INTRODUCTION

The processes by which algal carbon becomes available for bacterial growth have been identified and assessed in several studies in marine and freshwater environments (Larsson and Hagström, 1979, Mague et al., 1980; Brock and Clynne, 1984; Sondergaard et al., 1985; Baines and Pace, 1991; Sell and Overbeck, 1992; Hygum et al., 1997). Among these processes, phytoplankton exudation of organic molecules of various sizes represents a source of organic carbon readily available for bacteria (Cole et al., 1982). The importance of phytoplankton exudation across marine, estuarine and lacustrine systems was synthesized by Baines and Pace (1991). Their synthesis has shown that excretion is a normal process in algal cells, and that the rate of extracellular release is closely related to particulate production, with an average of 13% of total primary production being released. Recent studies [e.g. Rosenstock and Simon, 2001] investigated the relative importance of different molecular pools (e.g. free amino-acids versus protein), and demonstrated that phytoplankton and mesozooplankton were the main sources of total amino acids taken up by heterotrophic bacteria in Lake Constance. To our knowledge, nothing is known about the importance of phytoplankton exudation and its utilization by heterotrophic bacteria in lowland river systems.

In a previous study on the eutrophic River Meuse, Servais et al. (Servais et al., 2000) demonstrated the potential importance of the microbial loop in the carbon budget of this environment. Based on studies of the main processes involved in the organic carbon production and transfer, it was shown that, in this lowland river system, planktonic primary production represents the major carbon source, which largely exceeds allochthonous sources. For the studied period, bacterial consumption of carbon from phytoplanktonic origin was twice as high as algal carbon assimilation by metazooplankton. Moreover, the existence of a tight coupling between phytoplankton and bacteria development was observed, as in previous studies (Servais, 1989), with a peak of bacterial biomass occurring with a 2 week lag after the phytoplankton spring peak.

In the present study, we conducted an investigation on extracellular release of phytoplankton in the upper middle River Meuse (Belgium) in order to estimate the contribution of this process as a source of dissolved organic matter in the system. Particulate primary production, exudation and reassimilation of exudates by free-living and attached bacteria were estimated in parallel using 14C labelling and selective filtration techniques. The results showed that total phytoplankton exudation (EOCt) represented on average 7% of the particulate primary production. Of this exported organic carbon, 77% was taken up by bacteria, attached bacteria contributed 30% of this uptake. Calculations showed that in this river system, the excretion of organic carbon by algae accounted on average for 22% of the bacterial carbon demand.

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METHOD

Study site
The River Meuse rises in the East of France and flows through Belgium and the Netherlands, where it meets the lower Rhine, forming the Dutch Delta, which opens into the North Sea. The total length of the river is 815 km and its catchment is about 36,000 km², 40% of it in Belgium. Through all its Belgian course the River Meuse has been regulated for navigation, with weirs and locks distributed along its length. The study site, Tailfer, is situated 321 km from the source. At this site, the mean depth is 3.95 m and the mean width is 100 m. The River Meuse has alkaline, nutrient-rich waters. Some variations in the nutrient content occur over an annual cycle, due to inputs from the drainage area (N, Si), from sewage (mostly P) and to uptake by primary producers. However, in this stretch of the river, nutrients are not usually depleted to levels where they may be limiting for phytoplankton growth (Descy et al., 1987). Water samples were collected from the surface in the middle of the river with a 3 l opaque Van Dorn bottle. Measurements of activities (particulate primary production, exudation and bacterial re assimilation) were performed on the same river water samples on 15 occasions between April and October 2000, while measurements of phytoplankton and bacteria biomass were made more frequently between March and October 2000.

