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Organic carbon release by phytoplankton and bacterial reassimilation

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ABSTRACT

The release of organic carbon by phytoplankton and its reassimilation by bacteria were studied in Lake Geneva during four daily kinetics, using ^{14}C techniques. Gentamycin was used to suppress bacterial activity. Size fractionation was used to measure ^{14}C fixation in 2 particulate fractions ($\geq 1\,\mu m$ and 0.2 to $1\,\mu m$) and in a third fraction: dissolved organic carbon.
Measurements of bacterial biomass and ^{14}C glucose uptake as the $H^{14}CO_3^-$ uptake by samples prefractionated before incubation showed that the whole algae were retained on $1\,\mu m$ pore size Nuclepore filters, but bacteria were retained on $1\,\mu m$ and $0.2\,\mu m$ filters. Concentration of $20\,\mu g.ml^{-1}$ gentamycin resulted in incomplete bacterial inhibition while phytoplankton was affected.
Phytoplankton released less of 20 % of its photoassimilated carbon of which a large proportion was utilized by bacterioplankton.

Introduction

In aquatic ecosystems, 0 to 30 % (even more according to conditions and authors) of carbon photosynthetically fixed by phytoplankton is released as organic carbon (ROC) in the environment. It constitutes a source of dissolved organic carbon (DOC) in natural waters, a substrate and an energy source to heterotrophic organisms mainly bacteria. Chrost and Faust [8] calculated that bacterioplankton consumes 18 to 77 % of ROC. Bacterial uptake of up to 100 % of released algal products has been found [quoted in 11]. Bacterial populations associated to algae appear highly adapted for extracellular algal products utilization [25]. This explains that organic carbon excreted by algae constitutes a better tracer than radioactive compounds currently used, the latter given only a relative measurement of bacterial activity [9, 4, 7].
Methods used in a former work [14], gave under-estimated values of ROC and relative measurements of bacterial activity. Nevertheless they brought evidence that ROC from phytoplankton of Lake Geneva was, in trophogenic layers, an important substrate for bacterial secondary production. The purpose of the present study was

to quantify the gross release of extracellular carbon by phytoplankton of Lake Geneva and the subsequent uptake of these products by heterotrophic organisms.
In order to separate mechanisms of excretion and consumption of ROC by the natural planktonic communities in the lake, two methods were used. The size fractionation technique and the use of a bacterial antibiotic to inhibit selectively the assimilation of DOC by microheterotrophs. Berman [3] and Chrost [5, 7] have shown that, in adequate concentration and duration, gentamycin rapidly and efficiently inhibited aquatic bacterial activity without affecting phytoplankton metabolism, excepted that of blue-green algae. So this antibiotic was used.
Simultaneously heterotrophic activity was estimated by uptake of ^{14}C-labelled glucose.

Material and Methods

The study was conducted in Lake geneva. Kinetics have been done, in April, May, July, and October.

Water Sampling and Incubations

Procedures are summarized on figure 1.
Water was collected at the central station of the lake at a depth of two meters, one hour before sunrise and then it was immediately distributed into 120 ml pyrex flasks. Half of the flasks received raw water, the other ones receiving water filtered through 1 µm Nuclepore membranes (vacuum always lower than 100 mm Hg was systematically used during the present work).
Then three sets of samples were prepared with those two kinds of water. Half an hour before incubation the first set reveived 20 µg of gentamycin by ml^{-1}. At time zero each sample received 7.5 µCi $HNa^{14}CO_3$ from 50 µCi.ml^{-1} solution (made by dilution of 1 mCi Amersham solution) and was immediately incubated in *situ* at the sampling level. The set of samples with antibiotic was only incubated, in light. One of the two other sets without antibiotic was incubated in light while the other in dark.

Assimilation and Excretion Measurements

At chosen times intervals (3, 6, 9, 12, 15 and 24 hours) the flasks were raised and the samples were immediately filtered on Nuclepore membranes (Fig. 1). Raw water samples were successively filtered on 1 µm and 0.2 µm Nuclepore membranes. 1 µm prefiltered samples were only filtered through 0.2 µm Nuclepore membranes. Each membrane was rinsed with sterilized water. Measurements of filters and filtrates radioactivities (methods formerly described in Feuillade *et al.*, [14]) allowed calculation of particular carbon (PC) and filtrate organic carbon (DOC).
Activities of filters and filtrates from non incubated raw and 1 µm prefiltered sam-

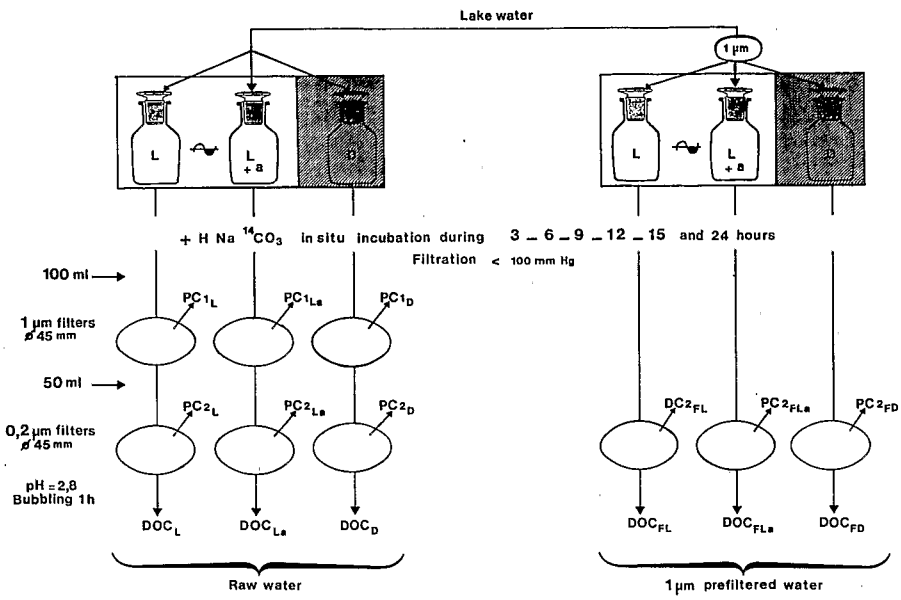

Figure 1. Illustration of different operations.

Incubated raw water:

$PC1_L$ = particulate carbon on 1 µm filter after light incubation without antibiotic.
$PC1_{La}$ = particulate carbon on 1 µm filter after light incubation with antibiotic.
$PC1_D$ = particulate carbon on 1 µm filter after dark incubation without antibiotic.
$PC2_L$ = particulate carbon on 0.2 µm filter after light incubation without antibiotic.
$PC2_{La}$ = particulate carbon on 0.2 µm filter after light incubation with antibiotic.
$PC2_D$ = particulate carbon on 0.2 µm filter after dark incubation without antibiotic.
DOC_L = dissolved organic carbon in filtrate through 0.2 µm filter after light incubation without antibiotic.
DOC_{La} = dissolved organic carbon in filtrate through 0.2 µm filter after light incubation with antibiotic.
DOC_D = dissolved organic carbon in filtrate through 0.2 µm filter after dark incubation without antibiotic.

