heterotrophs		
	NA	55	PO$_4$ (37); TURB (15); DO (3)
	Z-25	89	PO$_4$ (57); NO$_2$ (18); TEMP (14)
MAR	Total bacteria	89	S/O(82); TOM (7)
	Heterotrophs		
	NA	71	SS (63); DO (8)
	Z-25	82	S/O (59); PO4 (10); TEMP (8) TOM (5)

COND: Conductivity, DO: Dissolved oxygen, SS: Soluble sugar, S/O value: SS/TOM ratio, TEMP: Temperature, TOM: Total organic matter, TURB: Turbidity.

Turner (22) also noted that chemical analyses showed no significant trends over five years in acidification- increase in H$^+$, SO$_4^{2-}$, Mn^{2+}, Al^{3+}, Na$^+$, conductivity, and decrease in Cl$^-$, Fe, total-N, and suspended C. Harvey (10) reported that the effect of acidic deposition is to mobilize metals from watershed soils and lake sediments, both by displacement with H$^+$ and through increased metal solubility at lower pH. However, in this study, the pH value of the minimum in the reservoirs was 6.54. Thus, no significant correlation was found between water pH and heavy metal ion concentration.

Multiple regression analysis in seasons

The results of the multiple regression analysis on the seasonal change of the microbial population dynamics and pH are given in Table 2. Väätänen (26) reported that particularly high values were obtained for approximately 90% of the dependent variable, and good explanation was obtained from range 61 to 68%. The values obtained in the seasonal change were as follows; Particularly high values were obtained for the variation of heterotrophic bacteria (Z-25) in July, heterotrophic bacteria (Z-25) in December and total bacteria in March (89 to 97%), good explanation was obtained for the variation in others (64 to 82%). The plate counts of heterotrophic bacteria (Z-25) in July could be explained by NO$_2$-N and temperature (97%). In December, PO$_4$-P, NO$_2$-N and temperature explained for plate counts of

Table 3. Significant independent variables in the regression models for the parameters of bacterial community based on data in the stations.

Stations	Dependent variable	R^2(%)	Independent variables (P<0.2)
1S	Total bacteria	79	DO (79)
	Heterotrophs		
	NA	73	TEMP (73)
	Z-25	47	TOM (36); PO$_4$ (11)
1B	Total bacteria	98	NH$_4$ (74); SS (24)
	Heterotrophs		
	NA	93	TOM (71); TURB (22)
	Z-25	85	SS (59); TOM (26)
2S	Total bacteria	97	S/O (57); pH (40)
	Heterotrophs		
	NA	68	pH (68)
	Z-25	95	pH (59); TOM (36)
2B	Total bacteria	98	TOM (86); pH (12)
	Heterotrophs		
	NA	97	TOM (97)
	Z-25	94	S/O (66); TEMP (28)
3S	Total bacteria	95	S/O (95)
	Heterotrophs		
	NA	98	NO$_2$ (98)
	Z-25	98	NO$_2$ (98)
3B	Total bacteria	95	COND (95)
	Heterotrophs		
	NA	70	TOM (50); DO (20)
	Z-25	84	TOM (65); DO (19)
4S	Total bacteria	77	COND (77)
	Heterotrophs		
	NA	70	SS (70)
	Z-25	64	TOM (64)
4B	Total bacteria	61	COND (61)
	Heterotrophs		
	NA	53	COND (53)
	Z-25	57	COND (57)

heterotrophic bacteria (Z-25) (89%). In March, S/O value and total organic matter were explained for direct total counts of bacteria (89%). In the present study, conductivity (41~63%), NO$_2$-N (59~91%), and S/O value (73%) were significant in summer and autumn, which were affected by seasonal factor such as a drought. Dissolved oxygen (64%), PO$_4$-P (37~57%), soluble sugar (71%) and S/O (59~82%) were also significant in spring and winter. As a result, the relationship between the organic matters and microbial populations was significant in these seasons.

Multiple regression analysis in the stations

The ecosystem is partially composed of microorganisms and physico-chemical factors which are related to each others in functon. It is variously showed that de-

Table 4. Significant independent variables in the regression models for the parameters of bacterial populations based on data from all stations.

