

Similar bacterial growth on dissolved organic matter in anoxic and oxic lake water

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ABSTRACT: Anoxic metabolism yields less energy per unit substrate utilized than oxic respiration. In addition, substrate availability is believed to be reduced under anoxic conditions since oxygenases cannot be used. Consequently, it is generally assumed that bacteria grow slower in anoxic environments than in oxic environments. The results of the present study challenge this view. We compared the growth of bacterial assemblages under carbon-limited conditions in lake water under anoxic and oxic conditions. Bioassay experiments were performed with water from 3 lakes differing in nutrient concentrations and organic matter content. Among bacteria using the same source of organic matter, median anoxic growth rates were 84 to 110% of oxic growth rates. The total biomass yield during the experiments did not differ between anoxic and oxic treatments. We suggest that anoxic bacterial growth was regulated by substrate availability rather than by metabolic energy yield and that availability of organic matter under anoxic conditions was equal to or even greater than that in oxic treatments. This implies that anoxic decomposition rates may actually have been faster than oxic rates.

KEY WORDS: DOC · DOM · Anaerobic degradation · Bacteria

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INTRODUCTION

Bacterial growth and organic matter degradation is considered to be highly dependent on the presence of molecular oxygen (Schink 1988, Fenchel & Finlay 1995). Anoxic conditions are common in many water-logged ecosystems, such as poorly mixed water columns, sediments, and soils (Fenchel & Finlay 1995), and anoxic metabolism drives many biogeochemically important processes such as denitrification, sulfate reduction (sulfide formation) and methanogenesis. Further, anoxic metabolism may account for a substantial part (20 to 60%) of the carbon metabolism and the heterotrophic microbial production within lakes and wetlands (Hessen & Nygaard 1992, Mattson & Likens 1993, Boon & Mitchell 1995, Hamilton et al. 1995, Utsumi et al. 1998).

Anoxic conditions and organic matter accumulation tend to co-occur, and it is often argued that anoxic

decomposition is intrinsically slower and less extensive than oxic decomposition (Emerson & Hedges 1988, Schink 1988, Simon et al. 1994, Harvey et al. 1995, Andersen 1996, Nguyen & Harvey 1997, Sun et al. 1997, Hedges et al. 1999). Anoxic conditions are considered to significantly slow down rates of degradation and bacterial growth for at least 2 reasons:

(1) Anoxic metabolism yields less energy than oxic respiration per unit substrate (Zehnder & Stumm 1988, Fenchel & Finlay 1995).

(2) Absence of O₂ implies that the microbial availability of organic matter should be reduced. Many powerful enzymatic processes, such as those catalyzed by oxygenases, rely on the use of O₂ as a reactant in the primary attack of substrate molecules; hence, the array of possible initial degradation steps is restricted in anoxic environments (Zehnder & Colberg 1986, Schink 1988).

Interestingly, some studies addressing bacterial assemblages in their natural environment seem to contradict the findings of slower anoxic degradation rates. Several studies on marine environments suggest that

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rate differences between anoxic and oxic decomposition are small (Henrichs & Reeburgh 1987, Kristensen & Blackburn 1987, Pedersen & Calvert 1990, Lee 1992, Sun et al. 1993). *In situ* measurements indicate that bacterial production in anoxic freshwater may be as high as, or even higher than in oxic water (Lovell & Konopka 1985, McDonough et al. 1986, Pedrós-Alió et al. 1993, Cole & Pace 1995, Ochs et al. 1995, Tranvik 1998). Apparently, evidence for retarded decomposition of organic matter in anoxic environments is inconclusive. Considering metabolic energy yield and substrate availability, anoxic rates of decomposition and bacterial growth should be slower than oxic rates. On the other hand, studies on natural organic matter and natural microbial communities give a more complex picture, frequently suggesting little or no difference between anoxic and oxic rates.

Previous comparisons of bacterial degradation of organic matter in anoxic and oxic habitats typically focused on specific organic compounds or studied different organic matter under the different oxygen regimes. Much of the organic matter in anoxic environments has been subject to degradation for longer periods of time than organic matter in oxic environments, generally including exposure to oxic degradation before reaching the anoxic environment. Hence, such studies do not provide information about the inherent potential of bacteria under varying O_2 regimes. Similarly to many studies on the degradation of organic matter, estimates of *in situ* bacterial production in anoxic and oxic freshwater environments (reviewed by Tranvik 1998) are not directly comparable, since anoxic bottom waters are potentially richer in nutrients and organic matter than surface waters (Cole & Pace 1995).