Incubated 1 µm prefiltered water

$PC2_{FL}$ = particulate carbon on 0.2 µm filter after light incubation without antibiotic.
$PC2_{FLa}$ = particulate carbon on 0.2 µm filter after light incubation with antibiotic.
$PC2_{FD}$ = particulate carbon on 0.2 µm filter after dark incubation without antibiotic.
DOC_{FL} = dissolved organic carbon in filtrate after light incubation without antibiotic.
DOC_{FLa} = dissolved organic carbon in filtrate after light incubation with antibiotic.
DOC_{FD} = dissolved organic carbon in filtrate after dark incubation without antibiotic.

L: light
a: antibiotic
D: darkness

ples (= blanks t_o) were substracted. The filtrates activities (DOC = 26 ± 15 dpm. µCi $HNa^{14}CO_3$) were identical to the residual activity of the $HNa^{14}CO_3$ solution (measured in lake water prefiltered on 0.2 µm, acidified and bubbled for one hour); they represented about 0.001 % of added radioactivity. Activities of particulate frac-

tion 1, more than 1 µm, essentially algal was negligible (PC1 = 2 ± 1 dpm), whereas that of the fraction 2, less than 0.2 µm, essentially bacterial, was higher (PC2 = 15 ± 4 dpm).
To estimate molecular weight of the excreted organic ^{14}C, acidified and bubbled filtrates were neutralized ($7 < pH < 7.2$) and concentrated under vacuum in a rotatory evaporator at $t° < 40°$ C. Then the concentrated samples were fractionated on Sephadex G-15 fine grade, Dextran exclusion limit of 1,500 D (Pharmacia Corp.) on a 33 × 1 cm column. Two ml fractions were collected in scintillation vials and counted after addition of scintillation cocktail (Lumagel). The activity peaks in the elution sequences were characterized by the ratio Ve/Vt (Ve = volume used from start of elution to the peak, Vt = total gel volume). As a reference, ^{14}C glucose was eluted at Ve/Vt = 0.85.

Chlorophyll Analysis

The organisms were sampled on Nuclepore filters (1 µm then 0.2 µm) by the same filtration technique (identical vacuum and filtered volumes) than incubated samples and then freezed. Chlorophyll *a* concentrations (not corrected for degradation products) were determined in fractions 1 and 2 according to Strickland and Parsons [32] formula. Larger samples (1 l) were used to estimate chlorophyll *a* and degradation products (phaeopigments) according to Lorenzen [24] formula.
In both cases pigments were extracted by ultrasonic desintegration in 90 % acetone followed by a 15 h contact in darkness at 4° C. After filtration on GF/C fiber glass membranes the extracts were analyzed by spectrophotometry.

Glucose Heterotrophic Assimilation

Triplicates of 20 ml were inoculated with U-^{14}C glucose-labelled (final concentration 0.9 $\mu g.l^{-1}$). They were incubated *in situ* with and without gentamycin in transparent and in dark tubes. Then they were filtered on Nuclepore filters of 0.2 µm. The heterotrophic assimilation of glucose was calculated from the activity of filters counted by liquid scintillation. Activity of preformoled triplicates was deduced. In order to elucidate the contribution of the larger fraction to the heterotrophic uptake, other triplicates were filtered on 1 µm Nuclepore filters after their incubation.

Phytoplankton and Bacterial Standing Crops

Phytoplankton was preserved with Lugol's iodide solution and counted by the sedimentation method [33] with an inverted microscope. Bacterial cells were counted in the unfractionated water and in filtrates by epifluorescent microscopy of formalin-preserved samples after staining with acridine orange and collection on 0.2 µm Nuclepore filters [17].

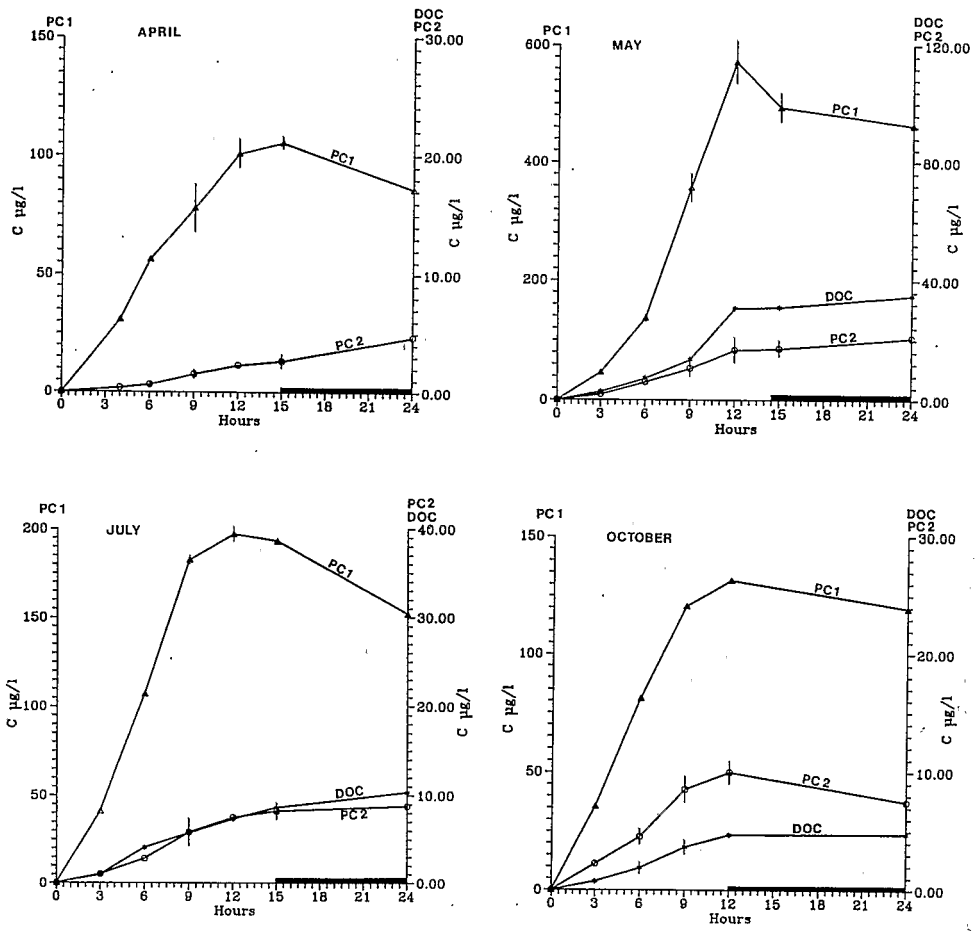

Figure 2. Time course of carbon fixation in larger fraction PC1 (≥ 1 µm), in fraction PC2 (0.2 to 1 µm) and in the dissolved fraction (DOC), during *in situ* incubations.

Statements on Calculations

The fractionation process resulted in measurements of radioactivity incorporated in two particulate fractions, fraction 1 (≥ 1 µm) and fraction 2 (0.2 to 1 µm), and of radioactivity recovered in filtrates passing the two filters.