Phytoplankton and bacteria biomass
Phytoplankton biomass was estimated through chlorophyll a (Chl a) measurements by high-performance liquid chromatography (Descy and Méten, 1996) and converted into carbon biomass using a C : Chl a ratio of 37 previously estimated for the River Meuse phytoplankton (Descy and Gouschka, 1994). Bacterial abundance was determined using epifluorescence microscopy at 1000× magnification, following the procedure proposed by Butler and Feig (Butler and Feig, 1980). After fixation with buffered formalin (2.5% final concentration) and DAPI (4,6 diamidino-2-phenylindole, final concentration 1 µg ml⁻¹), staining, at least 500 cells were counted in each sample. During microscopic observation bacteria were classified among 24 size classes by comparison with an eye-piece graticule and cell volume (V : µm³ cell⁻¹) in each class was calculated. Biomass was estimated from abundance and biovolume distribution using the relationship between carbon content per cell (C : fg C cell⁻¹) to biovolume (C = 92 × V⁰.598) which was determined from Simon and Azam’s data (Simon and Azam, 1985).

Particulate primary production, exudation and re assimilation by free-living and attached bacteria
Exudation of dissolved organic carbon (DOC) from phytoplankton and its utilization by bacteria were measured by ¹⁴C laboratory incubations followed by selective filtration, according to a protocol derived from Larsson and Hagström (Larsson and Hagström, 1979). A 300 ml sample of river water was inoculated with 250 µCi of NaH¹⁴CO₃ (Amersham CFA3, 50–60 mCi mmol⁻¹) and incubated for up to 6 h at in situ temperature under saturating light intensity (600 µE m⁻² s⁻¹). Ten milliliter aliquots were sampled at regular intervals for following labelling kinetics. The 10 ml aliquots were filtered on 2 µm pore-size filters (polycarbonate filter, Millipore Corp.); the resulting filtrate was then directly filtered on a 0.2 µm pore-size membrane (nitrile cellulose filter, Sartorius). Filtration was performed with vacuum lower than 50 mmHg. The filters were rinsed and acidified by addition of 250 µl of 2 M HCl to remove inorganic carbon. Radioactivity associated with the filters was estimated by liquid scintillation. The radioactivity associated to the 2 µm pore-size filter allowed calculation of particulate primary production (PP). Net uptake of algal ¹⁴C-exudates by free-living bacteria was measured as the radioactivity retained on the 0.2 µm filter (BRs). Net uptake of algal ¹⁴C-exudates by attached bacteria (radioactivity of the bacteria retained on the 2 µm filter (BRf)) was deduced from the selective filtration of [³H]thymidine-labelled samples (see below). The total amount of excreted organic carbon taken up by bacteria (EOCs) was calculated from net bacterial uptake of exudates assuming a bacterial growth yield of 0.3 (Servais, 1989). The 0.2 µm filtrate was acidified and bubbled with air to remove inorganic carbon; the residual radioactivity corresponded to the dissolved excreted organic ¹⁴C-carbon (EOCe) non-assimilated by bacteria. The total exudation (EOCt) rate was then calculated as the sum of EOCe and EOCa and the total primary production (TPP) as the sum of PP and EOCa; the percentage of extracellular release (PER) was calculated as: (EOCt/TPP) × 100.

Tritiated thymidine incorporation (Furhman and Azam, 1982) was used to estimate the contribution of attached bacteria (retained on a 2 µm filter) to total bacterial activity. Indeed, preliminary measurements of total bacterial activity had shown that a significant and variable part of thymidine incorporation in the River Meuse occurred in the particulate fraction >2 µm. This presumably resulted for thymidine uptake by heterotrophic bacteria attached to seston, composed of variable amounts of phytoplankton and of detritus. The experimental determination of the proportion of attached versus
free-living bacteria comprised incubations of three replicates of river water (10 ml) with [3H]thymidine (Amer sham, 37 Ci mmol⁻¹) for about 1 h in the dark at field temperature. After incubation, samples were fractionated on 2 µm and on a 0.2 µm pore-size membranes as for ¹⁴C incubations and the filters were rinsed with cold trichloroacetic acid (final concentration 5%). Radioactivity associated with the filters was measured by liquid scintillation and was used to assess the contribution of both bacterial fractions to bacterial production, considering that radioactivity of the 0.2 µm pore-size filters resulted from thymidine uptake by free-living bacteria, whereas that of the 2 µm pore-size filters resulted from thymidine uptake by attached bacteria. EOC uptake by both categories of bacteria was assumed to be proportional to the rate of thymidine incorporation.