To test the hypothesis that organic substrate availability (i.e. the amount of the organic matter that can be metabolized) is more important to heterotrophic bacterial growth rates than the oxygen regime, we experimentally compared anoxic and oxic growth potentials of bacterial communities in lake water under carbon-limited conditions. To our knowledge, no such direct comparison of anoxic and oxic bacterial growth in freshwater on identical natural organic matter has been performed previously.

MATERIALS AND METHODS

Sampling and experimental design. Bacterial biomass was monitored over time in batch-culture experiments with water and water-column bacteria from different lakes (Table 1). Water was collected during summer stratification from 3 small lakes of differing character, Illersjön (ILLER), Mårn (MÅRN) and Lillsjön (LILL) (Table 1). Hypolimnetic water in all lakes was anoxic (measured *in situ* with an Orion Model 835 oxygen electrode). Experiments in 1999 were conducted in all lakes using composite samples from 7 depths ranging from 0.5 m below the surface to 0.5 m above the sediment. During 1998, separate experiments were carried out using epilimnetic water and hypolimnetic water from one of the lakes, Illersjön. Lake water was pumped from the different depths into 10 l acid-rinsed polyethylene carboys with a submersible pump (Amazon 10, Awimex International). The filled carboys were then kept dark and cold until further processing in the laboratory. Throughout, experiment designations are based on the origin of the water (lake) as follows: ILLER, MÅRN, LILL, ILLER-EPI, and ILLER-HYP,

Table 1. Characteristics of the lake water used in experiments. All lakes were stratified and had anoxic bottom water. Tot-P, Tot-N, DIC, DOC and A_{420} denote total phosphorous, total nitrogen, dissolved inorganic carbon, dissolved organic carbon and absorbance at 420 nm, respectively. Values are averages of sampled depths

Expt	Water source (Lake)	Location	Sampling date	Sampling depth (m)	Tot-P ($\mu\text{g l}^{-1}$)	Tot-N (mg l^{-1})	DIC (mg l^{-1})	DOC (mg l^{-1})	A_{420}
ILLER	Illersjön	64° 94' 96" N 14° 52' 66" W	July 15, 1999	1–11.5	38	1.48	24.3	9.4	0.022
MÅRN	Mårn	64° 95' 96" N 15° 04' 15" W	July 28, 1999	0.5–13.5	52	1.71	9.3	17.9	0.057
LILL	Lillsjön	65° 04' 01" N 15° 19' 83" W	August 25, 1999	0.5–7.5	15	0.82	2.1	19.8	0.086
ILLER-EPI	Illersjön epilimnion	64° 94' 96" N 14° 52' 66" W	July 16, 1998	0.5	32	0.94	11.5 ^a	8.4 ^a	0.022
ILLER-HYP	Illersjön hypolimnion	64° 94' 96" N 14° 52' 66" W	July 16, 1998	8	35	1.23	15.6 ^a	6.6 ^a	0.022

^aSamples from experimental cultures and not from the lake directly

where EPI and HYP denote epilimnetic and hypolimnetic water, respectively. In each experiment there were 5 replicate cultures of each of 2 treatments—the absence or presence of O_2 . Expts ILLER-EPI and ILLER-HYP were begun upon arrival at the laboratory whereas the water was aged for 2 to 3 mo in the dark at 2°C before the start of the other 3 experiments.

