

SEASONAL CHANGES IN THE NUMBER OF SULPHATE-REDUCING
BACTERIA IN THE WATER, SOIL AND PLANT
OF THE WETLANDS NEAR OLSZTYN

STANISŁAW NIEWOLAK¹, RENATA BRZOZOWSKA²,
KAROLINA CZECHOWSKA¹, ZOFIA FILIPKOWSKA¹, EWA KORZENIEWSKA^{1*}

University of Warmia and Mazury in Olsztyn

¹ Department of Environmental Microbiology

² Department of Environment Protection Engineering

ul. R. Prawocheńskiego 1, 10-957 Olsztyn-Kortowo, Poland

*Corresponding author e-mail: ewa.korzeniewska@uwm.edu.pl

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Abstract: Seasonal changes in the numbers of sulphate-reducing bacteria in water, soil, the surface of sedge (*Carex acutiformis* Ehrb.) immersed in water and roots (dead and live) were studied. The study on one of larger wetland near Olsztyn (Masurian Lake District) was carried out in two annual cycles. Sulphate-reducing bacteria in the studied ecosystems occurred sporadically and generally in inappreciable count. Their count did not exceed 20 cells in 1 cm³ of water; in the soil and in different parts of sedge the number of bacteria ranged from several to over dozen thousands cells in 1 g of dry weight. In the first year of studies these bacteria were the most numerous in June, July and during first days of December, but in the second – in April (in soil and sedge immersed in water), in August (in soil and dead and live roots), in November (in water, soil, sedge immersed in water and dead roots) and exceptionally in other months.

INTRODUCTION

The sulphate-reducing bacteria (SRB) play an important role in many anaerobic processes, especially mineralization of organic substance, biodegradation of xenobiotics, mercury methylation, heavy metals removal from contaminated environments or metal reduction by insoluble sulphite precipitation [2, 5, 40, 42, 44]. SRB activity is conditioned by the presence of sulphate (electron acceptor) and easily soluble organic substance (electron donor). In anaerobic ecosystems, equally important are reaction (pH) and temperature. In natural ecosystems, the process of sulphate reduction has been observed in the range of pH 3.0–9.2 [11]. Low-molecule organic compounds utilized by SRB can be generated by the heterotrophic bacteria decomposing organic substance during fermentation of cellulose, pectin and other organic carbon compounds. The quantity and quality of organic substrate, as well as the vulnerability to degradation, are important factors regulating sulphate reduction also in wetlands. Some SRB oxidize these intermediary products of organic substance degradation/fermentation only partially, i.e., to acetate, and prefer simple products of fermentation, such as hydrogen, lactates or ethanol produced at the oxic/

anoxic interface in sediments (*Desulfovibrio*, *Desulfomicrobium*, *Desulfobulbus*), while other – oxidize them completely, i.e., into CO₂ (*Desulfonema*). SRB are very common in most of the anaerobic environments [1, 13–15, 17, 19, 20, 26, 29, 35, 36, 40, 46]. In surface waters (rivers, lakes, ponds), where aerobic conditions prevail, SRB quantities do not exceed several dozen, or less frequently – several hundred cells in 1 cm³ [25, 31]. They are more abundant in lake bottom deposits (up to several thousand cells in 1 g of fresh mass) [12–14, 30, 31]. The highest SRB numbers in the bottom sediments of some eutrophic and mesotrophic lakes have reached 2.0×10^3 – 4.7×10^5 cells in 1 g of fresh mass [25]. SRB occurrence and their role in mineralization of organic substance produced in wetlands have been scarcely studied [1, 9, 11]. Among the reasons for such little interest in this group of bacteria is the low sulphate content in the water (50–450 μM) needed as the acceptor of electrons in the process of organic substrate mineralization in the anaerobic conditions [11]. In such ecosystems nearly 11–14% of organic carbon is oxidized with participation of SRB whereas in salty marshes, where sulphate content is higher, as much as 50% of organic substance produced *in situ* can be degraded through dissimilative sulphate reduction. In such environments, the number of SRB in the bottom deposits can amount to 1.0×10^6 cells in 1 g of fresh mass [25]. Occurrence and seasonal variability of the number of these microorganisms in water, soil and rhizosphere of the plants growing in the wetlands of Mazurskie Lakeland is a missing subject in the reference literature. Therefore the aim of this study was determining the number of sulphate-reducing bacteria in different kinds of biotopes in one of the largest wetlands in the Olsztyn city area.

