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THE USE OF SIZE FRACTIONATION FOR DETERMINING THE BACTERIAL CONTRIBUTION TO OVERALL PELAGIC OXYGEN CONSUMPTION RATES

H.J. LAANBROEK* and J.C. VERPLANKE

Delta Institute for Hydrobiological Research, Royal Netherlands Academy of Arts and Sciences, YERSEKE, (THE NETHERLANDS),

* Present adress: Institute for Ecological Research, Boterhoeksestraat 22, 6666 GA HETEREN, (THE NETHERLANDS)

ABSTRACT - Size fractionations in combination with precise oxygen measurements, determinations of bacterial cell numbers and chlorophyll *a* analyses were used in the Oosterschelde basin and Lake Grevelingen to establish the bacterial contribution to overall pelagic oxygen consumption rates. On computation, 101 and 59 % of total respiration could be attributed to bacterial activity in winter in the basin and in the lake, respectively. In summer, the bacterial component of total activity was calculated to amount to 54 and 57 % in the basin and in the lake, respectively. Microbial contribution to *in situ* overall activity may slightly be overestimated by growth of the bacteria observed during incubation of the samples in oxygen bottles in the dark at *in situ* temperatures.

Key words : size fractionation, bacterial activity, oxygen consumption.

RÉSUMÉ - Afin de déterminer la contribution des peuplements bactériens dans la consommation d'oxygène totale en milieu pélagique, des études ont été effectuées dans le bassin de l'Oosterschelde et dans le lac de Grevelingen. Après fractionnement en différentes classes de taille, des dosages d'oxygène et de chlorophylle *a* et des dénombrements bactériens ont été réalisés. En hiver, la respiration liée à l'activité bactérienne représente, comparée à la respiration totale, 101 % dans le bassin et 59 % dans le lac. En été, la contribution bactérienne dans l'activité totale correspond à 54 % dans le bassin et 57 % dans le lac. La part des bactéries dans l'activité totale *in situ* peut être légèrement surestimée en raison de la croissance des cellules pendant l'incubation des échantillons. Celle-ci se fait en bouteille à oxygène, à température ambiante et à l'obscurité.

Mots clés : fractionnement en classe de taille, activité bactérienne, consommation d'oxygène.

Bacteria are more and more recognized as major components of the marine pelagic food web (Larsson and Hagström, 1982; Lancelot and Billen, 1984; Williams, 1981a). Determinations of oxygen consumption rates provide a simple and hence widely used method for measuring heterotrophic activity in aquatic ecosystems (Straskrbova, 1979). However, this method is little selective with regard to heterotrophic activity. In order to determine the specific bacterial component of total pelagic activity, Williams (1981b) introduced size fractionation. In a study concerning mineralization in the water bodies of the marine Oosterschelde basin and the saline Lake Grevelingen in the South-Eastern part of the Netherlands, precise oxygen measurements in combination with reverse filtration, has been used. Determinations of bacterial cell numbers and chlorophyll *a* concentrations were also done.

Description of the area and the sampling stations

Samples were taken in the Oosterschelde basin as well in Lake Grevelingen both located

in the south-western part of the Netherlands. Freshwater discharge into the Oosterschelde basin is regulated by sluices at the land-side, while at the sea-side the construction of a storm surge barrier is going on. Lake Grevelingen, is a former basin now surrounded by dikes. However its saline character is maintained by sluices at the sea-side. Sampling stations LGPK and Kr5 are situated in tidal channels in shallow parts of the Oosterschelde basin at mean depths of 19 and 8 m, respectively. At low tides, these stations are surrounded by intertidal mud-flats. Sampling stations Kats and KtA are located in the main tidal channels halfway the basin at mean depths of 34 and 22 m, respectively. Sampling station R14 is situated at the mouth of the basin at a mean depth of 35 m. Sampling station G17 is located in Lake Grevelingen at a depth of 20 m.