Experimental setup. To prepare for each experiment, the water was mixed and transferred from the carboys to a 10 l stainless steel vessel. The water was then spiked with phosphate (Na_2HPO_4) and ammonium (NH_4Cl) to make the organic carbon the limiting resource (additions gave a minimal final concentration of $350 \mu g P l^{-1}$ and $1.5 mg N l^{-1}$, respectively). This was followed by purging with N_2 (>99.9992% N_2 , <1 ppm O_2 , Air Liquide, Gas AB, Sweden) for 1 to 4 h. O_2 measurements with the oxygen electrode showed that the purging reduced O_2 concentrations in the vessel to $<0.005 mg l^{-1}$ within 1 h. Most of the water was then pressure-filtered (Gelman Supor 0.2 μm , 47 mm) into a 10 l glass bottle preflushed with N_2 , by applying overpressure with N_2 on the stainless steel vessel. A small proportion of the water was filtered through a glass-fiber filter (Gelman A/E for Expts ILLER, MÅRN and LILL, and Whatman GF/C for Expts ILLER-EPI and ILLER-HYP) according to the same procedure to yield an inoculum without bacterivores. All work after the purging of the water was carried out under N_2 atmosphere. The inoculum was then added to the 0.2 μm -filtered water in a proportion of 10% (1998) or 20% (1999). In the 1999 experiments 250 ml of water was transferred to 330 ml infusion bottles (Laboratory Service Provider BV, Beverwijk, The Netherlands) and in the 1998 experiments 900 ml of water was added to 1125 ml bottles. The bottles were then capped with 17 mm thick butyl rubber stoppers secured with aluminum caps. The stoppers were thoroughly cleaned by washing in both ethanol and detergents before use. Tests also confirmed that these stoppers remained gas tight after being pierced more than 100 times with syringe needles (Microlance 0.6 × 25 mm, Becton Dickinson, Franklin Lakes, NJ, USA) simulating samplings.

In a second oxygen removal step, the capped bottles were attached to a gas-exchanging device with which the bottles were evacuated until vigorous bubble formation occurred upon tapping the bottles with a plastic rod. Evacuation was followed by N_2 addition to about 2 atm pressure. This evacuation- N_2 -addition cycle was repeated at least 9 times. This procedure removed oxygen to below detection levels of both Winkler titration and the O_2 electrode, with little disturbance of the organic matter and bacteria compared to boiling at 100°C and addition of reducing agents such as sulfide. Five of the 10 bottles were then reoxygenated through subsequent evacuation and addition

of air while shaking the bottles. The air was added through a 0.2 μm filter initially using the underpressure created by evacuation, and then with a 50 ml syringe to apply overpressure. The evacuation-aeration procedure was repeated 4 times. Finally, the pressure in each bottle was adjusted to ambient air pressure by letting the overpressure out through a syringe needle, and the bottles were incubated in the dark at a temperature of 15°C (1998) or 12°C (1999). Temperatures were chosen to be intermediate compared to the natural temperatures at different depths in the lakes, ranging from 5 to 21°C. Bacterial samples (5 ml) were taken twice daily (Expts ILLER, MÅRN and LILL) or daily (Expts ILLER-EPI and ILLER-HYP). N_2 or air corresponding to withdrawn volumes was added through a disposable filter (0.2 μm) to maintain the pressure in the bottles. Prior to sampling of anoxic cultures, all syringes used were flushed with N_2 at least 3 times. Bacterial samples were preserved with borax-buffered sterile-filtered formaldehyde (final conc. 5%).

Dissolved oxygen. To determine whether sampling procedures had contaminated the cultures with O_2 , modified Winkler titrations (Carpenter 1965, European Committee for Standardization 1983) were carried out after the experiments. From each bottle, 50 ml (1998) or 40 ml (1999) of water was withdrawn using a 60 ml plastic syringe with a luer-lock-connected needle. The syringe had been flushed with N_2 at least 3 times prior to sampling in anoxic bottles. After sampling, the needle was removed and the Winkler reagents were added directly into the 60 ml syringe using 1 ml syringes with needles. After covering the small opening of the 60 ml syringe with the thumb (wearing a latex glove) the syringe was gently shaken to mix the reagents. After precipitation, concentrated H_3PO_4 was added and the syringe was shaken as described above. The sample was then transferred gently to a 100 ml beaker and colorimetric titration was carried out using an automatic burette (ABU 80 AUTOBURETTE, Radiometer Copenhagen, precision 0.001 ml).

Nitrate. Samples for measuring nitrate (NO_3^-) concentration were taken at the start of each experiment from the water remaining after transfer to infusion bottles. Samples were preserved by freezing and analyses were carried out by the method of Wood et al. (1967) on an autoanalyzer.

