ISSN 2449-8955

European Journal of Biological Research

Research Article

Dynamics of oxygen consumption during the formation of the anoxic zone in aquatic environment

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Received: 30 July 2018; **Revised submission:** 18 September 2018; **Accepted:** 02 October 2018

DOI: http://dx.doi.org/10.5281/zenodo.1443571

ABSTRACT

Anoxic environments and communities of anaerobic organisms are encountered in aquatic environments and biotechnological reactors. Because of their importance, they are continuously studied. In this study, the dynamics of oxygen removal were observed during experiments reproducing the formation of the anoxic zone. Seven experiments were performed in an aquarium (volume: 60 l) with bottom sediments and water collected from different aquatic environments (river, pond, eutrophic lake, sea). To exclude reaeration, the water was isolated from the air by a layer of liquid paraffin. Below the paraffin layer the water was periodically mixed with a stirrer and sampled for oxygen concentration. Initially, a high rate of oxygen consumption was observed. Later, at low oxygen concentrations, the oxygen removal rate switched to a much lower one. Anoxic conditions were observed after 4-20 days of incubation, depending on the experiment. The point at which the microbial community converted from aerobic respiration to anaerobic metabolism was distinct and was observed at an oxygen concentration of 0.26-1.41 mg/l, depending on the experiment. The experiments were accompanied by bacterial counts and analyses of ciliate communities. The study indicates how the disappearance of oxygen during anoxic zone formation should be modeled, and provides data on the oxygen removal rates associated with aerobic and anaerobic communities of microorganisms.

Keywords: Respiration; Bacteria; Ciliates; Anaerobic; Hypoxia.

1. INTRODUCTION

Anoxic environments and communities of anaerobic organisms are encountered in aquatic environments and biotechnological reactors. Because of its importance, the formation of anoxic zones is studied and modeled mathematically to predict environmental conditions and their impact on organisms [1-4], to improve the efficiency of biotechnological processes such as bioethanol, glutathione, and beverage production [5], and also to optimize wastewater treatment [6].

During the formation of the anoxic zone, the composition of the community of bacteria, archaea, protista, and metazoa changes [2, 7-9]. At first, sensitive aerobic organisms are excluded from the community due to unacceptably low oxygen concentrations. Simultaneously, microaerophilic organisms appear in high abundances. Later, microorganisms that are capable of both types of metabolism convert from aerobic respiration to anaerobic metabolism. Finally, anaerobic organisms appear. It should be

emphasized that the latter also take up oxygen [10]. This uptake is not associated with production of energy but with O_2-detoxification, which facilitates producing and maintaining a strictly anoxic environment [10].

Aerobic organisms differ according to their tolerance to low oxygen conditions [1, 11]. Different species or strains capable of both aerobic and anaerobic metabolism apply different molecular mechanisms of oxygen sensing [5, 12]; thus, it might also be expected that they convert between aerobic and anaerobic metabolism at different oxygen concentrations. Finally, obligate anaerobic organisms differ according to oxygen tolerance [8, 10, 13] and emerge at different oxygen concentrations. As a result, particular species response individually to decreasing oxygen concentration. Thus, one may expect that during the formation of the anoxic zone, oxygen consumption rate decreases gradually, what results in a curvilinear decrease in oxygen concentration. Consequently, oxygen removal is typically described with curvilinear models in mathematical ecosystem models [3, 4, 14]. However, our previous observations of the formation of anoxic zones indicate that the whole microbial community might sharply change the rate of oxygen uptake. Within the scope of this research we conducted studies aimed at determining the proper method for mathematical description of oxygen disappearance during anoxic zone formation. Our hypothesis was that two rates of oxygen disappearance should be assumed: (i) a higher one at a high oxygen concentration, and (ii) a lower one at a low oxygen concentration. This method of modeling assumed there was a switching point between the two rates of oxygen uptake (piecewise model). An alternative hypothesis was that the rate of oxygen consumption really decreased gradually, which could be described properly with a curvilinear model.

