

## HETEROTROPHIC CONSUMPTION BY PLANKTONIC BACTERIA AND ALGAE IN EMBALSE DEL RIO TERCERO (CORDOBA, ARGENTINA)\*

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**RESUMEN:** Consumo heterotrófico por bacterias y algas planctónicas en el Embalse del Río Tercero (Córdoba, Argentina).

La actividad heterotrófica de bacterias y algas planctónicas se estimó mediante los parámetros cinéticos obtenidos de la aplicación del método propuesto por Wright y Hobbie (1966). Este método nos permite diferenciar entre los dos mecanismos de consumo hallados en algas y bacterias, simple difusión y transporte activo respectivamente. Comparando las velocidades reales debido a difusión o transporte activo ( $vd/vt$ , %) se observó que las algas consumen menos del 10% de la cantidad removida por las bacterias en igual período, siendo éstas más efectivas para consumir sustratos orgánicos presentes en el agua en muy bajas concentraciones (menos de  $10 \mu\text{g} \cdot \text{l}^{-1}$ ). Los parámetros obtenidos por simple difusión sólo fueron significativos a fines del verano, sugiriendo que en esa época del año el heterotrofismo algal puede competir con el consumo bacteriano. Ambos mecanismos de consumo son sensibles a variaciones térmicas del ambiente ( $Q_{10}$  : 2,5 a 9,2) por lo cual el uso del agua por la Central Termonuclear de Embalse, pueda incrementar considerablemente el consumo heterotrófico.

### INTRODUCTION

Investigation of the kinetics parameters of natural populations has been widely used to study microbial heterotrophic activity in aquatic environments. Wright & Hobbie (1966) distinguished two different uptake mechanisms that operate simultaneously in the natural aquatic microbial populations. Bacteria uptake at low substrate (glucose, acetate) concentrations ( $0\text{-}500 \mu\text{g} \cdot \text{l}^{-1}$ ) follows Michaelis Menten kinetics and can be described by a rectangular hyperbola and exhibits saturation at higher concentrations. Another uptake at higher concentrations ( $0,5\text{-}4,0 \text{ mg} \cdot \text{l}^{-1}$ ) is due to simple diffusion kinetics and is normally associated with algae. Allen (1969, 1971) found simple diffusion in algae higher than  $5 \mu\text{m}$  of diameter, and active transport for glucose and acetate in nannoplanktonic algae and Cyanophyta (*Anabaena solitaria*).

We have applied this method for calculating turnover time, the affinity constant and the real velocity of uptake of bacteria and algae in Embalse del Río Tercero.

*Site description:* The Embalse del Río Tercero is a dam lake built for hydroelectric purposes in the Calamuchita Valley (Córdoba, Argentina)

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in 1930. The lake area is 54,26 km<sup>2</sup> and has a maximum depth of 40 meters. Its geographical position is: longitude 64°13'W and latitude 32°11'S, at 529,4 meters altitude above sea level. Mean values for 1980/1981 of primary production, chlorophyll a and Secchi disc were: 519 mg C m<sup>-2</sup> d<sup>-1</sup>; 44 mg m<sup>-2</sup> and 2 meters respectively. During summer periods there is an oxygen depletion in the deeper layers and blooms of dinoflagellates (*Peridinium sp.*) occur annually. Previously published papers (Bonetto *et al.*, 1976; Mariazzi & Conzonno, 1980; Mariazzi *et al.*, 1981a and Mariazzi *et al.*, 1981b) give detailed information about the characteristics of the lake.

## MATERIALS AND METHODS

The two uptake mechanisms described in the introduction, active transport by bacteria and simple diffusion by algae, could be differentiated controlling the quantity of substrate added to the water samples. The methodology used was that described by Wright & Hobbie (1966) and detailed in a previous paper (Romero & Mariazzi, 1983). The substrate used was D-(U-<sup>14</sup>C-glucose) with specific activity of 279 mCi/m mol (Radiochemical Centre, Amersham, England). The parameters obtained were:  $T_t$ , turnover time due to active transport (h<sup>-1</sup>);  $v_t$ , real uptake velocity ( $\mu\text{g l}^{-1}\text{h}^{-1}$ ) and  $K_t$ , active transport constant (h<sup>-1</sup>).

Wright & Hobbie (1966) showed that the uptake of organic substrates by planktonic algae at high substrate concentrations in relation to that found in the water, did not follow the first order Michaelis Menten kinetics. Resolution of the equation proposed by them, provides the kinetics parameters derived from simple diffusion:  $v_d = K_d (S_n + A)$  where  $K_d$  (h<sup>-1</sup>) is the diffusion constant;  $v_d$  ( $\mu\text{g l}^{-1}\text{h}^{-1}$ ) is the uptake velocity due to simple diffusion; ( $S_n + A$ ) is the natural and added substrate. And  $T_d = S_n/v_d$  or  $T_d = 1/K_d$ , where  $T_d$  (h) is the turnover time due to simple diffusion (time needed for consumption of substrate at real diffusion velocity,  $v_d$ ).