MATERIALS AND METHODS

Study area

The study covered the Lakeland area in the vicinity of the Stary Dwór forest's lodge. It is located among slopes afforested with pine and spruce in the upper parts and birch in the lower parts and with willow in coastal zone. The surface area of the Lakeland covers approximately 0.2 km², with a channel (6 m in width and 4–5 m in depth) running through in the center (a remnant of an old lake). The entire area is periodically flooded in spring and autumn, whereas in the summer the water count outside the channel zone falls to a dozen or so cm. The flora is dominated by sedges (*Carex*) and rushes (*Juncus*) that form a permanent cover or isolated tufts surrounded by water [32].

Materials

The number of sulphate-reducing bacteria was determined in water of the littoral zone overgrown with dense phytomass and between tufts of plants emerging from water; on plants emerging from water – separately on submerged stem and aerial leaves; in the root system (rhizosphere) – on old (the previous year's) and new (the current year's) roots, as well as in soil.

Collection of samples

Samples of water and tufts of sedge (*Carex acutiformis* Ehrb.) including the soil and root system were taken in 1 month intervals, in the vegetative seasons from March to December of the two following years. Water samples were collected from the depth of 0.3 m directly into sterile glass bottles. Site 1 was located 30 m from a forest tract leading along

the edge of wetland on its eastern side. Sites 2 and 3 were located on the edge of wetland, on the northeastern side, 80 m from a path leading to the Stary Dwór forest's lodge [32]. Under laboratory conditions, the material collected was separated into parts of plant immersed in water (stems), part protruding from water (aerial leaf), previous-year's roots (dead) and current year's roots (live), and soil from the root system. Aseptically weighed 10 g of soil samples and particular fragments of plants were transferred into flask with a 90 cm³ of sterile physiological NaCl solution and shaken in a shaker for 30 min. The obtained suspension of soil and plants and roots was diluted (1:10 ÷ 1:1000) and transferred in the volume of 1 cm³ on respective media appropriate for individual physiological group of bacteria. Simultaneously weighed 10 g samples of soil and particular fragments of plants were dried at 105°C to measure the dry matter.

Microbiological analyses

The number of sulphate-reducing bacteria was determined on Tauson's medium modified by Szturm [37]. The medium contained: 3.5 g calcium lactate, 4.0 g (NH₄)₂SO₄, 0.5 g K₂HPO₄, 1.0 g MgSO₄·7H₂O, 0.5 g CaSO₄·2H₂O, 0.5 g (NH₄)₂Fe(SO₄)₂·6H₂O (Mohr's salt), 1 dm³ distilled water, after neutralization by KOH to pH 7.0 and solidified by Difco agar (18 g agar per 1 dm³ medium). The medium (without Mohr's salt) was autoclaved in 121°C per 10 minutes. Mohr's salt was sterilized with using sterile membrane filters with pores of 0.22 µm in diameter and added to dissolved and cooled to 42°C agars medium before inoculation of samples. BRS inoculated in test tubes with rubber stoppers on Tauson's medium, were incubated in 25°C for 7, 14 and 21 days. The presence of BRS was identified by precipitation of black iron sulphide around colony of bacteria and hydrogen sulphide smell (after stopper removing).

Each measurement was done in three simultaneous repetitions of the same samples. MPN of BRS in 1 cm³ of water or 1 g of plant parts were measured according to the guidelines and read out from McCrady's charts [28].

RESULTS

In the water of the examined wetland SRB have been isolated rarely; the highest number observed in November, the second year of the study, at the sampling site 2 did not exceed 20 cells in 1 cm³. Periodically, they were present in larger numbers on the submerged in water parts of the sedge (to 7.5 x 10³ cells in 1 GWD at site 1 and to 9.0 x 10³ cells in 1 GWD at site 2), in the soil surrounding the root system of the plant (to 2.4 x 10³ cells in 1 GWD at site 1 and to 2.2 x 10³ cells in 1 GWD at site 2), and on the surface of dead roots (to 1.86 x 10³ cells in 1 GWD at site 1 and 11.6 x 10³ cells in 1 GWD at site 2) and live roots (to 645 cells in 1 GWD at site 1 and to 2.1 x 10³ cells in 1 GWD at site 2). On the surface of the emerged (aerial/aerated) parts of the sedge SRB were examined only at site 1. Throughout the study they were observed only twice and their quantity was lower than 15 cells in 1 GWD (Tab. 1).