The fraction 1 is mainly algal but with associated bacteria. So light-incorporated ^{14}C in this fraction consist of photosynthetic ^{14}C, dark anaplerotic-incorporated ^{14}C and excreted $DO^{14}C$ assimilated back by the bacteria retained on 1 µm filters.

Concerning the fraction 2, two cases may be considered. In the first one it was only bacterial without any algae, then its activity is due to heterotrophic assimilation of algal excreta. In the second case fraction 2 contained both small algae and bacteria, so an additional part of its activity in light is due to photosynthetic assimilation.

Being not measured, respiratory losses could only be calculated indirectly on data from dark incubations. Evident inaccuracy of these values led us to discard respiratory losses from the calculations. Consequently reassimilation of respiratory $^{14}CO_2$ have been neglected.
Calculations (see Figure 1 caption for abbreviations)
CO_2 assimilation by fraction 2: (it resulted of light incubation of 1 µm prefiltered water)

$$PC2_{FL} + DOC_{FL} \quad (a)$$

CO_2 photosynthetic assimilation by fraction 2: (CO_2 anaplerotic assimilation which was measured by dark incubation of 1 µm prefiltered water, is deducted from (a)).

$$PC2_{FL} + DOC_{FL}) - (PC2_{FD} + DOC_{FD}) \quad (b)$$

As we found photosynthetic assimilation by fraction 2 very low, the corresponding excreted part and therefore those reassimilated by bacteria would be all the more so negligible, then DOC = ROC (ROC = released organic carbon by algae).
Fraction 1 exudate incorporation into the particulate fraction 2: (it is constituted by total carbon incorporated into particulate fraction 2 after incubation of the raw water minus the CO_2 incorporated into this same fraction after incubation of the 1 µm prefiltered water).

$$PC2_L - PC2_{FL} \quad (c)$$

Released organic carbon in light (ROC_L) by fraction 1: (it is constituted by DOC originating from fraction 1 excretion and by the exudates incorporated into the fractions 1 and 2).

$$ROC_L = (DOC_L - DOC_{FL}) + (PC2_L - PC2_{FL}) + \text{OC incorporated by large bacteria} \quad (d)$$

(NB: OC incorporated by large bacteria is calculated by assuming that C uptake by the both fractions are in the same ratio than for glucose uptake).
Released organic carbon in dark (ROC_D) by fraction 1: (it is calculated according to the same formula as (d) with dark results).

$$ROC_D = (DOC_D - DOC_{FD}) + (PC2_D - PC2_{FD}) + \text{OC incorporated by large bacteria} \quad (e)$$

CO_2 assimilation by fraction 1: (it is constituted by the particulate carbon fixed in light and the organic carbon released, the part reassimilated by large bacteria of fraction 1 not included).

$$PC1_L + ROC_L \text{ (formula (d) without OC incorporated by large bacteria retained on 1 µm filter)} \quad (f)$$

CO_2 photoassimilated by fraction 1: (it is calculated by subtracting CO_2 assimilated by dark anaplerotic reactions from formula (f)).

$$(PC1_L + ROC_L \text{ without OC incorporated by large bacteria}) - (PC1_D + ROC_D) \quad (g)$$

Table 1. Dominant phytoplankton species during investigation periods. Measurement of the cells (diameter in µm, volume in µm³) and cell number per liter of raw water.

Species	Ø	Biovolumes	16 April	21 May	30 July (pl. 5 Aug.)	23 September	7 October
CYANOPHYCEAE							
Aphanizomenon flos-aquae	2.0	1 450			303	6	10
Oscillatoria rubescens	4.0	3 300				463	197
Anabaena macrospora	5.0	180				57	
DINOPHYCEAE							
Gymnodinium helveticum	30.0	15 000	12	1			
Gymnodinium hantzschii	12.0	5 000	27	6			
Ceratium hirundinella	40.0	75 000			34	28	9
CRYPTOPHYCEAE							
Cryptomonas sp.	10.0	2 500	87	204	9	41	4
Cryptomonas marsonii	8.0	2 000	37	2			
Rhodomonas minuta	4.0	250	711	882			
Rhodomonas minuta var. nannoplanctonica	5.0	100	775	2 999	605	999	204
CHRYSOPHYCEAE							
Erkenia subaequiciliata	4.5	50	30	1 662	4 601	216	707
Dinobryon sociale	8.0	800				110	
BACILLARIOPHYCEAE							
Melosira islandica	4.0	2 000	81	14			
Stephanodiscus binderanus	4.0	500	5				
Stephanodiscus alpinus	12.0	1 000	20	582			
Stephanodiscus minutula	4.5	100	185	389			
Fragilaria crotonensis	2.0	300	24	132	113	170	9
Asterionella formosa	1.0	200	145	24	15		
Synedra acus	5.0	1 000	20	136			
CHLOROPHYCEAE							
Chlamydomonas sp.	12.0	1 000	90				
Chlorella vulgaris	1.0	15	2 733	6 781	1 937	590	79
Ankyra judayi	3.5	100			19		
Phacotus lendneri	15.0	250				115	
Mougeotia gracillima	5.0	1 100				85	

Table 2. Chlorophyll distribution between the both size fractions, in $\mu g.l^{-1}$. Chlorophyll uncorrected (total chl) and corrected (active chl) for its degradation products.

	Fraction 1 (≥ 1 µm)		Fraction 2 (0.2–1 µm)	
	total chl.	active chl.	total chl.	active chl.
April	8.40 ± 0.34	8.06	0.99 ± 0.16	0
May	25.11 ± 0.45	20.84	1.19	0
July	10.53 ± 0.33	8.21	2.21 ± 0.04	0
October	5.60 ± 0.52	1.12	3.65 ± 0.05	0

Table 3. Carbon photosynthetic assimilation ($\mu gC.l^{-1}$), by the both fractions after light incubations during 12 hours (October) or 15 hours (the other months).
% = carbon photosynthetic assimilated by fraction 2 in percent of the carbon photosynthetic assimilated by fraction 1.

	Fraction 1 (≥ 1 µm)	Fraction 2 (0.2–1 µm)	%
April	106	0.2	0.2
May	539	0.3	0.1
July	204	1.8	0.9
October	142	0.3	0.2

Table 4. Time course of carbon fixation and excretion by the both fractions, without and with antibiotic, in $\mu gC.l^{-1}$.

