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Enzymatic activity in the surface microlayer and subsurface water in the harbour channel

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ABSTRACT

Hydrolytic activity of eight extracellular enzymes was determined spectrofluorimetric method in the surface microlayer and subsurface water in the harbour channel in Ustka. The ranking order of the potential enzyme activity rates in the studied water layers was as follows: lipase > phosphatase > aminopeptidase > β-glucosidase > α-glucosidase > xylanase > cellulase > chitinase. The level of activity of all studied hydrolases was higher in the surface microlayer than subsurface water. No clear gradients in the level of enzymatic activity were determined along the horizontal profile of the studied channel. Activity of extracellular enzymes was strongly influenced by the season.

1. Introduction

The surface microlayer (SML) is defined as a layer of the water surface tens to hundreds of μm deep, which is in direct contact with atmosphere and covers more than 70% of the Earth surface (Cunliffe et al., 2013; Astrahan et al., 2016; Chen et al., 2016). SML is a unique physical, chemical and biological habitat distinct from subsurface water (SSW) and plays a key role in global climate change, biogeochemical and ecological processes, energy transfer, organic matter transformation, degradation of pollutants, gases and aerosol exchange between the water and the atmosphere and also in regulation of microbial loops (Ya et al., 2014; Sarmento et al., 2015; Wurl et al., 2016). The surface microlayer is a specific and very dynamic ecotone which is susceptible to modification by wind, rain, water circulation, photochemical reactions and undulation which disintegrate its structure (Kuznetsova et al., 2005; Antonowicz et al., 2015; Engel and Galgani, 2016). Still, the surface layer is well fit for fast self-reconstruction of its original structure (Hale and Mitchell, 1997). The surface microlayer is also influenced by strong insolation associated with the emission of light in the visible and ultraviolet range (Santos et al., 2014).

Solar UV radiation (UVR 290–400 nm) has the potential to negatively effect because causes cellular damage on different cell targets, including nucleic acids, proteins and lipids, which may end up in mutations, cell inactivation and death and additionally can damage photosensitized reactions (Alonso-Sáez et al., 2006). Several physical and biological processes, including simple diffusion, flotation, convection, precipitation, anthropogenic pollution, turbulent mixing, scavenging, transport by bubbles and buoyant particles bubbles, primary and secondary production, biological degradation, convection and upwelling of subsurface water and atmospheric deposition contribute to the enrichment of this water layer with various inorganic and mainly organic chemical compounds (Cunliffe et al., 2013; Santos et al., 2013; Chen et al., 2016). According to Kuznetsova and Lee (2001), Gracia-Hor et al. (2005) and Stolle et al. (2010) the surface microlayer can be enriched up to 10^2-10^3 times compared to subsurface waters by the accumulation of dissolved (DOM) and particulate (POM) organic matter, mainly proteins, carbohydrates, lipids, nucleic acids, chitin, pectin and cellulose, which concentration is controlled by various complex processes.

More than 95% of DOM and POM accumulated in water basins have various heterogeneous polymeric structure and consist mainly of high (HMW > 10 kDa) molecular weight compounds (Raghul and Bhat, 2011; Celussi and Del Negro, 2012; Caruso, 2015). In both DOM and POM cycling, heterotrophic bacteria have been recognized as the major consumers and transformers of organic matter in water ecosystems (Bhaskar and Bhosle, 2008; Steinberg

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et al., 2008). High molecular weight polymers constitute a very important source of carbon, nitrogen, and energy used in biosynthesis or respiration processes of heterotrophic bacteria (Kolehmainen et al., 2009). As bacteria can only incorporate small molecules (<600 Da) via their cell-membrane permeases, polymers must be broken down to oligomers, dimmers and monomers that can easily diffuse into the periplasmic space and then be used to meet bacterial energy requirements and to build up their biomass (Baltar et al., 2010; Celussi and Del Negro, 2012). Water heterotrophic bacteria hydrolyze polymers using extracellular enzymes, both cell surface-bond and those released into the ambient water (Nagata, 2008). Many heterotrophic bacteria by high abundance, rapid growth and turnover, and high physiological diversity are known to carry genetic and metabolic potentials to synthesize a wide range of extracellular enzymes, mainly hydrolases with precise substrate specificities, which can degrade and modify a wide spectrum of organic polymers in water basins (Baltar et al., 2010; Raghul and Bhat, 2011; Caruso, 2015). These polymers are cleaved by a consortium of hydrolases: endohydrolases cleave off oligomers and exohydrolases cleave the terminal ends of the oligomers to produce monomers (Hoppe et al., 2002).