METHODS

From October 1983 till October 1984, samples were taken every three weeks at each sampling station from 1.00 m below the surface of the water. Sampling was done independently from the tide, since preliminary experiments did not show a correlation between the tidal period and the amount of oxygen consumed. Reverse filtration, described by Williams (1981b), started immediately after sampling. Only Nuclepore polycarbonate filters with a pore size of $3\text{ }\mu\text{m}$ and a diameter of 142 mm were used. The filters were used once, since multiple utilization led to a manifold increase in the oxygen consumption rates measured. The hydrostatic pressure was always kept below 10 cm water, yet filtration for respiration studies was always finished within 15 minutes. Filtration of larger volumes of samples for chlorophyll *a* analyses took more time. Samples for determination of oxygen consumption rates were incubated in the dark at *in situ* temperatures for 24 and 48 hours in summer and in winter, respectively. Oxygen concentrations were measured according to the method of Bryan *et al.* (1976). The number of free-living as well as attached bacteria and the mean cell volumes were determined by epifluorescence microscopy as described by Hobbie *et al.* (1977). The amount of bacterial cell-carbon was determined by multiplying the measured mean cell-volume by a conversion factor of $121\text{ fgC}\cdot\mu\text{m}^{-3}$ given by Watson *et al.* (1977). Chlorophyll *a* was determined by HPLC.

Bacterial contribution to overall pelagic oxygen consumption rates (B_c) was calculated according to the formula:

$$B_c = \frac{C - A}{B - A}$$

where A, B and C are the percentages of chlorophyll *a*, bacterial cells and oxygen consumption rates that passed a $3\text{ }\mu\text{m}$ polycarbonate filter respectively.

RESULTS AND DISCUSSION

Overall pelagic oxygen consumption rates were measured regularly every three weeks in water samples taken from 1 m below the surface of the water in the marine Oosterschelde basin and the saline lake Grevelingen. Oxygen uptake rates varied between 20 and $1000\text{ }\mu\text{g l}^{-1}\text{d}^{-1}$ in winter and summer, respectively. No significant differences were observed between the data from the basin and the lake (manuscript in preparation). Reversed filtration was applied to elucidate the role of bacteria in overall oxygen consumption rates. The percentages of overall oxygen consumption rates recovered in the $3\text{ }\mu\text{m}$ fractions varied between 10 % and more than 100 % in the individual samples, but the averages were rather constant over the year for both the marine basin and the saline lake studied (Tab 1).

Station	Percentage of oxygen consumption	
	Winter	Summer
LGPK	51 (8)	54 (9)
Kr5	90 (9)	75 (7)
Kats	35 (8)	34 (8)
KtA	46 (9)	65 (7)
R14	40 (7)	46 (8)
Oosterschelde average	54 (41)	54 (39)
G17	66 (9)	63 (7)

Table 1: Oxygen consumption rates in samples that passed a 3 μ m polycarbonate filter. Rates are presented as percentages of unfiltered samples. Number of samples in brackets.

The average percentages of overall oxygen consumption rates are of the same size as measured by Williams (1981b) in coastal waters and by Sepers *et al.* (1982) in the same Lake Grevelingen. However these last authors did not use polycarbonate filters. As already mentioned by Sepers *et al.* (1982), the oxygen consumption rates recovered in filtered water samples represent a minimum estimate of total bacterial activity. The measured activity in these filtered samples does not account for the activity of bacteria attached to particles which cannot pass the polycarbonate filter. For this reason determinations of percentages of total bacterial cell numbers recovered in the 3 μ m fractions were included in this study (Table 2).

Station	Percentage of filtered bacteria	
	Winter	Summer
LGPK	61 (8)	89 (9)
Kr5	53 (9)	90 (7)
Kats	50 (8)	91 (8)
KtA	51 (9)	89 (7)
R14	50 (7)	90 (8)
Oosterschelde average	53 (41)	90 (39)
G17	98 (9)	90 (7)

Table 2: Percentages of bacteria from surface samples that passed a 3 μ m polycarbonate filter. Number of samples in brackets.