Bacterial growth. To monitor bacterial abundance during the experiments and decide their duration, bacteria in samples from 1 to 2 replicates of each treatment was counted daily by epifluorescence microscopy of DAPI-stained cells (Porter & Feig 1980). When the stationary phase had been reached the experiments were terminated. When the experiments ended, bacterial abundance was determined in all samples using flow cytometry (Becton Dickinson FACSCalibur, CellQuest 3.1

software) and staining with Syto13 as described by del Giorgio et al. (1996). For each culture, the natural logarithm of bacterial abundance was plotted against time. The slope of this curve when $\ln(\text{abundance})$ increased linearly with time (i.e. during the exponential growth phase) yielded the maximal intrinsic growth rate, k (McManus 1993). At least 3 data points in the curve were used to determine k . To estimate bacterial biomass carbon at the beginning of the experiment, during exponential growth, and in the stationary phase, volumes (V) of 60 to 400 cells per replicate bottle and sampling time were determined using image-analyzed fluorescence microscopy as described by Bertilsson et al. (1999), followed by conversion to bacterial dry weight (m_b), using the equation $m_b = 435 \times V^{0.86}$ (Loferer-Kröbächer et al. 1998). The dry weight was then transformed to bacterial biomass carbon assuming that carbon comprised 50% of bacterial dry weight. Total bacterial carbon biomass was thereafter derived from flow cytometric bacterial counts times the average carbon content per bacterial cell as determined by image analysis. The final biomass minus initial biomass yielded the increase in bacterial carbon biomass during experiments.

Statistical analysis. Statistical comparisons of intrinsic growth rates and biomass increase in all experiments simultaneously were made by 2-way ANOVA, with the O_2 regime and the origin of water as the independent factors. Intrinsic growth rates in anoxic and oxic treatments were also compared within experiments using an unpaired t -test. In order to equalize the variance, data were log-transformed. All statistical analyses were performed using the software SPSS 8.0 for Windows.

RESULTS

Dissolved oxygen

At the end of the experiments, O_2 concentrations were below the detection limit of the Winkler titration in all bottles that were set up to be anoxic. This indicates that invasion of oxygen was avoided in anoxic treatments. The O_2 level in the oxic treatments after the experiments was $11.1 \pm 0.4 \text{ mg } O_2 \text{ l}^{-1}$ (mean ± 1 SD) in Expts ILLER, MÅRN and LILL, and $5.4 \pm 0.2 \text{ mg } O_2 \text{ l}^{-1}$ in Expts ILLER-EPI and ILLER-HYP. In the latter 2 experiments withdrawn sample volumes were consistently replaced with N_2 , resulting in dilution of the O_2 in oxic treatments. In the oxic treatments, the oxygen level never decreased below 50% of the atmospheric saturation.

Nitrate

Initial nitrate concentrations ranged from 0.06 to 0.76 mg l^{-1} , with the lowest value in Expt LILL and the highest value in Expt ILLER. In Expts MÅRN, ILLER-EPI and ILLER-HYP nitrate concentrations were 0.60, 0.26 and 0.44 mg l^{-1} , respectively.

Bacterial growth

Exponential bacterial growth was reached before the fifth day in all cultures (Fig. 1). In all cultures, the average cell volume of bacteria increased from the start of the experiments until the stationary phase by 3 to 100% (average 57%). The bacterial biomass increased by a factor of 2.3 to 230, with an average 25-fold increase during the experiments. In general, growth levelled off before the eighth day. Intrinsic growth rates in all experiments are shown in Fig. 2. A comparison of anoxic and oxic intrinsic growth rates within individual experiments reveals that oxic rates