In the water sampled from sites 1, 2 and 3, in soil and in individual parts of the sedge from sites 1 and 2 the differences in SRB numbers over the same period were less than 1 order of magnitude. In the first year of the study, in the soil surrounding the root system of the sedge, on the submerged in water parts of the plant and on the dead roots, more SRB were found in June, July and December, on the live roots – in July and December. In the

Table 1. The number (MPN·cm⁻³/MPN·GDW⁻¹) of sulfate-reducing bacteria in water, soil and the surface of sedge (*Carex acutiformis* Ehrb.) of natural wetland near Olsztyn in two following years of studies

Date		Water (in 1 cm ³)			Rhizosphere soil (in 1 GDW)		Sedge (in 1 GDW)							
							Surface							
		Submersed stem		Aerial leaf		Dead roots		Living roots						
		Site												
		1	2	3	1	2	1	2	1	2	1	2	1	2
April	First year of studies	–	–	–	0	0	–	–	–	–	0	0	–	–
May		0	0	0	0	0	4	45	–	–	0	0	–	–
June		1.5	0.4	2.5	1985	995	695	5035	–	–	1865	2145	–	–
July		0	0.4	0	445	250	210	635	–	–	245	370	250	330
August		0	0	0	0	0	0	0	–	–	0	0	0	0
September		0	0	0	0	0	0	35	0	–	35	0	0	0
October		0	0	0	0	0	0	0	0	–	0	0	0	0
December		0	0	4.5	1000	2270	7570	9000	0	–	450	11600	390	2170
March	Second year of studies	0	0	0	0	0	0	0	–	0	0	–	–	
April		0	0	0	0	250	1000	8000	0	–	0	0	–	–
May		4.5	0	0	0	0	0	30	10	–	0	85	–	–
June		0	0	0.3	0	0	0	0	0	–	23	20	0	0
July		0	0	0	0	0	0	0	0	–	0	0	0	0
August		2.5	2.5	2.5	2400	1055	0	0	15	–	1033	905	645	555
September		0	0	0	0	0	0	0	0	–	0	0	0	0
October		0	0	0	0	0	0	0	0	–	0	0	0	0
November	0	20	0	112	55	40	80	0	–	0	40	0	0	
Mean		0.4	0.2	0.5	350	285	560	1345	2	–	215	390	160	135
Range		0–4.5	0–20	0–4.5	0–2400	0–2270	0–7570	0–9000	0–15		0–1870	0–11600	0–645	0–2170

– not tested

second year of the study, in the soil surrounding the root system more SRB were counted on the dead and live roots in August whereas on the submerged in the water parts of the plant – in April. In other months of the study, they either did not occur in the examined soil mass and on the individual parts of the sedge, or their number was negligible. The exemption comprised the soil samples taken in April, the second year of the study, containing up to 250 cells in 1 GDW (Tab. 1).

DISCUSSION

Sporadic occurrence and very low numbers of SBR in the water of the examined wetland can be explained by the very good oxygenation during the vegetative period. Low depth of the water on the sampling sites has allowed for free oxygen diffusion from the atmosphere and its release through photosynthesis by the phytoplankton and hydromacrophytes. In the investigated wetland little sulphate content ($36\text{--}37 \text{ mg SO}_4 \cdot \text{dm}^{-3}$ – $380\text{--}390 \text{ }\mu\text{M}$) was probably the key factor in organic substance mineralization by SRB under anaerobic conditions. The data quoted by Feng and Hsieh [11] reveal that sulphate content in the fresh-water wetlands is generally low ($50\text{--}450 \text{ }\mu\text{M}$), unlike the salty marshes and marine sediments, and it also limits the proliferation of SRB. The water reaction (pH 6.0) of the examined wetland may have had no effect on the number of SRB. Although the optimum pH for these microorganisms is $7.0\text{--}7.2$ [35], the majority tolerates a broader range: $3.0\text{--}9.2$ [11]. Seemingly, temperature did not limit the SRB growth either, as they occurred even in November, the second year of the study, reaching a number higher than in the summer. As SRB occurred in the water of the wetland, their number did not vary from that given in the literature for the inland-lake water [12, 25, 31]. SRB occurrence in the water of the examined wetland should be associated with the higher number of these bacteria in the soil surrounding the root system of the sedge, on the submerged in the water parts of the plant and in the rhizosphere.