To verify the hypotheses, we conducted seven experiments in an aquarium using near-bottom water and bottom sediments taken from different aquatic environments (eutrophic lake, river, pond, sea). Material taken contained *in situ* microbial communities. We placed a layer of sediments in the aquarium to provide an inoculum of microaerophilic and anaerobic organisms and to accelerate the formation of the anoxic zone. To exclude reaeration, the water was isolated from the air by a layer of liquid paraffin. Experiments were performed at standard temperature of 20ºC. The water in the aquarium was mixed periodically with a stirrer and sampled for oxygen concentration. Changes in oxygen concentration were fitted to piecewise and curvilinear models and tested for higher statistical significance. To explain possible differences, experiments were accompanied with additional measurements like organic matter content, bacterial abundance, and also ciliate abundance and community composition. The latter were studied because they are the most important eukaryotic microbes inhabiting anoxic environments [7, 15]. The results of the study are discussed in the context of implications for environmental and biotechnological studies.

2. MATERIALS AND METHODS

Altogether, 7 experiments were performed in the fall of 2010 and in the spring of 2011 (Table 1) using near-bottom water and bottom sediments taken from different aquatic environments: the highly eutrophic Lake Gardno (54º38’N, 17º08’E), the Słupia River (54º27’N, 17º02’E), a pond located within the city of Słupsk (54º27’N, 17º02’E), and the coastal waters of the Baltic Sea (54º37’N, 16º58’E). Sampling sites were shallow and material was taken directly using buckets. Material collected for the experiments was characterized with respect to organic matter content. The biodegradable organic matter in the water was estimated as biochemical oxygen demand (BOD5, each time 3-5 simultaneous measurements were performed). In bottom sediments, organic matter was estimated as loss on ignition (duplicated measurements). Loss on ignition was estimated in grams per liter of wet sediment by drying a known volume of wet sediment at 60ºC and later combusting it at 550ºC for 4 h [16].

The experiments were performed one after another in a 60 l aquarium. First, we placed sediments on the bottom of the aquarium to a height of about 9 cm. Next, tubing necessary for water sampling was anchored in the sediments (inlet was placed 3 cm above the sediment and directed upward, Fig. 1), and the aquarium was filled with water. The sediments and water were acclimated to room temperature (20ºC) for up to one day.

Table 1. Origin and general characteristics of material collected for experiments in fall 2010 and spring 2011. Abbreviation BOD5 means biochemical oxygen demand measured after 5 days of incubation in the dark at 20ºC.

Exp.	Material origin	BOD5 - water (mgO_2/l)	Loss on ignition - sediments (g/l of wet sediment)
1	Lake Gardno - fall	2.60	4.77
2	Słupia River - fall	2.00	50.0
3	Pond - fall	4.74	4.24
4	Słupia River - spring	5.16	14.3
5	Baltic Sea - spring	2.56	1.13
6	Lake Gardno - spring	8.07*	8.60
7	Pond - spring	9.71*	3.69

* underestimated values, all oxygen was used up during the BOD5 measurements. No measurements with diluted water were performed.

After acclimatization, we placed a stirrer in the water and poured about 1 l of liquid paraffin onto the water, which created an isolating layer between the water and the air (Fig. 1). Application of paraffin oil in such experiments is a typical approach [17] and paraffin oil is degraded very slowly [18]. Finally, the aquarium was darkened to inhibit photosynthesis, which liberates oxygen therefore making it impossible to assess oxygen consumption properly. To sum up, incubations were carried out at room temperature (20ºC) and in the dark. Depending on the experiment, anoxic conditions in water were observed after 4-20 days of incubation.

Water for oxygen consumption measurements was collected once a day or more frequently, depending on the experiment. Each time, the water in the aquarium (below the liquid paraffin) was gently mixed with the stirrer (3 rpm, 5 min.) before sampling (Fig. 1). The water was siphoned into 5-6 Winkler flasks. Two or three of them were immediately taken for oxygen concentration measurement. The three remaining bottles were incubated for 24 h at room temperature in the dark before measurements of oxygen concentration were performed. This additional incubation period made it possible to compare decreases in oxygen concentration in the aquarium and in the bottles, which permitted assessing the importance of bottom sediments in oxygen consumption. All oxygen measurements were performed with the standard Winkler method using a Schott Titronic Basic piston titrator.

The dynamics of oxygen removal were described with two models: (i) curvilinear in which oxygen concentration values were logarithmically transformed prior to the calculation of linear regression, and (ii) piecewise regression, in which a breaking point separated two linear regression equations. For both models, coefficients of determination (R^2) and statistical significances were calculated with Statistica software (StatSoft). R^2 coefficients calculated for both models were compared with the non-parametric Wilcoxon's signed-rank test.