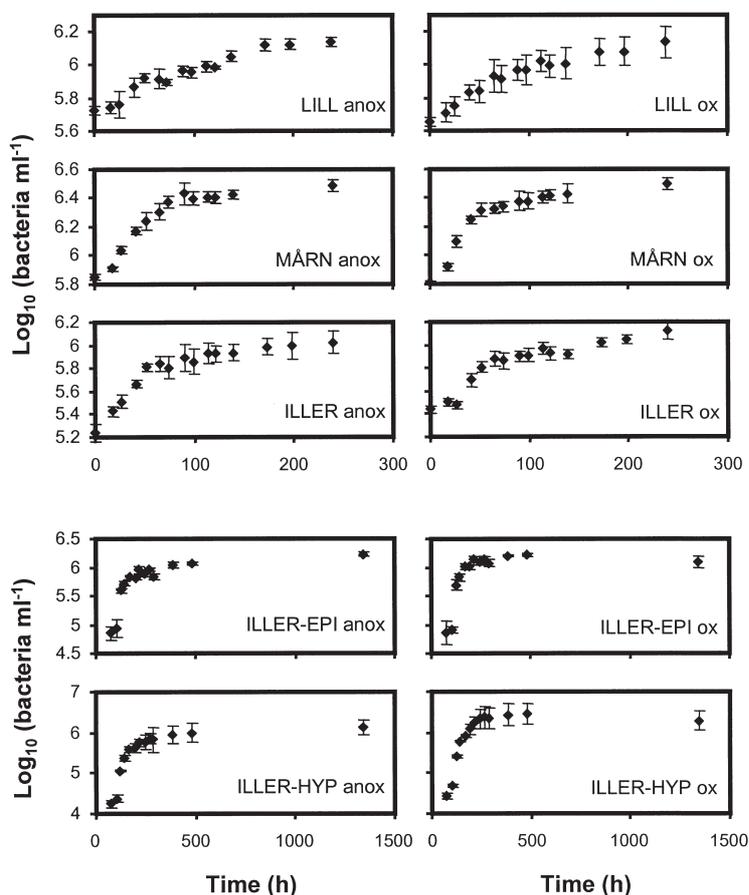


Fig. 1. Bacterial growth curves for the anoxic (anox) and oxic (ox) treatments in all experiments. Abbreviations as in Table 1. Error bars denote ± 1 SD ($n = 5$)

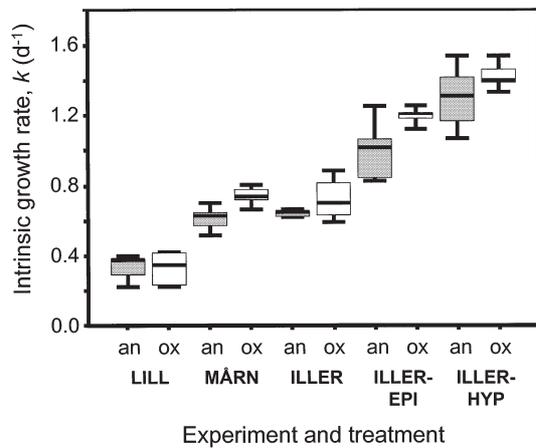


Fig. 2. Intrinsic growth rates (k) for anoxic (an) and oxic (ox) treatments in all experiments. Horizontal lines denote median, boxes denote interquartile range, and bars denote range of all values from a treatment ($n = 5$). Shaded and white boxes represent anoxic and oxic treatments, respectively

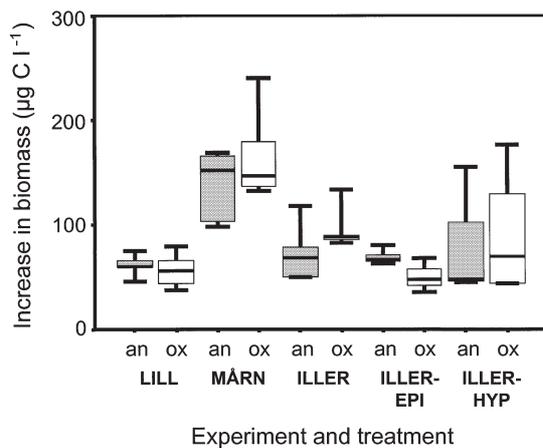


Fig. 3. Increase in bacterial carbon biomass during experiments for anoxic (an) and oxic (ox) treatments (shaded and white boxes, respectively). Horizontal lines denote median, boxes denote interquartile range, and bars denote range of all values from a treatment ($n = 5$)

were faster in Expts MÅRN and ILLER-EPI (t -test, $p = 0.01$ and 0.04 , respectively; $n = 5$). In the other experiments, rates did not differ significantly between anoxic and oxic cultures ($p > 0.05$). Comparison of intrinsic growth rates in all experiments simultaneously indicate that both the O_2 treatment ($p = 0.022$) and the origin of water ($p > 0.001$) affected growth rates (2-way ANOVA, $n = 5$), but there was no interaction between these 2 factors ($p = 0.49$). Differences between anoxic and oxic growth rates were small, and median values of anoxic intrinsic growth rates were within 84 to 110% of oxic rates. Growth was most rapid in Expts ILLER-EPI and ILLER-HYP

