HETEROTROPHIC BACTERIAL UPTAKE ON GLUCOSE AND GLYCOLLATE IN AN EUTROPHIC POND (CHASCOMUS, ARGENTINA)

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RESUMEN: Consumo bacteriano heterotrófico sobre glucosa y glicolato en una laguna eutrófica (Chascomús, Argentina).

El presente trabajo se realizó en la Laguna de Chascomús (Prov. Bs. As., Argentina), cuerpo eutrófico, poco profundo, de gran productividad y capacidad biogenética. La metodología aplicada fue la propuesta por Wright & Hobbie (1965), efectuándose las correcciones por la tasa de expiración de CO_2 (Hobbie & Crawford, 1969).

Los parámetros cinéticos del consumo de glicolato muestran una marcada fluctuación estacional y con la productividad. V_{max} y $K_t + S_n$ se incrementaron durante el verano en superficie (12,8 $\mu g.l^{-1}.h^{-1}$ y 588 $\mu g.l^{-1}$ respectivamente), y disminuyen en la muestra cercana al fondo en invierno, hasta valores no detectables con esta metodología. El T_t aumentó en agosto a 76 hs. Estos parámetros, para glucosa, no variaron ni estacional ni verticalmente tanto como los del glicolato; siendo sus valores máximos: 5,6 $\mu g.l^{-1}.h^{-1}$ (V_{max}); 49,5 $\mu g.l^{-1}$ ($K_t + S_n$) y 57,27 hs. (T_t) durante enero-febrero de 1986.

Las elevadas tasas de consumo, asimilación y excreción halladas durante la primavera y el verano, son producto de la presencia de una comunidad bacteriana heterotrófica adaptada a consumir los productos de excreción. El máximo porcentaje de mineralización observado, para el glicolato (85%) fue hallado en superficie durante enero de 1986; por el contrario el porcentaje de excreción de $^{14}CO_2$ proveniente de la glucosa no superó el 36% en igual época del año, siendo por lo tanto su rendimiento mucho mayor.

INTRODUCTION

The excretion of organic matter by phytoplankton plays an important role in the nutrition of heterotrophic bacterial communities. Glycolic acid is the main extracellular product (Fogg, 1966; Watt, 1966; Tanaka *et al.*, 1974; Wright, 1970), being carbon and energy sources for aquatic bacteria. Heterotrophic organisms consume a great variety of other compounds of low molecular weight (glucose, acetate, lactate, galactose, aminoacids).

The present paper attempts to understand and evaluate the trophic importance of glycollate and glucose, as mechanisms involved in the nutritional interrelation between phytoplankton and bacteria. Glucose uptake allows us to know the relevance of the heterotrophic potential of bacterial communities.

Chascomús pond

Chascomús pond belongs to the Salado River Basin, Bs. As. Province, forming the linked Ponds System of Chascomús, located in a geomor-

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TABLE I

Geomorphological, physical, chemical and primary production values of Chascomús Pond.

Geomorphological characteristics (Dangaus, 1976).

Area 3012,9 Ha
Volume 47.015.317 m^3
Max. depth 1,9 m
Mean depth 1,53 m
Length of shoreline 28,12 km

Chemical and primary production values (Conzonno & Fernandez Cirelli, 1985)

	x	s	% V.C
pH	8,72	0,16	1,8
Conductivity umho/cm (20°C)	756	43	5,7
mg Na^+/l	154,9	16,8	10,8
mg K^+/l	12,1	0,3	2,5
mg Ca^{2+}/l	22,2	4,0	18,0
mg Mg^{2+}/l	13,1	5,5	42,0
C-CO_3^- mg/l	20,3	7,7	37,9
C-CO_3H^- mg/l	264,2	24,0	9,1
mg Cl^-/l	114,4	11,4	10,0
mg SO_4^{2-}/l	56,3	12,3	21,8
µg N-NH_4^+/l	107	154	144,0
µg N-NO_3^-/l	32	42	131,0
µg N-NO_2^-/l	14	32	224,1
µg P-PO_4^{3-}/l	5	4	88,2
µ P total/l	259	118	45,4
Dissolved oxygen mg/l	9,4	1,3	13,8
Seston mg/l	105,2	66	62,7
POC mg/l	7,9	2,6	32,9
Chlorophyll-a µg/l	52	57	109,6
Pheopigments µg/l	25,8	16,5	63,9
Gross primary production mg O_2/m^3 .h	728	273	37,5
Si-SiO_2 mg/l	5,9	2,3	4,3