The method used for obtaining the parameters due to simple diffusion was the same of that of active transport using higher glucose concentrations: 405,5; 790,1; 1174,8 and 1559,4  $\mu\text{g l}^{-1}$ .

Measurements were made at monthly intervals from November 1980 to May 1981 with samples from a central station taken at 2,5; 7,5 meter depth and from 1 meter above bottom.

## RESULTS AND DISCUSSION

The experimental results are presented in table 1, which include correlation coefficients and the probability of error of the experimental equation

TABLE I  
Experimental results

Parameters	26-XI-1980			21-XII-1980			28-I-1981		
	2,5 m	7,5 m	bottom	2,5 m	7,5 m	bottom	2,5 m	7,5 m	bottom
$v_t; (\mu\text{g} \cdot \text{l}^{-1} \cdot \text{h}^{-1} \cdot 10^{-3})$	291,6	162,5	—	636,2	441,5	—	309,0	304,6	276,1
$v_d; (\mu\text{g} \cdot \text{l}^{-1} \cdot \text{h}^{-1} \cdot 10^{-3})$	7,6	13,0	—	64,5	23,5	—	36,4	22,8	5,6
$k_t; (\text{h}^{-1} \cdot 10^{-3})$	1950	1900	—	910	500	—	390	580	4110
$k_d; (\text{h}^{-1} \cdot 10^{-3})$	2,0	1,9	5,3	4,4	4,4	1,2	5,7	3,8	0,7
$T_t; (\text{h})$	13,1	42,2	—	23,0	12,1	—	20,5	19,4	28,9
$T_d; (\text{h})$	500,4	525,5	188,05	226,8	227,2	825,0	174,4	259,4	1409,7
$v_d/v_t; (\%)$	2,6	8,0	—	10,1	5,3	—	11,8	7,4	2,0
Chlor/pheopig; ( $\text{mg} \cdot \text{m}^{-3}$ )	12/9	12/10	12/9	6/6	4/8	4/7	10/75	4/27	0.5/10
Temperature; ( $^{\circ}\text{C}$ )	21	21	21	20,03	20,03	20,03	22	22	22
Coef. Pearson (r)/error (P)	.95/0.02	.94/0.02	.94/0.02	.89/0.05	.99/0.01	.99/0.01	.98/0.01	.97/0.01	.81/0.1

TABLE 1 (Continuación)

Parameters	26-II-1980			28-III-1980			28-IV-1981			29-V-1980		
	2,5 m	7,5 m	bottom	2,5 m	7,5 m	bottom	2,5 m	7,5 m	bottom	2,5 m	7,5 m	bottom
$v_t$ ; ( $\mu\text{g} \cdot \text{l}^{-1} \cdot \text{h}^{-1} \cdot 10^{-3}$ )	1194,5	-	70,9	1682,7	-	889,4	515,0	165,5	262,9	215,4	174,7	109,2
$v_d$ ; ( $\mu\text{g} \cdot \text{l}^{-1} \cdot \text{h}^{-1} \cdot 10^{-3}$ )	290,5	-	1,7	32,2	-	6,7	41,5	5,2	9,3	3,2	1,1	1,2
$k_t$ ; ( $\text{h}^{-1} \cdot 10^{-3}$ )	640	-	130	3410	-	80	430	2460	2650	1470	1150	3380
$k_d$ ; ( $\text{h}^{-1} \cdot 10^{-3}$ )	65,9	47,5	17,3	2,2	3,1	1,8	2,1	1,7	1,7	0,9	0,5	0,3
$T_t$ ; (h)	4,4	-	1,4	8,5	-	4,1	38,2	17,8	20,5	16,3	12,9	30,0
$T_d$ ; (h)	15,1	21,0	57,6	443,6	320,3	548,7	474,7	559,5	579,4	1069,9	1932,9	2606,4
$v_d/v_t$ ; (%)	24,3	-	2,4	1,9	-	0,7	8,0	3,2	3,5	1,5	0,6	1,1
Chlor/pheopig; ( $\text{mg} \cdot \text{m}^{-3}$ )	11/3	11/4	0.7/3	22/8	17/5	11/9	7/3	3/9	7/2	10/1	5/1	8/3
Temperature; (°C)	24	24	24	21,2	21,2	21,2	-	-	-	-	-	-
Coef. Pearson (r)/error (P)	.91/0.05	.95/0.02	.90/0.05	.97/0.02	.99/0.01	.90/0.05	.99/0.01	.99/0.01	.97/0.01	.84/0.1	.95/0.02	.96/0.02

solved. With the exception of one sample near the bottom on January and that of May at 2,5 m, the probabilities of error were lower than 0,05.