**RESULTS AND DISCUSSION**

**Time course of organic carbon release by phytoplankton during ¹⁴C incubation experiments**

In preliminary experiments, we observed that radioactivity increase in the final filtrate through 0.2 µm pore-size filters (EOCs) became maximal after 3 h and remained linear for up to 6 h after the start of the incubation (Figure 1). These kinetics were attributed to exudation of organic compounds by phytoplankton taking place between 2 and 3 h after the start of incubation; DOC release seemed to remain constant after that, while uptake by heterotrophic bacteria occurred at a constant rate. The delay between the start of labelled inorganic carbon uptake and the release of products from photosynthesis may be explained by the time necessary for the algal cells to replenish their carbohydrate pool.

Subsequent experiments were run for 5 h, and samples were taken every half hour, a linear increase of radioactivity in the final filtrate was always observed between 3 and 5 h of incubation, so that the rate of exudation could be determined from the slope of the regression plot dpm versus time.

**Estimates of total EOC and phytoplankton carbon utilized by heterotrophic bacteria**

Results of our exudation experiments are shown in Figure 2. Between 61% and 91% (on average 77.2%) of the total EOC was taken up by heterotrophic bacteria. Hence, the labelled DOC production, measured by the radioactivity recovered in the filtrate after acidification and sparging, was a rather small fraction of the total extracellular release of the algal cells (on average 22.8%). During our experiments under saturating light intensity, the percentage of extracellular release (PER), defined as the ratio of total EOC to total primary production, represented, on average, 10.2% but was usually lower than 10%, the mean being influenced by one unusually large value at the end of the growing season. At this moment, phytoplankton biomass and particulate production were low, and may have resulted in increased measurement errors. On the other hand, the phytoplankton was in a decrease phase, and may have comprised more senescent cells releasing larger amounts of material which sustained the heterotrophic activity. This may be indicated by the high proportion of Chl a degradation products, which amounted to >40% of total a phaeophorbides a (data not shown).

Bacteria retained by the 2 µm pore-size filter contributed for a variable but significant part of bacterial activity: this part was on average 30.2% of total heterotrophic activity. This may be indicated by the high proportion of Chl a degradation products, which amounted to >40% of total a phaeophorbides a (data not shown).

**Relation between total EOC and particulate production**

Correlations between EOCs, PER and different variables were investigated. Significant linear correlation (Pearson’s correlation coefficient, as calculated with the Microsoft
Excel software), were found between total EOC and particulate primary production (EOCt = 0.07 PP, r = 0.89, n = 15), as well as with Chl a (EOCt = 0.40 Chl a, r = 0.82, n = 15) (Figure 3). Other relations were not significant or could be explained via cross-correlations with primary production and Chl a. On the other hand, PER did not correlate with other variables, but was found to decrease with increasing particulate production and biomass (Figure 3). Given the very good correlation between EOCt and particulate production, and the actual dependence of DOC exudation upon photosynthetic production, the regression equation could be used to predict phytoplankton exudation (see below). Thus EOC appears as a link between phytoplankton production and bacterial production, which can at least partly explain the strong coupling between algal biomass and bacteria biomass in the River Meuse, found in previous studies (Servais, 1989; Servais et al., 2000) and observed again in this study (Figure 4). Figure 4 shows that bacterial biomass closely followed phytoplankton biomass during spring development, with biomass peaks appearing with a time lag of about 2 weeks.