Since Tables 2 and 3 contain average values, the sum of the percentage of bacteria that passed the 3 μ m polycarbonate filter (Tab 2) and the percentage of attached bacteria (Tab 3) may not exactly be 100 %. However, in the Oosterschelde basin in the winter period, a more pronounced discrepancy between the percentage of the total number of bacteria retained by the 3 μ m filter and the percentage of the bacteria attached to larger particles is observed. The exact reason for this discrepancy is still unknown, but may partly be found in the fact that the number of attached bacteria is likely to be underestimated since they have been hidden by the particles during the epifluorescence microscopy. The higher the relative number of attached bacteria, the higher the underestimation. The percentage of the bacteria attached to particles was relatively high in the basin compared with the saline lake. The percentages of bacteria attached to larger particles found in Lake Grevelingen are in agreement with observations done in coastal waters (Wiebe and Pomeroy, 1972; Zimmermann, 1978; Hodson *et al.*, 1981). In comparison to this, the

percentages of attached bacteria enumerated in the Oosterschelde basin were high, which might be due to the relatively high amounts of seston in this basin, specially in winter (Tab. 4).

Station	Percentage of filtered bacteria	
	Winter	Summer
LGPK	18 (8)	10 (9)
Kr5	20 (9)	12 (8)
Kats	22 (8)	11 (9)
KtA	23(9)	10 (8)
R14	21 (7)	13 (8)
Oosterschelde average	21 (41)	11 (42)
G17	4 (9)	3 (7)

Table 3 : Percentages of attached bacteria in surface samples of the Oosterschelde basin and Lake Grevelingen. Number of samples in brackets.

Station	Mean amount of seston	
	Winter	Summer
Oosterschelde	21 (40)	11 (42)
Grevelingen	4 (9)	4 (7)

Table 4 : Mean amounts of Seston (mg l⁻¹) in the Oosterschelde basin and in Lake Grevelingen in winter and in summer. Number of samples in brackets.

Station	Percentage of filtered bacteria	
	Winter	Summer
Oosterschelde	11 (41)	13 (32)
Grevelingen	19 (9)	28 (6)

Table 5 : Percentagés of chlorophyll-a from surface samples that passed a 3 µm polycarbonate filter. Number of samples in brackets.

Station	Percentage of filtered bacteria oxygen consumption rates			
	Winter		Summer	
	A	B	A	B
Oosterschelde	54	101	54	54
Grevelingen	66	59	63	37

Table 6 : Bacterial oxygen consumption rates in surface samples of the Oosterschelde basin and Lake Grevelingen presented as percentages of total oxygen consumption rates, A values not corrected for the presence of attached bacteria and small algae, B values corrected for the presence of attached bacteria and small algae.

A correlation between the percentages of attached bacteria and the amounts of suspended particles was also observed in the Humber estuary (Goulder, 1977; Bent and Goulder, 1981). The relatively low amounts of seston in the Oosterschelde basin in comparison to estuaries might be explained by the low discharge of freshwater into the basin. The higher percentages found in the winter period might reflect a larger freshwater discharge as well as more rough weather conditions at this time of the year.

The relatively high percentages of attached bacteria both in the Oosterschelde basin as well as in Lake Grevelingen during the winter period may also reflect poor growth conditions in the water. For example, carbon limitation may promote adhesion of bacterial cells to particles (Brown *et al.*, 1977).

The presence of active small phytoplankton cells in the bacterial size fraction may overestimate the bacterial contribution to overall pelagic oxygen consumption rates calculated from size fractionation studies. For this reason analysis of chlorophyll *a* was included in the studies. The percentages of the total amounts of chlorophyll *a* recovered in the 3 μm fraction are summarised in Table 5.