Wright & Hobbie (1966) considered the diffusion constant  $K_d$  as the diffusion rate of the organic solute due to planktonic algae and obtained values that ranged between  $3,2 \cdot 10^{-4}$  and  $10 \cdot 10^{-4} \text{ h}^{-1}$  in Lake Erken, finding the highest values at the end of summer. Our  $K_d$  values ranged between  $0,3 \cdot 10^{-3}$  and  $65,9 \cdot 10^{-3} \text{ h}^{-1}$ , found in May and February respectively (fig. 1). The diffusion rates were higher at the end of summer at 2,5 meters depth; during which period the maximum chlorophyll values observed (Mariazzi *et al.*, 1983). This also coincided with the dinoflagellates bloom (*Peridinium sp.*) which occurs annually during that period.

The active transport constant ( $K_t$ ) measures the bacterial substrate affinity being more effective at lower concentrations, when  $K_t$  is lower.  $K_t$  ranged between 80 and  $4110 \mu\text{g l}^{-1} \cdot 10^{-3}$  in March and January respectively, the lower values being obtained during February-March in samples

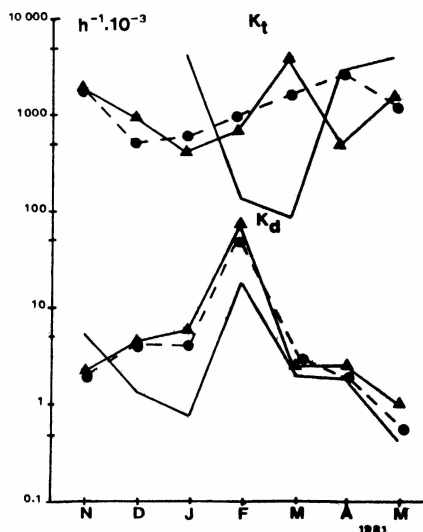


Fig. 1.— Seasonal variations of active transport constant ( $K_t$ ) and simple diffusion constant ( $K_d$ ). Data from 2,5 meters depth ( $\blacktriangle$ - $\blacktriangle$ ), 7,5 meters ( $\bullet$ - $\bullet$ ) and from one meter above the bottom ( $---$ ).

near the bottom. This suggested a higher glucose affinity during a period in which there is an input of primary production and phytoplankton biomass.

The turnover time due to simple diffusion ( $T_d$ ) ranged between 15,2 and 2606 hours, being faster at the end of summer (February) at 2,5 meters depth. Turnover time due to active transport and attributable to bacteria ( $T_t$ ) ranged between 1,4 and 42,2 hours. At 2,5 and 7,5 meters depth, turnover times were longer and showed less seasonal variation than samples near the bottom (fig. 2). The rapid consumption of glucose in the deeper zone was assumed to be due to a bacterial population with higher affinity for this substrate, as illustrated by the low  $K_t$  values.

If we compare the  $T_d$  and  $T_t$  values obtained in the study with those of Wright & Hobbie (1966) for Lake Erken ( $T_d$ : 715-3125 hours;  $T_t$ : 16-1000 hours) and Allen (1969) for Lake Löttsjön ( $T_d$ : 150-2500 hours;  $T_t$ : 0,85-300 hours) we can see that the values obtained for the eutrophic temperate dam lake (Embalse del Río Tercero) are significantly lower and positively correlated with algal biomass.

Another parameter that allows comparisons to be made between the heterotrophic activity of algae and bacteria is the uptake velocity. The

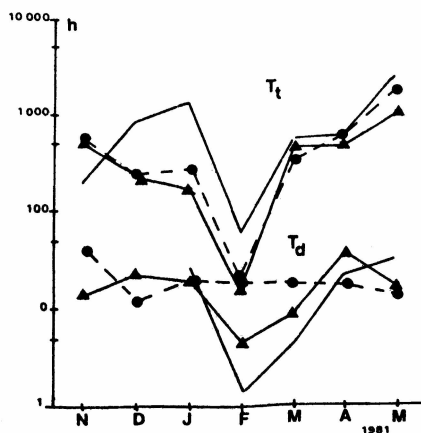


Fig. 2.— Seasonal variation of turnover time due to active transport ( $T_t$ ) and to simple diffusion ( $T_d$ ).

algal uptake velocity ( $v_d$ ) varied between  $1,1 \cdot 10^{-3}$  and  $290,5 \cdot 10^{-3} \mu\text{g l}^{-1} \text{h}^{-1}$  (fig. 3) and uptake velocities due to active transport varied between 10,9 and  $1682,7 \mu\text{g l}^{-1} \text{h}^{-1} \cdot 10^{-3}$ . Both  $v_t$  and  $v_d$  were higher at 2,5 meters at the end of summer.