Results obtained by the authors (period 1985-1986)

pH	8,76-8,16
Temperature °C	23,5-8,5
Secchi Disch cm	0,15
Dissolved oxygen mg O_2/l	15,9-5,22
Gross primary production mg O_2/m^3 .h	1922,5-469,5
Chlorophyll-a µg/l	49,61-16,01
Heterotrophic viable bacteria bact/ml	49.000-13.000
Glycollate (+) bacteria/ml	1.700-130

phological unit called "Pampa deprimida", where various limnological area can be recognized (Ringuelet, 1968). It is a shallow eutrophic pond, at its highest productive and biogenetical capacity stage, and with wide platform. Water colour varied from yellowish to brown, owed to the presence of humic substances and little transparency. With abundant inorganic nitrogen, phosphorus and carbon plus many other nutrients, and pH higher than 7. It should be remarked the great quantity of suspended particulate matter, mainly colloidal clays of 0,5 μm diameter and conspicuous litoral hydrophites.

Table I gives geomorphological, physical, chemical and biological parameters taken from Conzonno & Fernandez Cirelli (1985, in press), Dangaus (1976) and those obtained by us.

MATERIAL AND METHODS

Monthly samples at different levels (0,5; lm; and 0,5 m from bottom) were taken from a central station of the pond free from rooted vegetation. Heterotrophic bacterial activity was measured by means of kinetic parameters on ^{14}C glucose and ^{14}C glycollate (Amersham Radiochemical Centre), D - (U - ^{14}C specific activity glucose 279 m Ci/m mol, and 1 - ^{14}C sodium glycollate, specific activity 5,0 m Ci/m mol.

Kinetic parameters for the maximum velocity (V_{max}), the transport constant and natural substrate concentration ($K_t + S_n$) and the turnover time (T_t) were calculed from the equations reported by Wright & Hobbie (1965). Respiration corrections to uptake, for expiration of $^{14}CO_2$ were done according to Hobbie & Crowford (1969).

Evaluation technique for mineralization rate consisted in the incubation of a 5 ml sample in erlenmeyers of 250 ml, to which different sustrat concentrations were added (Table II). A piece of Whatman paper n° 1, soaked in 0,2 ml hyamine hydroxide (Beckman), was suspended with an adequate support from a rubber stopper. Once incubation time was over, 1-1,5 hs., 0,2 ml of SO_4H_2 2N were injected through a needle hanging from the stopper. The samples were fixed and acidificated, and

TABLE II

Experimental substrates solutions

		μg Glycollate/25 ml	μg Glycol./l	1 μg Glucose/25 ml	μg Gluc./l 1
Concentration	1	3	120	1,0	40
	2	4	160	1,8	72
	3	5	200	2,6	104
	4	6	240	3,4	136

TABLE III

Experimental results

	May 1985		June					
Depth	Sur		Sur		Mid		Bottom	
Substrate	gli	glu	gli	glu	gli	glu	gli	glu
V_{max} µg/l.h	4,30	1,80	3,70	0,95	0,85	0,85	0,26	0,50
$K_t + S_n$ µg/l	167	24,3	159,0	3,8	17,9	4,8	1,9	1,0
T_t hs.	39,0	13,5	43,5	4,1	21,0	5,6	7,5	2,0
V_{max} µg/l.h	7,82	2,17	6,07	1,25	1,42	1,06	0,44	0,62
$K_t + S_n$ µg/l	303,6	29,3	260,6	5,0	29,8	5,9	3,3	1,2
T_t hs	70,9	16,3	72,3	5,4	37,0	7,0	12,7	2,5
Expirated %	45	17	39	24	40	20	41	19