The higher numbers of SRB observed periodically on the surface of submerged in water parts of the sedge, in the rhizosphere and in the soil surrounding the root system of that plant may have been stimulated by the release to the environment of the soluble organic compounds (ethanol, lactates, presumably also acetates) by roots and root hairs, or the fatty acids and amino acids from the necrosis zone of the dead roots and/or through shedding of the tips of live roots and root hairs [39]. In the ecosystems, like soil and rhizosphere, simple organic carbon compounds (formates, lactates, ethanol, fatty acids, secondary alcohols, isobutyrate, propionate, hydrogen and some other) can be made available to SRB by fermenting bacteria or acetogenic bacteria [43]. Mineralization processes of organic substance by heterotrophic bacteria use up oxygen, therefore, conditions are created for the SRB occurrence. The maximum numbers of SRB determined in the soil and rhizosphere (on the surface of dead and live roots) and on the submerged in water parts of the sedge occurred in parallel with the maximum numbers of heterotrophic bacteria in July, the first year of the study, amounting to a few million up to a billion and more cells in 1 GDW [23]. Assuming that live roots of sedge release much oxygen to the environment which allows for existence of nitrifying bacteria [34] in the rhizosphere and in the soil surrounding the plant's root system (literature data deal with the amounts of oxygen released only by *Phragmites* – the values range from $0.02 \text{ g} \cdot \text{m}^{-2} \cdot 24 \text{ h}^{-1}$ to $5\text{--}12 \text{ g} \cdot \text{m}^{-2} \cdot 24 \text{ h}^{-1}$ depending on the applied measurement technique [3]), niches must be created

for the suitably of low redox potential ($E_h = -150$ to -200 mV) needed for SRB survival [6–8, 10, 41], although they do not reduce sulphate [16, 21, 22]. SRB are strict anaerobes vulnerable to oxygen. The critical concentration of oxygen which still allows for sulphate reduction is $0.1\text{--}1.0$ mg·dm⁻³ [18]. Provided the level is higher than 1 mg O₂·dm⁻³, the reduction will not occur due to increased redox potential and inhibition of SRB. However, if water, sediment or soil contain larger amounts of soluble organic substance SRB become active in the anaerobic micro-niches, although these comprise the elements of an aerobic environment [45]. It is believed that oxygen inactivates or inhibits enzymes or proteins active in the sulphate reduction process [4]. Literature data of the past few decades revealed that most of the known SRB may periodically survive in contact with oxygen; some cause oxygen reduction, however the growth in such conditions is rather slow [42]. SRB can also migrate from the aerobic zone or make aggregates of cells providing for their survival [24].

The individual cases of small SRB amounts detection on the surface of the emerged from water (aerated) parts of the sedge may have been the result of accidental contamination caused by moose, roe deer, or elk using the wetland as drinking reservoir in the near shore area [33]. Some SRB strains living in the rumen of ruminants together with autogenic bacteria can interact with fermentative bacteria decomposing carbohydrates to organic acids (e.g., acetic acid) and molecular hydrogen, oxidized then by SRB [29].