The ratio  $v_d/v_t$  (%) (table 1) was at its maximum in February (24,33%) and excluding this, values were always lower than 10% at all depths (fig. 4). This relation ( $v_d/v_t$ ) allowed us to evaluate the relative importance of the two populations in the uptake of organic substrates (in this case, glucose). Wright & Hobbie (1965) stated that in their experiments, algae consumed a maximum of 8% of substrate consumed by bacteria in the same period of time. At the low glucose concentrations found in the Embalse del Río Tercero (less than  $10 \mu\text{g l}^{-1}$ , Romero & Mariazzi, 1983), the bacterial active transport system is more effective than diffusion attributable to algae in the consumption of organic substrates. In consequence, the natural substrate concentration is one of the factors which prevents competition between algae and bacteria for organic solutes in nature.

Values observed for bacteria of three kinetics parameters (active transport constant, uptake velocity and turnover time) show that they are so effective in removing the organic substrate (glucose) that they maintain the natural concentrations below  $20 \mu\text{g l}^{-1}$ , thus controlling algal heterotrophic uptake. The results obtained with algae at high glucose concentra-

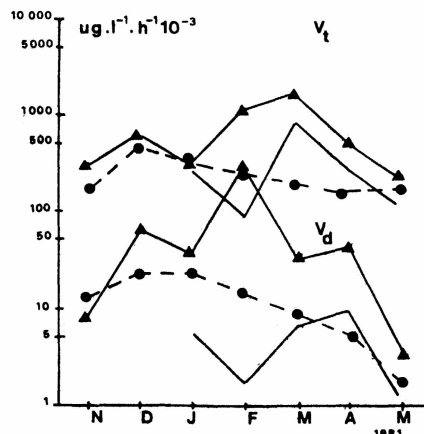


Fig. 3.— Seasonal variation of real uptake velocity due to active transport ( $v_t$ ) and to simple diffusion ( $v_d$ ).

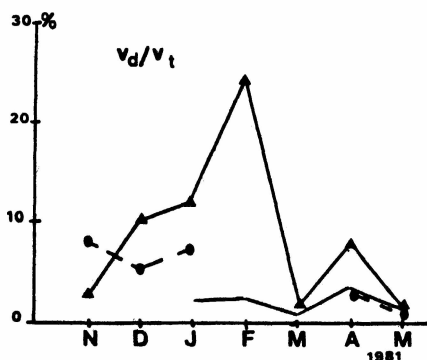


Fig. 3.— Seasonal variation of  $v_d/v_t$  (%).

tions only demonstrate their ability to grow heterotrophically, but this does not prove that such activity occurs under natural conditions. Different authors have proposed different membrane transport mechanisms for algae, Wright & Hobbie (1965) supported the idea that algae consume organic substrate heterotrophically by the simple diffusion mechanism. However, Danforth (1962) suggested that algae could grow heterotrophically by specific transport mechanisms analogous to those in bacteria. Wright & Hobbie (1966) and Allen (1971) mention that nannoplanktonic algae (3-8  $\mu\text{m}$ ) could have active transport uptake systems. Rhode (1955) found that some phytoplanktonic species possessed both autotrophic or heterotrophic growth mechanisms and their use depended on the environmental conditions. Such experiments with algal cultures, the use of differential filtration combined with the examination of kinetics parameters method, have provided evidence of the existence of a mechanism of heterotrophic algal uptake, which would be conditioned by cellular size, the concentration level of the organic substrates and environmental conditions (light, temperature, etc.).

Temperature effect on heterotrophic algal uptake can be analyzed by  $Q_{10}$  values found for the diffusion constant ( $K_d$ ) in natural populations; Wright & Hobbie (1965) give  $Q_{10}$  values for glucose of 3,1; Wartiovaara (cited by Wright & Hobbie, 1966) gives values that range between 2,5 and



9,2. These coefficients indicate the sensitivity of the passive diffusion mechanism to temperature. An increment of water temperature of 10°C could thus increase the rate of passive diffusion three times. This fact is very significant considering that the water of the Embalse del Río Tercero will be used for cooling purposes in a Thermonuclear Power Plant. The artificial thermal increment might favour algal heterotrophy although the final balance will depend upon the interaction of temperature and substrate concentration.

## CONCLUSIONS

Through a comparison of uptake due to simple diffusion and active transport it was possible to state that algae were able to consume less than 10% of the substrate utilized by bacteria in the same period of time.

Parameters due to simple diffusion found at the end of summer (February) coincidental with a dinoflagellate bloom (*Peridinium sp.*) suggest that algal heterotrophy might be significant during that period.

Under natural conditions, competition between algae and bacteria would not exist, because heterotrophic algal uptake would be controlled by low natural glucose concentrations and temperature. An artificial increase in temperature could favour algal heterotrophy.

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