July						August					
Sur		Mid		Bottom		Sur		Mid		Bottom	
gli	glu	gli	glu	gli	glu	gli	glu	gli	glu	gli	glu
5,30	0,70	0,98	0,55	0,25	0,35	9,70	1,65	1,34	0,43	0,86	0.28
280,9	1,3	18,2	1,6	2,4	1,6	73,7	3,8	3,4	3,0	15,1	2,7
52,8	1,8	18,6	2,9	9,6	4,5	76,0	2,3	2,5	6,9	17,6	9,6
9,14	0,82	1,92	0,66	0,39	0,42	26,20	2,04	2,23	0,53	1,56	0,34
484,3	1,5	35,8	1,9	3,8	1,9	199,2	4,7	5,6	3,7	27,5	3,3
91,0	2,1	36,5	3,5	15,0	5,4	105,4	2,8	4,2	8,6	32,0	11,6
42	15	49	17	36	16	63	19	40	19	45	17

September						December					
Sur		Mid		Bottom		Sur		Mid		Bottom	
gli	glu	gli	glu	gli	glu	gli	glu	gli	glu	gli	glu
10,70	2,85	2,10	1,53	–	1,75	10,50	1,75	5,30	1,86	0,85	1,50
350,5	17,6	22,3	4,9	–	7,5	469,5	38,6	79,9	25,7	49,3	26,3
76,0	6,2	10,1	3,2	–	4,3	44,7	22,1	15,1	13,8	58,0	17,5
66,9	3,9	10,0	2,0	–	2,4	45,6	2,5	21,2	2,5	2,8	2,2
2190,0	24,4	101,4	6,5	–	10,4	2041,0	55,9	319,6	34,7	164,3	39,3
204,7	8,6	46,1	4,3	–	5,9	194,4	32,0	60,3	18,6	193,3	26,1
84	28	78	25	–	28	77	31	75	26	70	33

January 1986						February					
Sur		Mid		Bottom		Sur		Mid		Bottom	
gli	glu	gli	glu	gli	glu	gli	glu	gli	glu	gli	glu
12,80	5,60	6,50	3,80	–	0,90	10,50	6,80	4,15	2,80	0,50	0,44
588,0	49,5	83,0	45,4	–	20,8	459,5	36,7	73,7	37,3	4,6	25,2
45,9	8,8	12,8	11,9	–	23,1	43,8	5,4	17,8	9,8	9,3	57,3
85,3	8,7	46,4	5,1	–	1,3	45,6	8,9	13,8	3,5	1,7	0,5
3920,0	77,3	592,9	60,4	–	28,9	1997,8	48,3	245,7	34,1	15,5	30,4
306,3	13,8	91,2	15,9	–	31,9	145,9	7,1	59,2	12,2	31,0	69,0
85	36	86	25	–	28	77	24	70	20	70	17

April						May					
Sur		Mid		Bottom		Sur		Mid		Bottom	
gli	glu	gli	glu	gli	glu	gli	glu	gli	glu	gli	glu
8,70	5,60	0,85	2,80	0,27	0,35	2,40	1,36	0,15	0,95	–	1,05
22,6	17,5	38,5	14,3	1,6	2,8	52,8	5,6	5,4	4,3	–	5,2
25,9	3,1	45,3	5,1	5,8	8,0	22,0	4,1	36,0	4,5	–	4,9
18,1	6,9	1,7	3,5	0,6	0,5	6,0	1,6	0,2	1,1	–	1,2
470,4	21,6	75,5	17,7	3,3	3,5	132,0	6,7	8,3	5,1	–	5,7
54,1	3,9	84,3	6,3	12,1	10,1	55,0	4,9	55,4	5,3	–	5,7
52	19	49	19	52	21	50	16	35	15	–	13

the liberate CO_2 was absorbed by the paper. Bottles were shaked and then left at ambient temperature during 2 hs., afterwards the erlenmeyers were opened, the paper placed in a vial filled with toluene scintillating solution (4 g 2,5-diphenyloxazole (PPO), 50 ml 1,4-2 (5)-phenyloxazolylbenzene (POPOP) in 1000 ml toluene.