(Fig. 2). These experiments were run at 15°C with non-aged water while the other experiments were conducted at 12°C using aged water, in which the concentration of labile organic matter was probably reduced. The increase in bacterial biomass (Fig. 3) did not differ between anoxic and oxic treatments ($p = 0.70$) but was clearly affected by the origin of water ($p < 0.001$, 2-way ANOVA, $n = 5$).

DISCUSSION

The O_2 level was checked using O_2 electrodes during preparation of the cultures, and by Winkler titration of water in infusion bottles prior to and after experiments. Oxygen concentrations in the anoxic bottles were always below the detection limit, regardless of the method used. In addition, we calculated the theoretical amount of O_2 needed to attain the observed biomass yield in the anoxic cultures, using the stoichiometry of oxic respiration of glucose and a bacterial growth efficiency of 26% (median of published values on bacterial growth efficiency in lakes; del Giorgio & Cole 1998). This calculation shows that any O_2 contamination sufficient to support significant bacterial growth in the anoxic cultures would easily have been detected during O_2 measurements, confirming that anoxic conditions were reached and maintained during the experiments.

In this study, we used both the intrinsic growth rates and the biomass yields to investigate the carbon metabolism in anoxic and oxic treatments. Bacterial growth rates and biomass yields are related to carbon mineralization rates through growth efficiency, which represents the fraction of the substrates metabolized that turns into new biomass (growth efficiency = biomass carbon produced/substrate carbon consumed). Given similar organic substrates, a reduced energy yield implies that more substrate is needed just to obtain the basic maintenance energy, and that a reduced fraction of metabolized substrate can be allocated to growth. Thus, a reduced energy yield results in diminished growth efficiency. This should cause decreased growth rate and biomass yield unless more of the substrate can be metabolized.

Anoxic growth rates of bacteria were similar to or only slightly slower than oxic rates (Fig. 2). This is consistent with several studies on carbon degradation or bacterial growth using natural mixtures of organic matter (Foree & McCarty 1970, Westrich & Berner 1984, Pedersen & Calvert 1990, Cole & Pace 1995, Thunell et al. 2000). Presumably, bacterial growth rates in the experimental bottles could have been regulated by metabolic energy yield and by the substrate availability (see above). If the metabolic energy yield

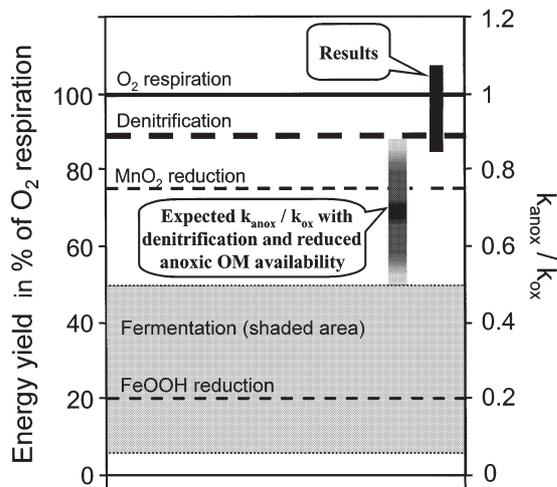


Fig. 4. Theoretical energy yields for different metabolic processes versus bacterial growth rates. Left axis: energy yield of different anoxic metabolic processes expressed as % of oxic respiration; energy yield of denitrification was calculated at ambient concentrations of nitrate ($\Delta G'$); standard conditions were applied in calculations concerning processes other than denitrification (ΔG°); organic matter was represented by CH_2O in all calculations (chemical reactions and data from Zehnder & Stumm 1988). Right axis: ratio between anoxic and oxic intrinsic growth rates ($k_{\text{anox}}/k_{\text{ox}}$), representing expected anoxic growth relative to oxic growth if energy yield alone regulates growth rates. Black bar at top right shows range of $k_{\text{anox}}/k_{\text{ox}}$ in experiments in this study. Shaded bar shows expected $k_{\text{anox}}/k_{\text{ox}}$ when denitrification is the dominating metabolic process in combination with reduced availability of anoxic organic matter