Over recent years, more and more hydrobiological studies have focused on the role which the surface microlayer plays in the marine ecosystems functioning. However, only a few quantitative studies (Kuznetsova and Lee, 2001; Mudryk and Skórczewski, 2004; Santos et al., 2013) on enzymatic activity in the surface layer of the estuarine and marine environment were carried out. Therefore, the aim of this paper was to determine (1) extracellular enzymatic activity in SML and SSW of the harbour channel, (2) horizontal and seasonal variation in the level of enzymatic activity in the studied channel.

2. Material and methods

2.1. Study area and sampling

This study was carried out in the harbour channel, which is the estuarine part of the Słupia River. Over 60% of the catchment area of the river covering 1 623 km^2 (Perliński, 2015) is exploited for agricultural purposes (Jarosiewicz and Obolewski, 2013). This river carries 15.5 m^3 s^{-1} of water into the Baltic Sea, as well as 200 000–300 000 m^3 y^{-1} of natural and anthropogenic sediments (Zawadzka, 1996). The studied channel is 40.5 m wide and about 6 m deep, and is located in the vicinity of the port in Ustka (54°35.2N, 16°21.2E) (Fig. 1). The port in Ustka covers the area of 0.3 km^2 and its main functions are fishery, transport and marine tourism (Christowa et al., 2007). The port in city Ustka is limited by two breakwaters of about 300 m length, which are also the final part of the Słupia River, and where this river enters the sea. Some values of selected chemical and microbiological parameters in the harbour channel are presented in Table 1.

The water samples from the harbour channel in Ustka were taken from four sites for further enzymatic analyses (Fig. 2).

Site 1 – located on the border between the Słupia River and the studied channel,
Site 2 – located in the central part of the channel,
Site 3 – located in one of the water basins called the coal basin,
Site 4 – located at the site where the channel enters the sea, i.e., near the heads of breakwaters.

During every sampling procedure the precise location of each sampling site was taken with a GPS receiver.

Water samples were taken quarterly in 2010–2013. The surface microlayer (SML) samples (thickness 250–300 μm) were collected with a 75 × 75 cm Garrett net (Garrett, 1965) of 0.14 mm mesh size. The water collected in the net was scrapped off with the wiper and the sample was collected in a sterile bottle. The subsurface water (SSW) was collected at about 0.5 m depth with a horizontal Van Dorn water sampler adapted for the collection of the samples in flowing water (Mudroch and MacKnight, 1994). The water samples collected with the Van Dorn sampler were transferred to sterile bottles using drain valve. Prior to sampling, the Garret net and Van Dorn sampler were rinsed with distilled sterile water and ethyl alcohol. The collected samples of water were transported to the laboratory in the ice containers at the temperature that did not exceed 7 °C. The time between the collection of the samples and enzymatic analyses usually did not exceed 3 h.

2.2. Estimation of enzyme activity

The level of enzymatic activity was estimated following the method by Hoppe (1993). In order to determine the activity of hydrolytic enzymes following fluorescently labelled with MUF (4 methylumbelliferyl) and MCA (4 methylcoumarinyl-7 amide) organic substrates were used:

4-methylumbelliferyl-α-d-glucopyranoside to determine the activity of α-glucosidase (α-GLU) (E.C. 3.2.1.20),
4-methylumbelliferyl-β-d-glucopyranoside to determine the activity of β-glucosidase (β-GLU) (E.C. 3.2.1.21),

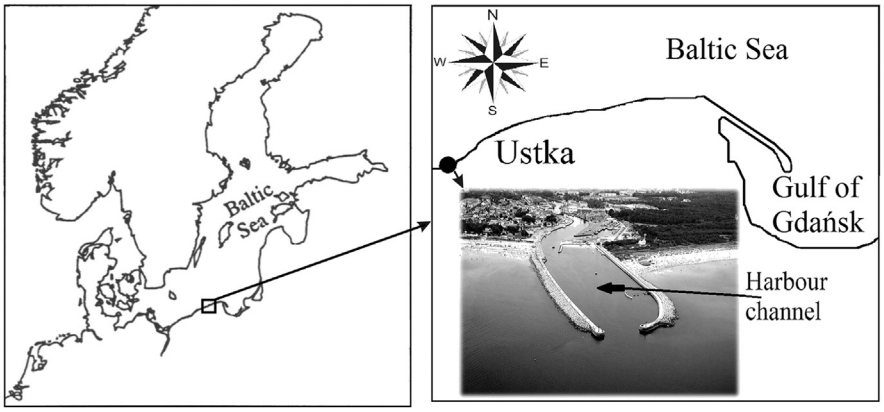

Fig. 1. Map of localization the study marine harbour channel in Ustka.