Column 1	Carbon incorporated into particulate fraction 1 after light incubation.
Column 2	CO_2 assimilated by fraction 1 (by photosynthesis and anaplerotic reactions), calculated by formula (f).
Columns 3–4	Released organic carbon in light by fraction 1, calculated by formula (d) without (col. 3) or with (col. 4) organic carbon incorporated into the bacteria ≥ 1 µm.
Columns 5–6	Percent of the CO_2 assimilated which is excreted, without (col. 5) or with (col. 6) organic carbon incorporated by bacteria ≥ 1 µm.
Column 7	Released organic carbon incorporated into the bacteria retained on 1 µm.
Column 8	Carbon incorporated into particulate fraction 2 after light incubation of raw water.
Column 9	CO_2 assimilated by fraction 2 (by photosynthesis and anaplerotic reactions, calculated by formula (a)) after light incubation of 1 µm prefiltered water.
Column 10	Released organic carbon incorporated into fraction 2 calculated by formula (c).
Column 11	Dissolved organic carbon in filtrate through 1 µm then 0.2 µm after light incubation of raw water. It represents the unused organic carbon.
Columns 12, 13, and 14	give the values, in presence of antibiotic, of the same parameters of columns, 1, 9, and 11 respectively.

		WITHOUT ANTIBIOTIC									WITH ANTIBIOTIC		
		FRACTION 1 (≥ 1 µ)					FRACTION 2 (0.2–1 µm)			DOC	FR.1	FR.2	DOC
	Hours	$PC1_L$	CO2 assim.	ROC_L	PER	ROC incorp.	$PC2_L$	CO2 assim.	ROC incorp.		$PC1_L$	$PC2_L$	
April	4	31	31	0.4–0.5	1.3–1.6	0.1	0.4	0.1	0.3	0.1	31	nd	nd
	6	57	58	0.7–1.0	1.2–1.7	0.3	0.7	0.1	0.6	0.1	54	0.7	0.2
	9	78	79	1.4–2.0	1.8–2.5	0.6	1.6	0.2	1.4	0.0	77	1.3	0.3
	12	101	103	2.1–3.0	2.0–2.9	0.9	2.3	0.2	2.1	0.0	93	nd	nd
	15	105	107	2.4–3.4	2.2–3.2	1.0	2.7	0.3	2.4	0.0	85	2.2	0.9
	24	86	90	4.3–6.1	4.8–6.8	1.8	4.7	0.4	4.3	0.0	77	3.0	0.4
May	3	47	52	4.5– 7.1	8.7–13.7	2.6	2.0	0.3	1.8	2.8	48	2.7	2.5
	6	137	150	12.9–21.3	8.6–14.2	8.4	6.1	0.5	5.7	7.3	118	6.2	6.2
	9	357	380	23.1–37.5	6.1– 9.9	14.4	10.6	1.0	9.7	13.5	337	11.3	14.6
	12	572	618	46.3–69.7	7.5–11.3	23.4	16.7	1.1	15.8	30.7	577	19.0	32.5
	15	494	541	47.2–71.5	8.7–13.2	24.3	17.3	1.1	16.3	31.0	507	15.7	35.6
	24	462	516	53.5–81.4	10.4–15.8	27.9	20.8	2.2	19.2	34.9	427	13.0	46.0
July	3	41	42	1.3– 1.5	3.1– 3.6	0.2	1.0	0.7	0.3	1.0	40	0.9	1.5
	6	107	112	5.3– 6.1	4.7– 5.4	0.8	2.8	1.6	1.2	4.1	100	1.8	4.3
	9	182	190	8.1– 9.7	4.3– 5.1	1.6	4.6	2.4	2.4	5.7	151	2.1	4.8
	12	198	210	11.6–14.5	5.5– 6.9	2.9	7.5	3.2	4.6	7.3	162	3.0	7.0
	15	193	206	12.8–15.6	6.2– 7.6	2.8	8.2	4.0	4.7	8.6	144	2.5	9.0
	24	152	166	14.4–17.1	8.7–10.3	2.7	8.8	4.7	4.8	10.3	122	2.8	13.4
October	3	35	38	2.5– 5.0	6.6–13.2	2.5	2.2	0.4	2.1	0.7	33	1.9	1.1
	6	81	87	5.6–10.7	6.4–12.3	5.1	4.5	0.8	4.2	1.9	55	2.6	2.3
	9	121	132	10.9–21.0	8.3–15.9	10.1	8.5	1.2	7.8	3.6	63	3.6	4.4
	12	132	145	13.0–24.5	9.0–16.9	11.5	10.0	1.7	8.9	4.7	67	4.5	4.7
	24	119	130	11.3–19.3	8.7–14.8	8.0	7.5	1.0	6.9	4.8	51	3.2	8.3
Column number		1	2	3 4	5 6	7	8	9	10	11	12	13	14

Table 5. Bacteria distribution between fractions 1 and 2. Number per ml (nb.ml^{-1}). Biomass (µgC.l^{-1}). Percent of the total (%).

	NUMBER						BIOMASS					
	Fraction 1 (≥ 1 µm)				Fraction 2 (0.2–1 µm)		Fraction 1 (≥ 1 µm)				Fraction 2 (0.2–1 µm)	
	Attached		Free-living		Free-living		Attached		Free-living		Free-living	
	nb.ml^{-1}	%	nb.ml^{-1}	%	nb.ml^{-1}	%	µgC.l^{-1}	%	µgC.l^{-1}	%	µgC.l^{-1}	%
APRIL	49987	3	213998	13	1370725	84	0.72	6	6.15	49	5.73	45
MAY	84859	8	133351	13	845733	79	0.61	12	1.52	29	3.11	59
JULY	44582	2	277405	13	1788275	85	0.58	4	5.41	34	9.92	62
OCTOBER	6605	0.3	105678	6	1651222	94	0.27	2	3.65	29	8.54	69

Table 6. Size fractionation of C. glucose uptake in ng C glu.l^{-1}.h^{-1}. Percent of C. glucose uptake by the both fractions. Measurements in light (L) and dark (D), with and without antibiotic. Percent inhibition with antibiotic.
* Values for fraction 1 + fraction 2.

		WITHOUT ANTIBIOTIC				WITH ANTIBIOTIC				% inhibition on glucose uptake	
Hours		FRACTION 1 (≥ 1 µm)		FRACTION 2 (0.2–1 µm)		FRACTION 1 (≥ 1 µm)		FRACTION 2 (0.2–1 µm)			
		C-glu uptake	% of total uptake	C-glu uptake	%of total uptake	C-glu uptake	% of total uptake	C-glu uptake	% of total uptake	FR. 1	FR. 2
APRIL 12.4	L			6.4*		0.9	30	2.1	70	53*	
	D			10.1*		1.1	30	2.6	70	63*	
MAY 6.0	L	9.7	61	6.2	39	5.2	66	2.7	34	46	56
	D	9.8	63	5.7	37	4.8	64	2.7	36	51	53
JULY 4.2	L	14.1	38	23.5	62	9.9	28	25.3	72	30	≈ 0
	D	13.1	40	19.6	60	6.8	31	16.4	69	48	16
OCTOBER 4.0	L	3.8	51	3.7	49	2.1	40	3.1	60	45	16
	D	3.3	55	2.7	45						

Results

1. *Kinetics* (Fig. 2, Tab. 4)

We followed the 24 hours time course of activity in intracellular and extracellular pools of fractions 1 (≥ 1 µm) and 2 (1 – 0.2 µm). It can be seen that incorporation in fraction 1 is negative during night. Except in April, incorporation in fraction 2 slowed down during night. So, even particulate carbon on 0.2 µm pore filters, *a priori*

mainly due to heterotrophic assimilation, showed the daylight effect. This last effect was maximal in October in which we have observed absence of excretion during the night. A similar phenomenon was noted by Lancelot and Mathot [23].