(i.e. the amount of energy gained per unit substrate utilized) was the primary regulator of bacterial growth, denitrification (nitrate reduction) could explain the similarity between anoxic and oxic growth rates in the experiments. Denitrification yields almost the same amount of energy as respiration with O_2 , while other electron acceptors yield significantly less energy (Fig. 4). In addition, nitrate was present in all experiments. However, denitrification follows first-order kinetics relative to nitrate concentrations below 1 mg l^{-1} (Kadlec & Knight 1996), i.e. within the concentration range of this study. That is, the potential for denitrification should have been larger in experiments with high nitrate concentrations such as Expts MÅRN and ILLER (Fig. 5). Accordingly, if metabolic energy yield was the primary regulator of bacterial growth, anoxic growth rates should have been highest compared to oxic rates in these experiments. This was not the case. On the contrary the ratio of anoxic to oxic rates ($k_{\text{an}}/k_{\text{ox}}$) was highest in Expt LILL, which had the lowest nitrate concentration (Fig. 5). Consequently, the metabolic energy yield was probably not a major regulator of intrinsic growth rates.

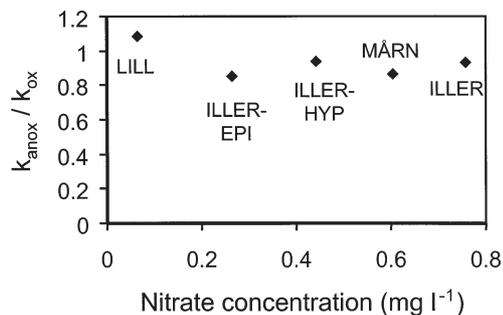


Fig. 5. Ratio of anoxic to oxic intrinsic growth rates ($k_{\text{anox}}/k_{\text{ox}}$) versus nitrate concentrations in experiments of present study

If the energy yield did not control bacterial growth, then substrate availability remains a potentially controlling factor. Inorganic N and P were added in surplus to the cultures, and limitation of bacterial growth by inorganic nutrients other than N or P is unlikely, since bioassay experiments with lake water have demonstrated limitation of only N, P or organic carbon (Elser et al. 1995, Tranvik 1998). If both N and P are added in batch-culture experiments (such as in the experiments of this study), the availability of organic matter should become increasingly important to growth rates with time, assuming that bacteria will preferentially degrade labile fractions initially, leaving recalcitrant organic matter behind. If so, the proportion of recalcitrant organic matter would increase during the experiment and with carbon being the limiting nutrient, additional growth would depend on availability of organic matter. The total fraction of the organic matter available for bacterial utilization would limit the increase in biomass, while the rate at which organic matter could be accessed and metabolized by bacteria (i.e. the substrate availability *per unit time*) would limit growth rates. Hence, both intrinsic growth rates and the increase in bacterial biomass during the experiments were probably limited by the availability of organic matter.

Both anoxic and oxic growth rates reflected lake productivity, being higher in the eutrophic lakes (Mårn and Illersjön) than in the oligotrophic lake (Lillsjön), and there was a greater difference in growth rate among experiments (lakes) than among treatments (O_2 regimes) (Fig. 2, Table 1). In addition, the increase in bacterial biomass was equal in anoxic and oxic treatments. This indicates that the oxygen regime was not a major regulator of substrate availability, and that enzymatic degradation of substrate molecules was as efficient in anoxic water as when oxygen was present. In fact, anoxic growth rates were higher than oxic rates in the water that contained the lowest amount of nitrate and presumably also had the largest proportion of recalcitrant organic matter (Expt LILL). Consequently,