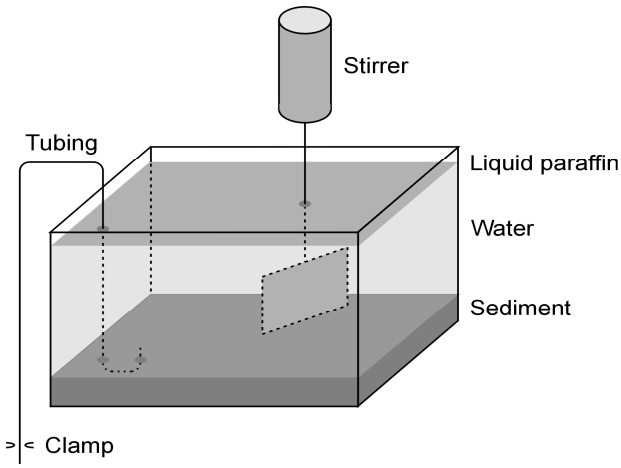

Figure 1. An aquarium of 60 l contained sediments and water taken from the environment and isolated from air by a layer of liquid paraffin. Water was sampled through gas-tight tubing the inflow of which was directed upward 3 cm above the sediments. Before sampling, water was gently mixed with the stirrer. Incubations were carried out at room temperature and in the dark. Depending on the sediments and water used, the formation of the anoxic zone lasted from 4 to 20 days of incubation.

At the end of each experiment, after the formation of the anoxic zone was complete,

microbial communities were analyzed. Bacterial abundance was estimated with direct counts of bacteria under an epifluorescence microscope after concentrating bacteria on polycarbonate filters and staining with acridine orange [19]. Ciliates were fixed with acid Lugol's solution, and were concentrated with the Utermöhl [20] technique and observed under an inverted microscope. They were identified according to Foissner and Berger [21] and other guides. Ciliates were later divided into (i) aerobic pelagic ciliates, (ii) aerobic benthic ciliates, (iii) microaerophilic ciliates, and (iv) specialized anaerobic ciliates. This classification was performed according to Fenchel et al. [7], Fenchel and Finlay [8], and Foissner and Berger [21].

3. RESULTS

The material collected for the experiments was described in Table 1. Depending on the experiment, anoxic conditions in water were observed after 4-20 days of incubation (Fig. 2). Each time, high rates of oxygen consumption were observed initially. Later, at low oxygen concentrations, rates of oxygen removal were much lower. Simultaneous incubations of water in bottles (see Materials and Methods) indicated that the sediments initially consumed oxygen, but after a few days they became anoxic and further decreases in water oxygen concentration were predominantly caused by pelagic organism respiration (not shown). Changes in oxygen concentration were fitted to curvilinear and piecewise models (Fig. 2). In six of seven experiments, R^2 values for both models were statistically significant. In the shortest experiment (the second one, material collected from the Słupia River in fall 2010) oxygen was removed within 4 days and the number of measurements was too low to demonstrate statistical significance (Fig. 2). In this experiment, the high consumption rates in this experiment were a consequence of the very high organic matter content in the sediments: 50 g per liter of wet sediment (Table 1) and a low initial oxygen concentration (Fig. 2). The direct comparison of curvilinear (logarithmic) and piecewise models demonstrated that the piecewise model offered higher coefficients of determination (Wilcoxon's signed-rank test, $p = 0.028$). Thus, the hypothesis posed in the introduction was confirmed. The point at which the microbial community converted from aerobic respiration to anaerobic metabolism was distinct and was observed at an oxygen concentration of 0.26-1.41 mg/l, depending on the experiment (Table 2). Its variability could not be explained by the organic matter content in the water or sediments (proxies of organic matter are listed in Table 1).

Table 2. The point at which the microbial community converted from aerobic respiration to anaerobic metabolism in seven experiments reproducing the formation of the anoxic zone. Switching points were calculated with the piecewise model.

Exp.	Switching point (piecewise model) (mgO_2/l)
1	0.76
2	Non-significant
3	1.41
4	1.39
5	1.20
6	0.26
7	0.37

After the formation of the anoxic zone, the abundance of bacteria and ciliates was analyzed. Bacterial abundance ranged from 3.19×10^6 to 22.8×10^6 cells/ml (Table 3), and abundances were generally inversely related to the time necessary for the formation of the anoxic zone. Ciliate abundance ranged from 3.30 to 34.0 cells/ml (Table 3). Ciliate abundances were not proportional to those of bacteria. Bacteria and ciliates are the dominant oxygen consumers during the formation of the anoxic zone; however, no dependence was demonstrated between their abundances and oxygen consumption rates measured after the community converted from aerobic respiration to anaerobic metabolism.