2. *Separation Techniques Efficiencies*

2.1 Differential filtration

Its efficiency is estimated on the one hand by comparing the pigments, phytoplankton and bacterial biomass of both fractions and on the other hand by the measurements of their photosynthetic and heterotrophic activities.
Sizes of the main phytoplanktonic identified species (Tab. 1) should not allow these organisms to pass through 1 µm filters, and therefore they must be exclusively found in fraction 1.
Chlorophyll distribution between both particulate fractions is shown on Table 2. In fraction 2 it didn't exceeded 10 % of total chlorophyll in April and May but it respectively reached 17 % in July and 40 % in October. Measurements according to Lorenzen [24] method and pattern of the absorption spectra of acetonic extracts before acidification, showed both that fraction 2 chlorophyll was 100 % degradation products. On the contrary, in fraction 1 chlorophyll was mainly in its active form, except in October.
These results were confirmed by measurements of carbon photosynthetic assimilation (Tab. 3). Whole CO_2 fixation is nearly recovered in the fraction 1, on 1 µm pore size filters. The photosynthetic activity of fraction 2, measured in 1 µm prefiltered water is only 0.1 to 0.9 % of that of fraction 1. Therefore it is likely than fraction 2 chlorophyll consists mainly of small particles containing degraded pigments, chloroplasts or fragments of chloroplasts from damaged cells, not very able or unable to fix CO_2 photosynthetically. So fractionation has been efficient for algal partition.
On the contrary, bacteria were found in the two fractions (Tab. 5). 6 to 21 % were recovered on 1 µm filters, but as a result of their large size, they represented 31 to 55 % of the total bacterial biomass; more of 70 % of this biomass was free-living large bacteria, others were fixed on particles.
Similarly, the proportion of assimilated glucose retained on 1 µm filters (Tab. 6) was high, varying from 30 % (April) to 63 % (May). It was connected roughly to the important proportion of bacterial biomass recovered on the same filters (Tab. 5). The heterotrophic assimilation of ^{14}C.glucose by the bacterial biomass of this fraction 1 was about 3 times less efficient in April, nearly just as efficient in July and about 2.5 more efficient in May and October than the one of fraction 2.
It must be noticed that successive filtrations did not lead to radioactivity losses. The mean sum of radioactivities fixed from glucose heterotrophic assimilation recovered on 1 µm then 0.2 µm filters amounted to 98.8 % (± 15.7) of radioactivity recovered after filtration of the samples across one single 0.2 µm filter.
The two particulate fractions compositions and activities can be summarized (Fig. 3):

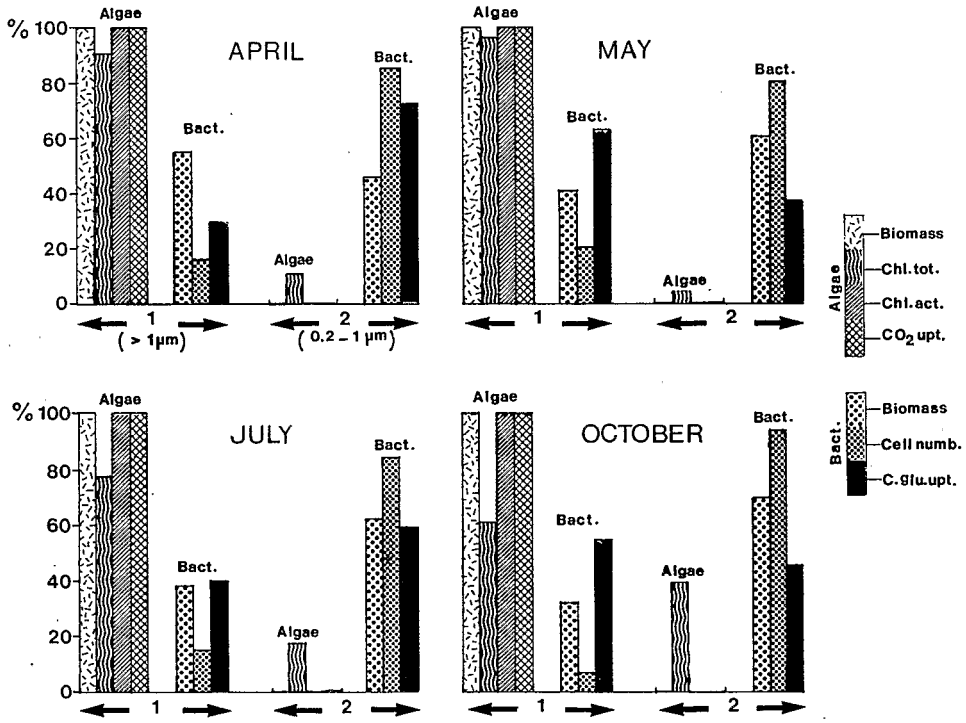

Figure 3. Distribution between fractions 1 and 2 of: algal biomass, total and active chlorophyll, phytosynthetic uptake of CO_2, bacterial biomass, bacterial cell number, heterotrophic uptake of ^{14}C.glucose; expressed as percentage of the total.

Fraction 1 = 100 % algae + 30 to 60 % bacteria
Fraction 2 = 40 to 70 % bacteria

2.2. Gentamycin Effects

In fraction 1, incorporation of carbon in light by bacteria is very low compared to its incorporation by algae. So, it is possible to deduce the effect of gentamycin on phytoplankton from the difference between the light incorporation of mineral carbon by this fraction with and without gentamycin (Tab.7). In April, inhibition appeared after 12 hours and did not reach 20 %. In July and October it was noticeable after 3 hours incubation and increased with duration of this incubation. It reached maxima of 25 % in July and 57 % in October. High inhibition of phytoplankton photosynthesis appears to be related to the presence of blue-green algae, organisms reacting to gentamycin. There were effectively a large biomass of *Aphanizomenon flos-aquae*

in July and an exceptional (for Lake Geneva) population of *Oscillatoria rubescens* in October.
Variations in carbon incorporation in fraction 2 reflects the gentamycin effect on bacteria alone only if phytoplanktonic excretion has not been modified by the antibiotic. The inhibition in this fraction is never total. However, it is always higher than in the fraction 1. The low inhibition in May appearing doubtful we have used a new gentamycin lot for the following experiments.
Direct effect on bacteriaplankton is deduced by the decrease of glucose heterotrophic assimilation with the antibiotic. Inhibitory effect of gentamycin on net glucose assimilation is never total whatever time or fraction (Tab. 6). It is higher in April and May when incubations were longer. The partial inhibition of carbon incorporation by fraction 2 (Tab. 7) strengthens the observation that gentamycin suppresses only partly bacterial heterotrophic activity.