In order to obtain the value of assimilated sustrate, 25 ml aliquots of sample were poured into bottles containing different concentrations of labelled sustrate, covered with aluminium foil and incubated together with the erlenmeyers. Control of each substrates and concentration were immediately fixed with SO_4H_2 before incubation. At the end of this period, they were filtered through a membrane of 0,2 μm pore size, and the filters placed in vials containing 10 ml of scintillating solution. Cpm were corrected for quench by an efficiency curve obtained by leaking different volumes of water from the pond. The uptaken substrate was found adding the assimilated to the mineralized or expired. To correct for adsorption on detritus, the activity of the controls were subtracted. It is specially important in this pond because of the great amount of suspended particulate matter, for which reason high controls were obtained.

We worked with a probability error of less than 0,05 ($P < 0,05$) disregarding those experiences that showed higher error. Another correction was that performed on the observed efficiency of $^{14}CO_2$ freed from acidific ated aqueous sample. A control experience was done by adding knowing quantities of labelled bicarbonate with destilled and sterile water, and following the same process of the samples, to evaluate possible sources of error and loss of the liberate $^{14}CO_2$. Efficiency proved to be between 46-67%.

RESULTS AND DISCUSSION

Kinetics parameters of glycollate uptake evidence a marked seasonal fluctuation as well as with depth. Maximum theoretical velocity of assimilation ($V_{max.as}$, table III) is increased during summer months with a highest value of 12,8 $\mu g.l^{-1}.h^{-1}$ in January 1986. The lowest value, on the surface, was observed during May (2,4 $\mu g.l^{-1}.h^{-1}$). Vertical variation of this parameter is also important, detecting no assimilation in the sample close to the bottom during September 1985, January and May 1986. On the contrary, highest assimilation rates were observed in the euphotic zone.

A similar seasonal and vertical variations was noticed for the affinity constant and natural glycollate concentration ($K_t + S_n$) with summer increments on the surface and decrease at the bottom during winter, Turnover time ($T_{t,as}$) was longer in winter (July and August).

We must remark that the value of kinetic parameters observed at Chascomús pond were different from the results other researchers had verified using the same methodologies, in Lake Biwa (Tanaka *et al.*, 1977)

and in Gravel Pond (Wright, 1970) (Table IV). Only Wright, in Klamath Lake observed a maximum theoretical velocity of glycollate assimilation of 2,45 $\mu g.l^{-1}.h^{-1}$ (14 – 65 $\mu g.l^{-1}.day^{-1}$) similar to ours. Photosynthetic activity, geomorphological, physical chemical and biological characteristics of this pond would explain these differences.

TABLE IV

Results obtained by other researchers

	V_{max} $\mu g.l^{-1}.h^{-1}$	T_t hs.	$K_t + S_n$ $\mu g.l^{-1}$
Tanaka, Nakanishi & Kadota (Lake Biwa, 1974)	0,510	540	275
Tanaka, Nakanishi & Kadota (Lake Biwa, 1975)			
maximum values September	0,769 (7 m)	1798 (sup)	290 (sup)
November	0,119 (3 m)	5443 (3 m)	648 (3 m)
Wright (Gravel Pond, 1970)			
maximum values	0,830 (sup)	1300 (10 m)	100 (sup)

Observed glucose parameters indicate not so marked seasonal variations, with increases in turnover time, assimilation velocity, assimilation constant and natural glucose concentration during summer (Table III, fig. 2). $K_t + S_n$ for glucose are lower than those for glycollate, therefore concluding that glycollate natural concentration or affinity constant of bacteria for this compound is higher. These variables fluctuates, for glucose between 1,0 to 49,5 $\mu g.l^{-1}$, similar to those found in PluBsee by Overbeck (1975) (3,8-46,9; mean 19,3 $\mu g.l^{-1}$).

Because of the seasonal increase of the affinity constant and natural substrate concentration, the inverse relation between assimilation time and velocity noted by us were not verified as it was when the technique was applied in Río III, Ramos Mexía and Pellegrini reservoirs, where constant and concentration fluctuated around the mean value, withouth a clear seasonal patterns.