Roughly half of ciliates (range: 39-68%, mean: 51%, Fig. 3) were microaerophiles (scuticociliates, *Prorodon* spp., and others). Specialized anaerobic ciliates (*Metopus* spp., anaerobic *Lacrymaria* sp., and others) contributed 4-30% of ciliate numbers (Fig. 3). On average, 8% and 10% of ciliate abundance was contributed by aerobic forms typical for pelagic (e.g., *Urotricha* spp., *Monodinium* sp., and others) and benthic zones (e.g., *Litonotus* spp. or hypotrichs), respectively.

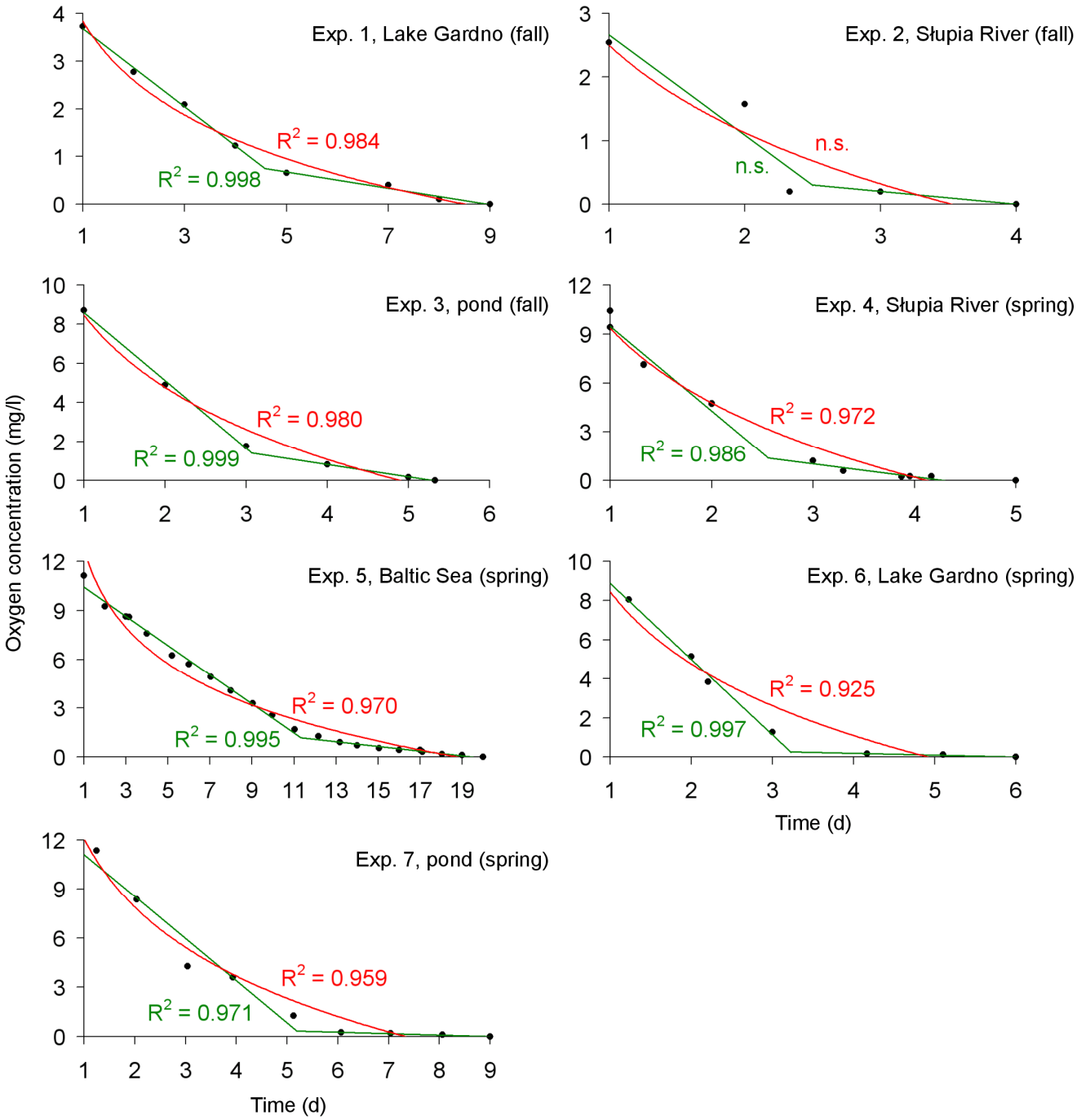

Figure 2. Oxygen removal rates observed during seven experiments performed with near-bottom water and bottom sediments taken from different aquatic environments: the highly eutrophic Lake Gardno, the Słupia River, a pond located within the city of Słupsk, and coastal waters of the Baltic Sea. Oxygen removal rates were fitted to logarithmic (R^2 value above curves) and piecewise (R^2 value below curves) models. R^2 values are highly statistically significant ($p < 0.01$). Non-significant regressions in the second experiment (Słupia River - fall) are indicated as n.s. R^2 values calculated for the piecewise model are significantly higher (Wilcoxon's signed-rank test, $p = 0.028$).

They remained intact even though oxygen was depleted. At the end of the shortest experiment (the second one, the Słupia River, fall), in which oxygen was already depleted after 4 days, aerobic ciliates still comprised 32% of ciliate abundance and anaerobic forms contributed only 5% to ciliate abundance (Fig. 3). In contrast, at the end of the longest experiment (the fifth one, the Baltic Sea, spring), which lasted 20 days, only 4% of ciliates were aerobic and 30% of ciliates were anaerobic (the highest value observed). The relationship between (i) time necessary for the anoxic zone