it is possible that the microbial availability of organic matter was actually higher under anoxic than under oxic conditions. If anoxic growth rates are only limited by the metabolic energy efficiency per substrate unit, i.e. assuming equal substrate availability, the ratio between the anoxic and the oxic intrinsic growth rates ($k_{\text{anox}}/k_{\text{ox}}$) is expected to correspond to the energy gain of the metabolic process in use (Fig. 4). If anoxic substrate availability is reduced, anoxic growth rates are expected to be even lower than oxic rates as illustrated by the shaded bar in Fig. 4. Accordingly, if denitrification were the dominating metabolic pathway in the anoxic treatments, the results could be explained by roughly equal organic matter availability under both oxic and anoxic conditions. On the other hand, if metabolic processes other than denitrification were responsible to a significant extent in the anoxic treatments, our results can only be explained by greater organic matter availability under anoxic conditions leading to a more extensive degradation during anoxia than in the presence of O_2 . An estimate based on the median of published lake-water bacterial growth efficiencies (ca 26%, del Giorgio & Cole 1998) indicates that reduction of the nitrate initially present could have accounted for 8, 39, 97, 32 and 60% of the biomass yield in Expts LILL, MÅRN, ILLER, ILLER-EPI, and ILLER-HYP, respectively. Due to lack of data, we can only speculate on the use of other electron acceptors.

The idea of high anoxic organic matter availability compared to availability in presence of O_2 , is consistent with *in situ* measurements of bacterial abundance and growth in freshwater and thermodynamic considerations. Due to thermodynamic constraints, the energy gain per unit substrate is probably reduced in anoxic environments. Nevertheless, microbial biomass and growth rates in anoxic environments are frequently found to be as great as, or greater than in oxic environments (Lovell & Konopka 1985, McDonough et al. 1986, Cole et al. 1993, Pedrós-Alió et al. 1993, Cole & Pace 1995, Ochs et al. 1995). To maintain such biomass or growth levels, and given a reduced growth efficiency, anoxic bacteria would have to degrade more organic matter than bacteria in oxic environments (Howarth & Hobbie 1982). Obviously, specific substrates will be utilized at different rates (Lee 1992), but anoxic conditions *per se* do not necessarily imply that overall bacterial metabolism of complex mixtures of organic matter is hampered by anoxia. These results are consistent with the hypothesis that oxic and anoxic bacteria have a similar potential to degrade most organic compounds, except when steric configuration of substrate compounds only allow small molecules, such as H_2O_2 produced by oxic bacteria, to take part in the initial degradation steps (Hulthe et al. 1998). According to this hypothesis, anoxic degradation of organic

matter tightly adsorbed to particles should be limited simply because of the size of the hydrolytic enzymes.

Accumulation of organic matter tends to be most pronounced in anoxic environments. This may seem incompatible with the idea of extensive anoxic degradation. However, anoxia may just be the consequence of high organic matter loading and subsequent O_2 consumption during degradation. Organic matter accumulation may therefore be a function of the load, with anoxia being merely a secondary effect of extensive microbial metabolism (Henrichs & Reeburgh 1987, Emerson & Hedges 1988, Pedersen & Calvert 1990). Furthermore, anoxic environments are surrounded by oxic environments, where most of the primary production occurs. Therefore, most of the organic matter reaching anoxic environments has previously been subjected to oxic degradation (Henrichs & Reeburgh 1987, Hedges et al. 1999). This would reduce the substrate quality and increase the recalcitrance of the organic matter in anoxic environments. Recalcitrance of the organic matter also increases with its age (Emerson & Hedges 1988). Thus, the age and previous degradation history of organic matter are important regulators of its microbial availability. Accordingly, the oxidation state and the elemental composition of organic matter may be key factors determining potential bacterial metabolism in both anoxic and oxic environments (Vallino et al. 1996, L. Sun et al. 1997, Hunt et al. 2000).

In conclusion, when exposed to identical sources of dissolved organic matter, bacterial assemblages developing in anoxic and oxic batch cultures grew at a similar rate, and attained a similar stationary-phase biomass in the absence of predators and when inorganic nutrients were available in excess. We suggest that the bacterial growth rates were regulated by the rate at which the organic matter could be accessed by the bacteria, e.g. by the enzymatic capacity of bacteria, which was largely independent of oxygen regime. Accordingly, the overall microbial organic matter availability in anoxic environments may be equal to or even greater than that in oxic environments. Differences in bacterial activity between anoxic and oxic environments may thus not be due to different inherent abilities of the bacteria to use the substrates available, but may rather be an effect of differences in factors such as predation, diagenetic stage of the substrate, and supply and concentration of organic and inorganic nutrients.

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