3. *Heterotrophic Transfer of Released Organic Carbon by Algae*

It appears from Table 4 that carbon incorporation by fraction 2 was less when organisms were incubated alone (1 µm prefiltered water) (col. 9) than when they were incubated in presence of fraction 1 organisms (col. 8). Consequently large part of light incorporated carbon by fraction 2 bacteria (about 90 % in April, May, October and 50 % in July) originated in organic extracellular products (ROC) issuing from fraction 1 organisms photosynthetic activity.
It appears from Table 4 (Col. 10) that a large quantity of the ROC was reassimilated by the bacteria. After 24 hours of incubation the bacteria of the fraction 2 have reassimilated the totality in April, 36 and 33 % in May and July, and 61 % in October (Tab. 4; Col. 10/Col. 3). The ROC assimilated by bacteria retained on 1 µm filters was not measured: but it was estimated (Tab. 4, Col. 7) by assuming that ROC uptake of both fractions were in the same ratio than for glucose uptake. By accounting this calculated assimilation of fraction 1, ROC reassimilated by total bacteria, after 24 hours of incubation, reached respectively 100, 58, 44 and 77 % (Col. 10 + Col. 7/Col. 4).

Table 7. Percent inhibition attributable to gentamycin on carbon incorporation into fraction 1 (PC1) and fraction 2 (PC2) during the light periods (3 to 15 h incubations) and during 24 h incubations.

Incubation	April		July		October	
h^{-1}	PC1	PC2	PC1	PC2	PC1	PC2
3	0	nd	2	10	6	14
6	≈0	0	7	36	32	42
9	≈0	19	17	56	48	58
12	8	nd	18	60	49	55
15	19	19	25	70		
24	11	36	20	68	57	57

Table 8. Total night losses of particulate carbon by fraction 1 (≥ 1 µm) and parts attributable to excretion and respiration, as a percentage of carbon assimilated during the light period.

	April	May	July	October
Night losses	17.8	5.9	19.9	9.8
Excretion	2.5	1.8	0.7	0
Respiration	15.3	4.1	19.2	9.8

So the whole phytoplanktonic ROC has not been generally consumed, the surplus was recovered in filtrates from 0.2 µm filters (= DOC; Tab. 4, Col. 11). This DOC was larger in May; in July and October it was 3 to 5 times less (in April without direct measurement of DOC it was calculated by subtracting the PC2 from carbon organic recovered in the 1 µm filter filtrate; it was practically zero).

Molecular gel fractionation of DOC showed (Fig. 4) in May higher quantities of both large and small molecules. However, higher values of DOC observed in May were mainly due to products with molecular weights greater than 1,500 D. Comparison of extracellular products at 6, 9 and 12 hours incubations showed (Fig. 5) an important increase of high molecular weight products in the course of time and from 9 to 12 hours almost stability for small ones. Either only large molecules were excreted or small ones preferably consumed by heterotrophs.

Simultaneous existence in May of both high excretion and low bacterial biomass (2.5 to 3 times less than in other months) is in accordance with these observations and suggests that for this month the phytoplanktonic ROC was superior to bacterial needs.

It is noticeable that, even when fraction 1 photosynthetic fixation is inhibited by gentamycin, the DOC (= net excretion) is often higher (particularly after 24 hours incubations) when ROC bacterial incorporation is inhibited by the antibiotic (Tab. 4, Col. 11 and 14). Expressed as percent of carbon incorporated in fraction 1 the DOC is also higher when ROC uptake was inhibited, difference being also maximal after 24 hours incubation (values were successively for the 4 periods, 0 – 7.6 – 6.8 – 4.0 % without gentamycin (Col. 11/Col. 1) and 0.5 – 10.8 – 11.0 – 16.3 % with gentamycin (Col. 14/Col. 12)).

These results give some confirmation that ROC is at least partially reassimilated by bacteria and that gentamycin inhibits this reassimilation. Chrost [7], using the same gentamycin content (20 $\mu g.ml^{-1}$), but with a *Ceratium hirundinella* plankton found a markedly higher percent of net excretion for the sample incubated with antibiotic : 5 to 16 % versus 4 to 7 % for the sample incubated without antibiotic. Associated to a large difference in recovered ROC with and without gentamycin (after 24 hours recovered ROC was about 40 $\mu g.l^{-1}$ with gentamycin versus only 20 $\mu g.l^{-1}$ without), these results allowed Chrost to conclude that bacteria had used a large amount of algal ROC.

4. *Extracellular Release and Respiration*

On a daily 24 hours cycle the phytoplankton excreted 6 to 81 $\mu g.l^{-1}$ dissolved organic carbon (Tab. 4, Col. 3 and 4). This ROC represents 1.2 to 10 % of photosynthetic

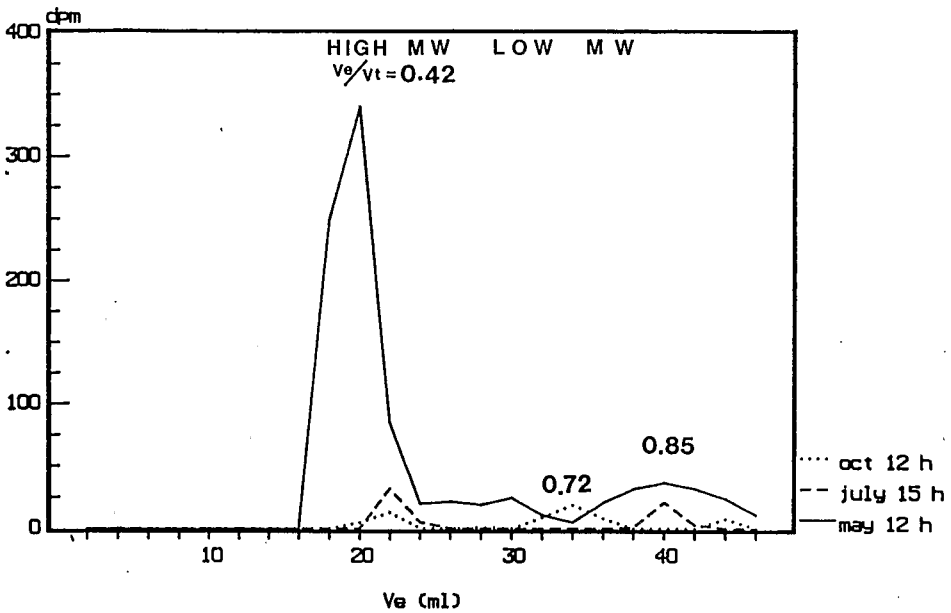

Figure 4. Sephadex gel (G-15) fractionation of dissolved organic carbon recovered after *in situ* incubation with inorganic ^{14}C during 12 hours (May–October) and 15 hours (July).