formation and (ii) the fraction of anaerobic forms within ciliate communities was statistically significant (R^2 = 0.91, p = 0.0008). Some of ciliates (mean 18%) remained unidentified (Fig. 3), because they were damaged during fixation with Lugol's solution.

4. DISCUSSION

During the formation of the anoxic zone, oxygen is initially depleted in bottom sediments and later in the near-bottom water [8]. In our experiments, we observed the escape of meiofauna from anoxic sediments to the pelagic water, which took place during the initial few days of the experiments. Simultaneous incubations of water in bottles also indicated that after a few days the sediments became anoxic. During all the experiments, the sediments became anoxic before the occurrence of the switching point, at which the rate of oxygen removal shifted to a much lower one (Fig. 2). This indicated that oxygen consumption by sediments did not explain the occurrence of the switching point.

In aquatic environments, an oxygen concentration below 2.85 mg O_2/l (= 2 ml O_2/l) is considered to be hypoxia [2, 9, 22]. Comparable value (3 mg O_2/l) was also reviewed and used in the mathematical model by Liu et al. [4]. However, different aerobic organisms obviously tolerate different levels of oxygen depletion.

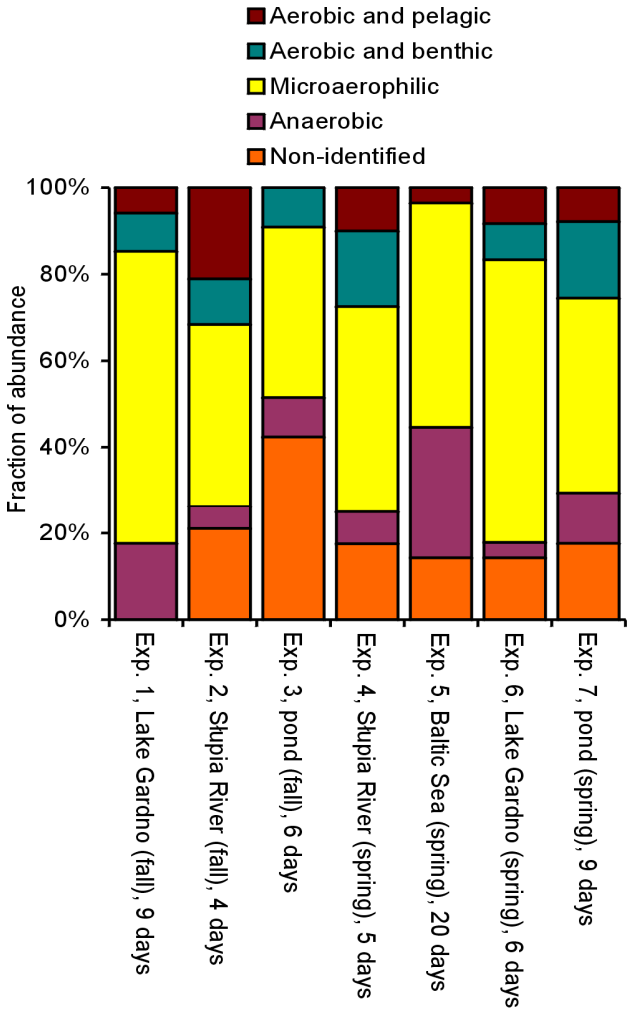

Figure 3. Composition of ciliate communities observed at the end of experiments. Each description of experiment was supplemented with the number of days that elapsed before anoxic zone was developed.