Wright (1973) emphasises the problem of the competitive effects of utilized substrates, pointing that glycollate and lactate share a common enzimatic transport system either in natural populations or pure laboratory cultures. As a consequence, kinetic parameters ($K_t + S_n$) and T_t are increased by any lactate present, while V_{max} would be unaffected. On the other hand, acetate shows as inhibition effect not competitive with glycollate. This competitive inhibition between glycollate and lactate metabolisms would explain the higher values of these parameters, and the different results obtained by Mariazzi & Romero (1983).

When comparing the variable attained for each substrate one deduces the existence of a complex bacterial community frankly adapted to consuming algal excretory products over other compounds in the euphotic zone. Seasonal fluctuation of glycollate parameters also shows us the

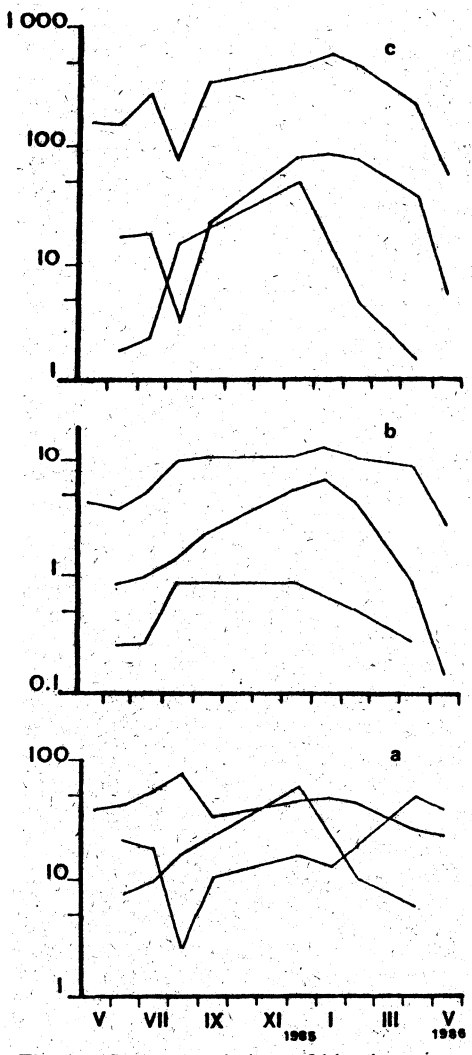

Fig. 1.– Seasonal variations of kinetic parameters for glycollate. (a) Turn over time (hs), (b) assimilated velocity (µg/l. hs);(c) natural glycollate concentration and affinity constant (ug/l).

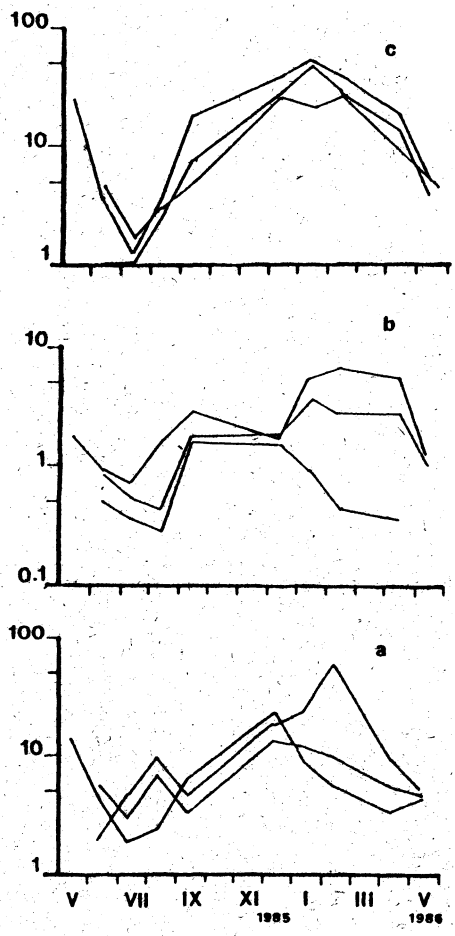

Fig. 2.– Seasonal variations of kinetic parameters for glucose. (a) Turn over time (hs); (b) assimilated velocity (ug/l.hs); (c) natural glucose concentrations and affinity constant (ug/l).

existence of a relevant relationship of heterotrophic organisms with primary producers.