**Contribution of EOC to bacterial carbon demand**

As reliable data on heterotrophic bacterial production were not available for the River Meuse in 2000, we used data from an earlier study performed in the River Meuse in 1996 (Servais et al., 2000) to evaluate the extent to which EOC may constitute a significant fraction of the total demand of organic carbon by heterotrophic bacteria (bacterial carbon demand, BCD). In the 1996 study, particulate primary production was estimated for 11 sampling dates [for complete experimental procedure, see (Servais et al., 2000)]. On the basis of the data of daily particulate production (DPP), daily EOCt (DEOCt) was calculated by the following relationship: DEOCt = 0.07/DPP considering that a constant fraction (0.07) of the primary production was excreted. This calculation assumed that the percentage of excreted organic carbon did not vary with light intensity as mentioned by Petit et al. (Petit et al., 1999) so that the PER determined at saturating light intensity (Figure 3) can be applied to the daily primary production. For the same dates, BCD was estimated on the basis of the bacterial production measurements estimated from thymidine and leucine
incorporation rates [for complete experimental procedure, see Servais et al. (2000)] considering a bacterial growth yield of 0.3 (Servais, 1989). BCD and DEOCt are compared in Figure 5. Excretion of organic carbon by algae accounted for 7 to 49% (on average 22%) of the bacterial carbon requirement. The fact that on average only one-fifth of the carbon utilized by bacteria came from phytoplankton excretion indicated that this process alone cannot explain the coupling between phytoplankton and bacteria in the River Meuse.

Conclusions

As in other studies on exudation of DOM by phytoplankton, our experiments demonstrated that short-term reassimilation by bacteria must be accounted for when measuring total exudation flux. However, obtaining correct measurements depends on the experimental procedure, particularly on the care with which filtrations are carried out, as breakage of algal cells results in apparent DOM release and reassimilation. In our case, the fact that DOM release increased through time suggests that we were measuring a process related to increasing 14C uptake by phytoplankton during the course of the experiments (see Figure 1).

Correspondingly, the rate of EOCt by the River Meuse phytoplankton was correlated with the rate of light-saturated photosynthesis and phytoplankton biomass. The strong correlation with algal production may also have resulted from the highly homogeneous composition of the River Meuse phytoplankton, which was dominated by diatoms all over the study period. Green algae, which have the ability to photorespire (Reynolds et al., 1985), i.e. to oxidize photosynthetic intermediates back to CO2, represented on average only 11.4% of phytoplankton biomass, barely exceeding 22% in one series. PER appeared not to be related to any variable; it seemed to increase in the lower range of biomass and production, but this might be explained by DOM release from lysis of senescent or dying phytoplankton cells in the low biomass samples (as indicated by the higher percentage of Chl a degradation products in those samples). Therefore, as in studies in other aquatic systems, phytoplankton exudation appears as a process which occurs in normal algal cells and which is related to photosynthetic production rather than to algal abundance (Maupé et al., 1988; Baines and Pace, 1991).

A question raised by many authors is whether dissolved organic carbon released in the excretion process can meet carbon demand of heterotrophic bacteria (BCD). Most estimates from a variety of aquatic ecosystems pointed to significant EOC, but insufficient to fuel bacterial production (Brock and Clynne, 1984; Søndergaard et al., 1985; Chrzaniowski and Huberard, 1982; Baines and Pace, 1991; Pirt et al., 1999) whereas others have found EOC exceeding BCD, at least in some instances (Sundh and Bell, 1992). In a eutrophic lowland river, such as the River Meuse, bacterial production is high (Servais, 1989), and despite significant inputs of degradable organic matter (Servais et al., 2000), is clearly coupled to phytoplankton production (Servais, 1989; this paper). This is hardly surprising, given the level of phytoplankton production in this lowland river, which reaches, in the studied stretch, the range reported for eutrophic freshwater ecosystems (ca. 600 g C m–2 year–1, Descy et al., 1985, i.e. to oxidize photosynthetic products for growth of epilimnetic bacteria. Appl. Environ. Microbiol., 36, 1078-1090).


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REFERENCES