Seasonal changes in SRB numbers in the rhizosphere and in the soil surrounding the root system of the sedge and on the surface of the submerged in water parts of the plant may have been stimulated by the release of photosynthesis products (soluble organic C compounds). The example of card grass *Spartina alterniflora* Loisel, inhabiting salty marshes (Chapman Marsh) in the south-eastern area of New Hampshire, USA, has shown that larger numbers of SRB may occur in the rhizosphere in the first months of growth (May and June) and then drop when the plant starts blooming [19, 27, 38, 39]. These authors share the opinion that as soon as the first phase of the vegetative growth plant “re-mobilizes” the non-structural carbohydrate accumulated in root hairs and transfers the new products of photosynthesis to the fast-growing roots and root hairs. An increase of the store of soluble carbohydrates together with the products of the root tips lysis is the reason for larger leakage of soluble organic carbon compounds from the roots and root hairs. Once the plant enters the phase of the generative (reproductive) growth, organic carbon is transferred to the blooming structures, carbohydrates are immobilized in root hairs, and in consequence, the amount of the released organic carbon compounds in the rhizosphere rapidly decreases. Simultaneously, the number of SRB drops. It is possible that similar phenomena have occurred in the case of the sedge inhabiting the examined wetland in the Olsztyn city area. As in the case of *Spartina alterniflora*, the maximum numbers of SRB occurred in the rhizosphere of the plant during the vegetative growth (in June and July of the first year of the study, and in August of the second year). Next, the SRB number decreased or the bacteria were absent from the examined root mass. The repeated increase of the SRB number in the rhizosphere of the sedge observed in early December of the first year of the study or in late November of the second year may have been caused by the mineralization of the dead parts of the plant occurring with the participation of the heterotrophic bacteria and saprophytic fungi [23, 32] and by the environment’s enrichment in the intermediate products of the decomposition, utilized by SRB.

CONCLUSIONS

1. Sporadic occurrence of small amounts of SRB in the water of the examined wetland can be caused by the low depth (between ten and several dozen cm) on the individual sampling sites which enables constant oxygen diffusion to the atmosphere.
2. The higher SRB numbers observed periodically on the submerged in water parts of the sedge, in the rhizosphere and in the soil surrounding the root system of the plant can be related to the larger amount of the intermediary products of the organic substance decomposition of the dead plant parts or secreted by the plant.
3. The increased number of SRB on the surface of the submerged parts of the sedge, in the rhizosphere and in the soil surrounding the root system of the plant in the summer (in June and July of the first year of the study and in August of the second year) can be related to the release of organic carbon substance by the roots and root hairs, while in the autumn (December of the first year of the study and end of November of the second year) to decomposition of organic remnants by the heterotrophic bacteria and fungi.
4. The differences in the number of SRB in the water samples, in the samples of various parts of the sedge and in the samples of soil surrounding the root system, collected from different sampling sites in the wetland were usually negligible.
5. The single cases of SRB detection on the surface of the emerged from water (aerated) parts of the sedge may be due to accidental contamination caused by ruminants (moose, roe deer, elk) living in the wetland area.

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SEZONOWE ZMIANY LICZEBNOŚCI BAKTERII REDUKUJĄCYCH SIARCZANY W WODZIE,
GLEBIE I ROŚLINNOŚCI ŚRÓDLEŚNYCH MOKRADEŁ W OKOLICY OLSZTYNA

Badano sezonowe zmiany liczebności bakterii redukujących siarczany w wodzie, glebie, zanurzonych w wodzie i wynurzonych z wody (napowietrznych) częściach turzycy błotnej (*Carex acutiformis* Ehrb.) oraz jej korzeniach (obumarłych i żywych). Badania przeprowadzono na jednym z większych mokradeł śródleśnych w okolicy Olsztyna (Pojezierze Mazurskie) w dwóch kolejnych latach badawczych. Bakterie redukujące siarczany występowały sporadycznie w badanych ekosystemach i z reguły w niewielkich ilościach. W wodzie ich liczba nie przekraczała 20 komórek w 1 cm³, w glebie i różnych częściach turzycy błotnej – kilku (wyjątkowo kilkunastu) tysięcy komórek w 1 g suchej masy. W pierwszym roku badań występowały one głównie w czerwcu i lipcu oraz w pierwszych dniach grudnia, w drugim zaś – w kwietniu (w glebie i zanurzonych w wodzie częściach turzycy błotnej), w sierpniu (w glebie i na korzeniach martwych i żywych) oraz w listopadzie (w wodzie, glebie, zanurzonych w wodzie częściach turzycy błotnej oraz korzeniach martwych), wyjątkowo w innych miesiącach.