Primary production and chlorophyll concentration highest values (1922,5 mg $O_2.m^{-3}.h^{-1}$ and 32,25 mg chlor.m^{-3}, respectively) were reached in August-September 1985 and January 1986, agreeing with highest glycollate uptake. In Lake Biwa, Tanaka *et al.* (1974) noted a maximum in numerosity of bacteria using glycollate as carbon and energy sources together with the highest organic carbon release rates of

phytoplankton; having bacterial kinetics a similar magnitude to this compound production.

Kinetic parameters relation between uptake and assimilation for both sustrates, is seen in fig. 3. Organic substrate uptake is the sum of the assimilated or fixed in the bacterial cell plus that mineralized part of it. Seasonal CO_2 expiration rate increase to frankly relevant values, specially in glycollate kinetics.

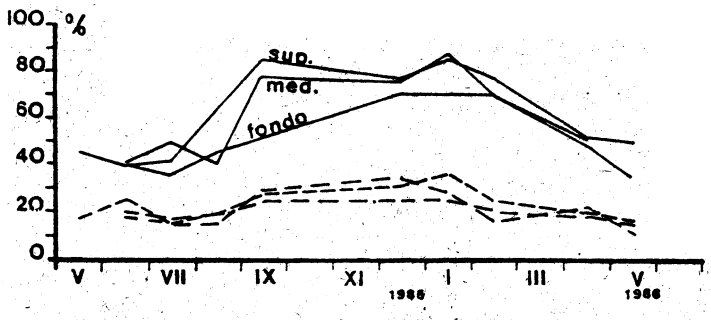

Fig. 3.– Mineralized percentage for both substrates. Glucose (– – –) and glycollate (——).

Observed results in respiration experiences for both substrates are expressed as porcentages of expired organic matter, CO_2 with respect to the total incorporated substrate. This percentage is the average of the obtained for each of the four concentrations of the experience, fluctuation was of the order of the unit, and the highest expiration rate was obtained at lower substrate concentration. This variation is understood as product of the higher ^{14}C isotope proportion with respect to the ^{12}C aggregate in lowest concentrations.

Expired glycollate varied between 39% uptaken on the surface in winter up to 85% in spring and summer. Depth variation was almost null. So high respiration percentage show the need of correcting kinetic parameters according to mineralization rates, not to get underestimates uptakes (Hobbie & Crowford, 1969). These same authors give means of 49% mineralized glycollate (Dairy Pond, Raleigh, North Carolina); Wright (1974) obtained 72% in Klamath Lake, thus a growth yield of only 25%.

On the other hand, mineralized glucose percentage was 15% during winter and 36% in summer, with a clear seasonal variation and constant with depth. Williams (1970) found 33%; Wright (1974) 11-45% in Klamath Lake, Oregon, USA (mean 26%), according with us with higher values as temperature increased. Crawford (1971) found mineralization percentage averaging at 13% (8-17%) in Palmico Estuary, North Carolina.

CONCLUSIONS

Seasonal and depth fluctuation of bacterial glycollate uptake evidences its interdependence with the photosynthesis and algal excretory products. By means of this mechanism, heterotrophic microorganisms liberate nutrients from organic molecules, and they can be used by algae again.

Uptake, assimilation and excretion rates observed during spring and summer evidence the existence of an heterotrophic bacterial community adapted to consume excreted products.

Glucose kinetic parameters do not show so conspicuous fluctuations because glucose is not subject to so clear seasonal variations and its lack of vertical variation comes from the considerable mixture of the water pond.

So high mineralization glycollate percentage indicate very low income or assimilation respect to glucose and other organic compounds. The significant concentration of this excretory product would explain its uptake in spite of its low income.

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