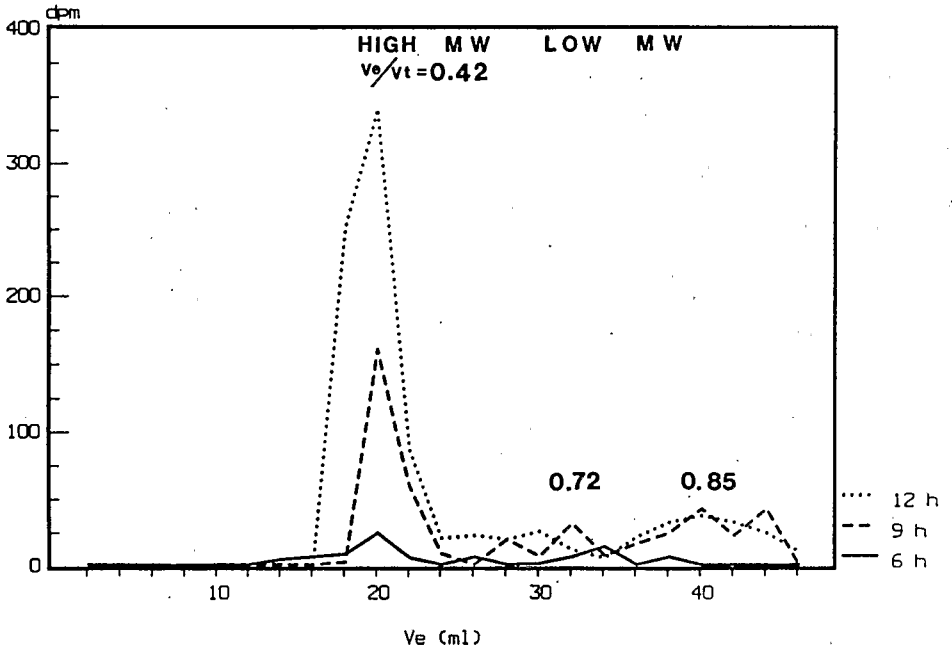

Figure 5. Sephadex gel (G-15) fractionation of dissolved organic carbon recovered after *in situ* incubations with inorganic ^{14}C during 6, 9, and 12 hours (May samples).

carbon, but taking in account ROC reassimilated by bacteria retained on 1 µm filters, it reached 1.6 to 17 %. Values of this percent extracellular release (PER) were maximal for 24 hours incubations, release continuing (October excepted where they were maximum after 12 hours) during the night whereas photosynthetic assimilation stopped. These values are underestimated for ROC respired by bacteria. This underestimation can be important. Lower PER values were in April when phytoplankton growth was fast, whereas higher values in October correspond to phytoplankton decrease (Tab. 1).
Measured night carbon losses of particulate fraction 1 are due to excretion and respiration; they amounted from 5.9 to 19.9 % of the carbon assimilated at the end of the day (Tab. 8), 0 to 2.5 % were due to excretion, 4.1 to 19.2 % could be attributable to respiration. These values confirm that excretion carbon losses were generally low compared to those from respiration.

Discussion and Conclusion

The size fractionation technique by filtration through 1 µm Nuclepore filter allowed the obtention of two fractions, on one hand, fraction 1 mainly constituted of the phytoplankton with 6 to 21 % of bacteria total number (corresponding to 31 to 55 % of total bacterial biomass) and on the other hand fraction 2 almost exclusively bacterial. A percentage from 5 to 40 % of total chlorophyll had been found in fraction 2; but photosynthetic carbon fixation by this fraction never reached more than 0.1 to 0.8 % of algal fraction 1 photosynthetic carbon fixation. Berman [3] found a surprisingly high percentage ($\approx$ 25 %) of chlorophyll and radioactivity apparently passing through 1 µm Nuclepore filters, but according to this author these particles were presumably chloroplasts or chloroplasts fragments released from broken algal cells, unable of photosynthesis; because the lack of substrates for enzymatic chains. The amount of such particles may be dependent upon vacuum pressure and the volume of sample filtered. These observations showed that despite of filtration under low vacuum ($<$ 100 mm Hg) some cells disruptions occured. According to Jones *et al.* [21] even the slightest vacuum resulted in the appearance of chloroplasts in the filtrate. Only with filtration under gravity through a 1 µm Nuclepore filter the algae were retained on the filter and most ($>$ 80 %) of the bacteria were found in the filtrate. But this procedure is time consuming and inversely more bacteria may be retained in the algal fraction. Recently Stockner and Antia [31] thoroughly reviewed the plankton size fractionation procedure and Feuillade et al. [14] have discussed the problem. Stoeckli [30] presented a new experimental system working in a continuous way and liable to improve size fractionation and extracellular products recovery.
Our analysis have shown that pigments of the fraction 2 were degraded. These pigments originated probably from delicate cells at end of growth therefore less active. However, a contamination of the bacterial fraction with labelled algal cell fragments is not excluded, particularly in October at the end of the growing season.
A high apparent bacterial ^{14}C due to photosynthetic fixation by the small size particulate fraction ($<$ 1 µm) was therefore excluded; although Fahnenstiel *et al.* [13] demonstrated the contribution made by 0.22–1 µm algal phytoplankton to carbon

production in lakes. Presence of picoplankton (photoautotrophic microorganisms of particle size 0.2–2.0 μm diameter, [31]) was also unlikely. Microscopic examination confirmed this conclusion; freshwater picoplanktonic species, reviewed by Stockner and Antia [31] have not been recovered in Lake Geneva.

Within the context of our experiments, use of 20 $\mu g.ml^{-1}$ gentamycin as a bacterial selective inhibitor was inadequate. In any case bacterial inhibition was total without affecting the phytoplankton. The effects on photosynthetic carbon fixation are consistent with results obtained by Chrost [5], who found an inhibition of 5 % after 6 hours and 24 % after 8 hours incubation with 20 $\mu g.ml^{-1}$ gentamyin, for plankton dominated by Diatoms. The inhibitory effect of gentamycin on plankton, in which blue-green algae dominated, was faster (evident after 2 hours exposure). But the bacterial susceptibility did not agree with results of Chrost [5, 7] for which incubation of the water sample with 20 $\mu g.ml^{-1}$ gentamycin for 90 minutes caused complete inhibition of bacterial activity and growth. Likewise, Berman [3] observed, with natural marine phytoplankton that, one or more (4) hours of incubation with 20$\mu g.ml^{-1}$ reduced the number of bacterial colonies by 80 to 95 % and significantly reduced (70 to 90 %) glucose uptake in most cases (40 % only after 4 hours for one experiment). On the other hand with natural marine plankton, Iturriaga and Zsolnay [19] found at gentamycin concentrations between 40 to 80 $\mu g.ml^{-1}$, that 4 hours incubation resulted in glucose uptake inhibition ranging only from 10 to 46 %, and that at 500 $\mu g.ml^{-1}$ heterotrophic activity was inhibited only by 68 % (probably after 1 hour incubation), whereas concentrations higher than 100 $\mu g.ml^{-1}$ affected the photosynthetic rate.

Moreover, in our experiments, inhibitory effects on glucose uptake and on ROC uptake were not identical. Likewise Schleyer [28] found the inhibition of glucose assimilation to be 3 to 37 % larger than the inhibition of bacterial assimilation of an algal extract.