Relatively high amounts of chlorophyll *a* passed the polycarbonate filter in water samples of Lake Grevelingen during the summer period. At least a part of the chlorophyll-*a* in the small, bacterial fraction belonged to active phytoplankton cells, since phytoplankton production was observed in this fraction (de Visscher, unpublished results). The percentages of chlorophyll-*a* and bacterial cells that passed a 3 μm polycarbonate filter were used to calculate the bacterial contribution to overall oxygen consumption rates (tab 6). Respiration of other organisms such as zooplankton was neglected, since their biomasses are small compared to those of bacteria and algae in both water bodies (C. Bakker, pers. comm.). Since oxygen consumption rates were determined in five-fold in bottles, a possible inclusion of zooplankton into these bottles could be observed and excluded from calculations. The oxygen consumption rate per organism was assumed to be independent upon free-living of attached, or upon size class.

Hence, without taking into account the activity of attached bacteria and the small algae, the bacterial contribution to overall, pelagic oxygen consumption rates may be under- or overestimated when using size fractionation alone. The presence of many bacteria attached to particles had a large impact on the calculated bacterial contribution to overall pelagic activity in the Oosterschelde basin in the winter period. In Lake Grevelingen, the measured bacterial activity was slightly overestimated by the presence of phytoplankton cells in the small, bacterial fraction. Considering the corrected values for bacterial contribution to overall oxygen consumption rates from Table 6 in more detail, the difference between the marine Oosterschelde basin and the saline Lake Grevelingen during the winter period is most striking. Since the levels of primary production are almost the same in winter in both water bodies (de Visscher, unpublished results), the difference in bacterial contribution to overall oxygen consumption rates could largely be explained by resuspension of organic matter from the bottom by the tide in the open Oosterschelde basin. The calculated bacterial contribution to overall pelagic activity may have been overestimated due to growth of the bacteria in the oxygen bottles during the incubations in the dark at *in situ* temperatures. Changes in bacterial biomass were calculated from microscopic changes in cell numbers and mean cell volumes during these incubations (Table 7).

In Lake Grevelingen, a large difference between unfiltered and filtered samples was never observed with regard to increases of biomass. During the summer period, a large diffe-

	Total sample	3 μm fraction
Oosterschelde winter	- 0.4 (39)	0.5 (39)
summer	2.9 (40)	7.7 (37)
Grevelingen winter	- 0.6 (9)	1.3 (9)
summer	6.1 (7)	5.9 (7)

Table 7 : Changes in bacterial cell-carbon ($\mu\text{g l}^{-1}$) during incubation in oxygen bottles in the dark at *in situ* temperatures. Number of samples in brackets.

rence in increase of biomass between unfiltered and filtered samples was observed in the Oosterschelde basin. The reason for this phenomenon is still unknown. Since almost all bacterial cells passed the polycarbonate filter at this time of the year, a relative increase in available substrates in the filtered samples could be excluded. On the contrary, the presence of larger phytoplankton cells in the unfiltered samples may even stimulate the growth of bacteria in these samples. The absence of grazing zooplankton in the filtered samples may explain the increase in bacterial cell-carbon in filtered samples of the Oosterschelde basin. However, this would mean a difference in grazing pressure between the marine Oosterschelde basin and the saline Lake Grevelingen, since no increase was observed in filtered samples compared to unfiltered samples of the lake in summer (Tab. 7). Since the biomasses of bacteria varied from 5.6 to 25.0 $\mu\text{g C l}^{-1}$ at the time of sampling, the observed growth in the oxygen bottles may have a large impact on calculations of bacterial contribution to overall oxygen consumption rates. However, due to the relatively long incubation times in the dark, it is not possible to estimate the extent of the influence of bacterial growth on these calculations. In winter, the difference between the unfiltered and the filtered samples was less pronounced and the total increases of biomass were not as large as in summer, presumably due to the lower *in situ* temperatures. Mean cell volumes tended to increase during the incubations. This increase was always due to growth of rod-shaped bacteria. An increase in mean cell volume was also observed in seawater cultures by Ammerman *et al.* (1984). The increase was most pronounced in the Oosterschelde basin in the summer period. In this period mean cell volume increased from 0.046 μm to 0.051 μm . Intermediate increases were observed in the basin in winter and in Lake Grevelingen in both seasons.

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