Table 3. Abundances of bacteria and ciliates, the two main components of anaerobic communities, which were observed at the end of seven experiments reproducing the formation of the anoxic zone.

Exp.	Material origin	Bacterial abundance (10^6 cells/ml)	Ciliate abundance (cells/ml)
1	Lake Gardno - fall	17.1	34.0
2	Słupia River - fall	22.8	12.7
3	Pond - fall	18.1	3.30
4	Słupia River - spring	11.9	8.00
5	Baltic Sea - spring	3.67	6.72
6	Lake Gardno - spring	12.0	33.6
7	Pond - spring	3.19	10.2

Marine crustaceans change their behavior below 4 mg O_2/l and are gradually excluded after oxygen concentration decreases to about 1 mgO_2/l [1]. Similar value of tolerance limit of benthic fauna to low oxygen concentration (1.0-1.1 mg O_2/l) was reported by Rosenberg et al. [9]. In the case of aerobic ciliates, their grazing, growth, and respiration decrease below 2.5-1.5 mg O_2/l [11, 22]. When

bacteria are considered, conversion from aerobic respiration to anaerobic metabolism takes place when the oxygen concentration decreases to about 0.1 mg O_2/l [8, 23, 24]. This value is much lower than those reviewed for larger organisms, because bacteria are the smallest organisms and have the most favorable ratio between cell surface and volume, which facilitates the aeration of the cell [8]. The switching points observed in this study for the whole community ranged from 0.26 to 1.41 mg O_2/l (Table 2) and were lower than values considered as hypoxia for larger organisms and higher than oxygen concentration at which the bacterial community changes its metabolism. Thus, they are reliable as points at which the whole community converts from aerobic respiration to anaerobic metabolism. The considerable variability in the moment of the switching point was not explained by accompanying measurements of organic matter content in the water or sediments.

It was demonstrated that the disappearance of oxygen during anoxic zone formation could be successfully modeled using the piecewise model. Thus, the hypothesis posed in the introduction was confirmed. This has implications for models that simulate the onset and recovery from hypoxia in the near-bottom zones of different water bodies such as those formulated by Livingstone and Imboden [14] or Liu et al. [4]. Similarly, the piecewise model of the disappearance of oxygen during anoxic zone formation could be applied in models describing biotechnological processes like that by Martínez-García et al. [6]. Variability in values of the switching point observed in this study was not explained. Thus, we recommend using a mean value of 0.9 mg O_2/l in models.

Bacterial abundances observed at the end of the experiments were within ranges reported elsewhere [7, 8, 25]. Ciliate communities in the anoxic environments produced in our experiments were typical both in terms of abundance and composition [7, 8, 15, 25]. The presence of some aerobic ciliates and many microaerophilic ones within the community demonstrated that the succession from an aerobic to an anaerobic community was not completed in any experiment. Bacterial and ciliate abundances were not correlated with the oxygen concentration at which oxygen consumption rates switched to lower rates or oxygen consumption rates measured after the conversion from aerobic respiration to anaerobic metabolism. Most probably, this correlation could be demonstrated after analyzing the functional composition of the bacterial community. It should be also emphasized that, typically, only a fraction of the bacteria observed in the environment is metabolically active, and it is difficult to relate microbial processes to the general abundance of bacteria [26].

AUTHOR'S CONTRIBUTION

KR designed research, analyzed the data and wrote the manuscript; KR and BM performed experiments, measured oxygen consumption rates and accompanying parameters; BM analyzed bacterial abundance; AK analyzed abundance and composition of ciliate communities. All authors read and approved the final manuscript.

SOURCE OF FUNDING

This work was supported by the Polish Ministry of Science and Higher Education through the statutory activities of the Pomeranian University in Słupsk (Poland), project number 11.6.15. The sponsor was not involved in the study design and execution.

CONFLICTS OF INTEREST

The authors have no conflict of interest to declare.

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