Dependent variable	R^2(%)	Independent variables (P<0.2)
Total bacteria	45	S/O (18); pH (14); TEMP (6); COND (4); TURB (3); DO (1)
Heterotrophs		
NA	40	pH (14); DO (11); SS (6); TEMP (4); TURB (4)
Z-25	40	TEMP (14); S/O (6); DO (6); pH (6); TOM (4); TURB (4)

gree of complexity by the combination of each ecosystem. The multiple regression analysis for the stations are presented in Table 3. Jones (14) found that about 65% of the variation in plate counts of bacteria and 75% of that in direct counts could be explained by chorophyll a, temperature, inorganic nutrients, and pH. From the result of general analysis in the station, surface water could be explained by pH, S/O value, NO_2-N and turbidity, and bottom water explained by TOM and SS. However, independent variables for the total and heterotrophic bacteria were differentiated by physico-chemical environmental factor at the stations. At station 1, was explained by DO (79%) and NH_4-N (74%), at station 2, pH (68%) and TOM (97%), at station 3, S/O (95%), NO_2-N (98%) and TOM (65%), at station 4, conductivity (77%). At the station 2, heavily eutrophied lake was affected significantly by hydrogen ion concentration. At the surface water of station 2, the independent variable which was particularly high were obtained for the variation of the total and heterotrophic bacteria (94 to 98%). The variation of the total direct counts of bacteria was explained by the S/O (57%) value, pH (40%), and that of the plate counts of heterotrophic bacteria (Z-25, NA) was explained by pH (59% and 68% in each). At the bottom water of station 2, the variation in the total direct counts of bacteria and the plate counts of heterotrophic bacteria (NA) were explained by the TOM (86%, 97%), and that of plate counts of heterotrophic bacteria (Z-25) was explained by the S/O (66%) value and temperature (28%). In the surface water of station 2, the total and heterotrophic bacteria was explained by pH (40~68%), while the bottom water showed a distinct difference in the change of pH by 12%. The result of the variation at station 2 was related to seasonal change of pH, which had a broader range, from 7.33 to 10.24, than those at other stations. Result of multiple regression analyses by environmental factors based on the data from all stations are given in Table 4. The proportion of the variation explained for total and heterotrophic bacteria was only 40 to 45%. This result was related to the fact that most
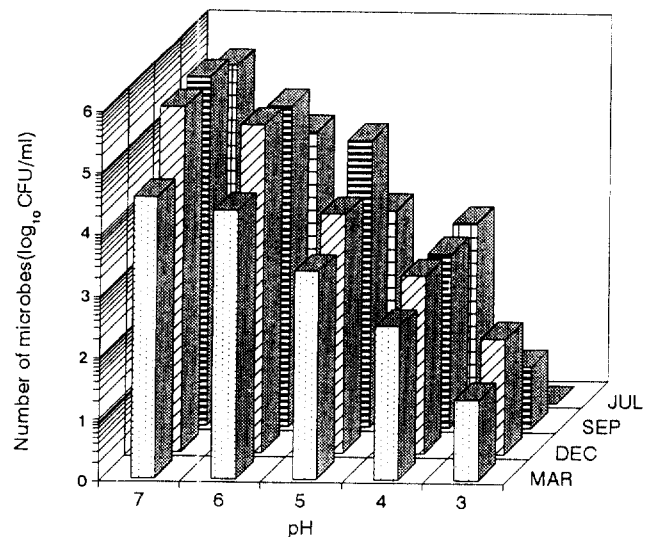
Fig. 2. Heterotrophic bacterial plate counts at various pH values at the Wangsong reservoir.

of independent variables affected on the microbial population. The total bacteria was controlled by S/O (18%) value, pH (14%), temperature (6%), conductivity (4%), turbidity (3%), and DO (1%), and the heterotrophic bacteria (NA) by pH (14%), DO (11%), SS (6%), temperature (4%), and turbidity (4%). The heterotrophic bacteria (Z-25) was explained by temperature (14%), pH (6%), S/O (6%), DO (6%), TOM (4%), and turbidity (4%). Water temperature and pH were significant in microbial population at most of the stations.