Another reason to limit use of antibiotics is the large variations in algal susceptibility between different localities. Jensen and Søndergaard [20] observed, in a lake that the plankton dominated by blue-green algae, which although prokaryotic, showed greater resistance to the antibiotics than the Diatoms dominating in another lake.

Despite inadequacies of separation methods for algal and bacterial activities, data allow us to conclude that part of phytoplanktonic excreted DOC had been consumed by the bacteria. This part reaches after 24 hours incubation 44 to 77 % with or 33 to 61 % without large bacteria assimilation, percentage being probably larger in April. During a *Ceratium hirundinella* bloom, Chrost [7] found between 23 and 46 % of the excreted algal ROC was utilized by bacteria. Coveney [11] found the ROC in an eutrophic lake during autumn to support between 32 and 95 % of bacterial needs while it was about one third of these needs in an oligotrophic lake during summer [10]. By the fractionation technique, Jensen and Søndergaard [20] found, in 5 lakes, that 18 to 71 % of ROC was taken up by bacteria, and that 20 to 97 % of the bacterial production could be explained by ROC uptake. These authors reported that high values (77 and 97 %) found by the fractionation technique were unrealistically high considering other published results, probably because of the presence of algal picoplankton in the bacterial fraction. In calculating uptake after correction for algal picoplankton crossing filters, they found only 42 and 47 %. In our experiments, we

do not exclude that contamination of the bacterial fraction (0.2–1 μm) by labelled algal cell fragments was responsible of October high values.

The whole ROC has not been consumed. We observed in the course of time an increase in unconsumed molecules; particularly important in May for which there were mainly large molecules. Several authors (reported in [22]) have shown that algae liberate macromolecules. Moreover according to Iturriaga and Zsolnay [19] the smaller molecules might be polymerized extracellularly by bacteria. The predominance of large molecules may be also explained by the rapid utilization of the small ones by the heterotrophs [18, 26]. However Chrost [6] observed that molecules from 500 to 10,000 D were consumed as rapidly as molecules inferior to 500 D; the utilization of large molecules (> 100,000 D) was also observed [27, 8]. So, these results, with the presence in our experiments of unused small molecules, suggest that organic carbon was not limiting the heterotrophic growth (April excepted).

In April, total utilization of the ROC may be due, to the presence of a relatively important bacterial biomass, compared to the autotrophic activity. Moreover it may be due to the presence of phytoplankton in exponential growth for which excretion was relatively low. At last, probably the excreted substances were mainly small molecules easily usable, as Nalewajko and Lean [26] had already shown there is predominance of small molecular weight compounds in young cultures.

Our values are however rough ones because, on one hand respiration has not been considered and on the other hand, the bacterial uptake of DOC by fraction 1 (> 1 μm) has been calculated considering that bacterial populations in both fractions assimilated glucose and ROC in the same ratios. In fact different bacterial populations respond differently to various substrates. Berland *et al.* [2] have shown from a set of 200 bacterial strains that most of them used a large number of substrates but not one appeared as universal. About 50 % strains used amino acids and only 28 % sugars; so glucose is not a good choice to estimate bacterial heterotrophic activity. It must be pointed out that uptake measurements by fraction 2 small bacteria gave different values for glucose and ROC. ROC uptake being from 9 to 250 times that of glucose, these differences may appear excessive. Chrost [7], comparing estimates of bacterial heterotrophic activity with several substrates, found that glucose or acetate uptake was 5 to 8 times lower than phytoplankton ROC uptake by bacteria. For Hoffle *et al.* [16], C.glucose assimilation lays between 3 to 30 % of total carbon organic assimilation by bacteria. So, we conclude that assimilation of the algal ROC provide a better estimate of natural bacterial activity than uptake of single substrates. Because respiration was neglected, values of bacterial and algal assimilations and of algal excretion, were underestimated. Herbst [15] has investigated the metabolic coupling between *Oscillatoria redekei* and several strains of accompanying bacteria, he found that the greater part (70 %) of the dissolved organic substances liberated by the algae is respired by the bacteria. But according to Bell [1] marine bacteria adapted to the algal phytoplanktonic population respire around 40 % of the algal DOC they have assimilated. During our experiment, we found that 5 to 48 % of C. glucose uptaken by bacteria was respired after 4 to 12 hours of incubation. But the mineral carbon issuing from this respiration was negligible compared to the natural mineral carbon. So the photo-

synthetic reassimilation of this respired C.glucose could never be, in any case, more than 1/300,000 of the photosynthetic assimilation of natural carbon, and more than 0.5 % of the heterotrophic assimilation of C.glucose. For Wiebe and Smith [34], values for bacterial respiration of algal products amounted from 2 to 4 % of the total bacterial uptake whereas for Coveney [11] wide variations of the published values for bacterial respiration of algal products (0 to 97 % of total uptake), make choice of a respiration value arbitrary, so no respiration was applied to his data.

DOC release by bacteria have been also neglected, there are few existing data on this subject; Dunstall and Nalewajko [12] found that natural bacteria showed low release after 24 h incubation.

In conclusion, separations of bacterial and phytoplanktonic activities by antibiotic use and differential filtrations whereas not perfect, allowed us to estimate the C organic release by phytoplankton and its assimilation by bacteria during four characteristic periods in Lake Geneva.

Summary

The purpose of the present study was to quantify the gross release of extracellular carbon by phytoplankton of Lake Geneva and the subsequent uptake of these products by heterotrophic organisms. Two methods were used. On one hand the size fractionation technique through 1 µm and 0.2 µm filters gave two particulate fractions and a dissolved organic one. On the other hand a bacterial antibiotic was used to inhibit selectively the assimilation of DOC by microheterotrophs. The heterotrophic activity was simultaneously estimated by uptake of ^{14}C glucose. Four times in the year, 24 hours kinetics of carbon fixation in the different fractions were followed with start at sunrise.

Fractionation through filters has been efficient for algal separation but bacteria were found in both particulate fractions. Up to 21 % of bacterial cells and 55 % of bacterial biomass were recovered on 1 µm filters. More of 88 % of the total bacterial biomass was free-living bacteria, others were fixed on living or dead particles. Some pigments found in the particulate fraction between 0.2 µm and 1 µm were degraded forms, unable to sustain photosynthesis. Use of 20 $\mu g.ml^{-1}$ of the antibiotic gentamycin was inadequate as bacterial inhibitor, bacterial inhibition being never total but with algal photosynthesis being strongly affected.

On a 24 hours cycle the phytoplankton excreted between 6 to 81 $\mu g.l^{-1}$ of dissolved organic carbon. This amounted to 1.6 to 17 % of photosynthetic carbon fixation. 33 to 100 % of this phytoplanktonic excreted DOC was consumed by the bacteria.

Gel fractionation of the recovered DOC displayed an increase of large molecules in the course of time. Among the possible explanations, the most attractive is the preferential use of small ones by the bacteria.

Measurements of uptake by the smaller fraction essentially bacterial gave values largely higher and variable, from 9 to 250 times, for algal excreta than for glucose. So glucose would not be convenient to estimate bacterial uptake capacities in this aquatic environment.

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