pH adaptation

The results of pH adaptation at the various pH values are shown in Fig. 2. The number of bacteria was counted as 4.1×10^5 cells/ml on the pH 7.0 media, 2.1×10^5 cells/ml on the pH 6.0 media, 7.8×10^3 cells/ml on the pH 5.0 media, 7.8×10^2 cells/ml on the pH 4.0 media, and 25 cells/ml on the pH 3.0 media at the St. 2. All the colonies grew the best on the media of pH 7, but started to decrease on pH 5 media (positive correlation). Boylen et al. (5) obtained the same number in the population of bacteria isolated from the water of pH 7.0, and when subsequently recultured at pH 3.0, 4.0, 5.0, 6.0, and 7.0, it started to decrease at pH 5.0.

Discussion

In all the stations except for station 2, the result of multiple regression analysis of organic and inorganic matter and microbial population showed a broad range from 47 to 98%. This is probably due to the shallow depths (5 m in average) and freshwater fluctuation of the reserviors. Change of abundance of phytoplankton by eutro-

phication may also be related with the variations of all the physico-chemical environmental factor in summer. Seasonal pH values were higher in summer than in winter at all stations. By multiple regression analysis at the station 2 surface water, the total bacteria and heterotrophic bacteria (Z-25 and NA) were explained by pH. Therefore, the results of the stepwise multiple regression analysis at station 2 were made to ascertain the effects of hydrogen ion.

Schindler et al. (23) observed that NH_4^+, Fe, Mn^{2+}, Zn and Al^{3+} concentrations increased accordingly as pH became lower from 6.7~7.0 to 5.7~5.9. Hall et al. (8) also reported that Al^{3+}, Ca^{2+}, Mg^{2+}, K, Mn^{2-}, Fe and Cd concentration is increased, while dissolved organic carbon (DOC), Na^+, NO_3^-, NH_4^+, Ni, Pb, Cu^{2+} and Zn are not changed at the stream where the pH is maintained at 4.0 by the added sulfuric acid. In the report of Schindler et al. (23), about the resistance of a lake against the acidification suggested that bicarbonate in sediments and weak organic acid in which pKs are higher than that of sulfuric acid acting as buffering sources in water column. Thus, organic matter and heavy metals are considered to be important elements to know the extent of acidification in lakes. However, there is no change of organic matter and heavy metals with pH change in this study. Heavy metal ions form complexes with fluoride, phosphate, and organics are ionized only under the condition below pH 4.7 (11). Thus the dynamics of metal concentration and inorganic matters in this study had no change, because the pH of reservoirs was always above 6.0.

In the experiment in the adaptation of bacteria to acidification, the bacteria grew the best on the media of pH 7.0, but started to decrease on pH 5.0 media. Rattray and Logan (18) reported that the water pH had no apparent effect on viable counts of heterotrophic bacteria. But, our observations agree with those of Boylen et al. (5), who found that regardless of the original isolation (pH 5.0 or pH 7.0), less than 10% of the isolate grew at pH<5.0. Traaen (25) reported that the acidified lakes were chemically neutralized, the planktonic bacterial flora responded and rose to resemble in the nonacidic lakes. In all the studies above, the change of microbial population dynamics were interpreted by the change of microbial composition and ecological function. So, the development of acidification-process model is very important.

Various studies of acidification have been described from the many viewpoints. Henriksen et al. (11) has interpreted the chemical state of lakes. Acidic lakes are pH<4.7; lakes in transition have pHs between 4.7 and 5.3, and bicarbonate lakes are those of pH>5.3. Thus, during acidification, a lake depend on rates of cation

recruitment. Several attempts have been made to derive an acidification model to identify the shift to acid-tolerant periphytic diatom species and chrysophytes (10). We didn't expect the study to show the various dynamic functions of microbial ecology, but instead ascertained the pattern of acidic proceeding. Almost, the reservoirs in Korea were mostly close to alkaline state due to eutrophication. So, freshwater acidification by acidic precipitation was not reported with the exception of thermal acidic waters and acidic mine drainage streams. It may be concluded, therefore, that degree of freshwater acidification and various function of microbial ecology were expected by multistage microcosm investigation with respect to certain natural environmental parameters.

Acknowledgement

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