BALTIC COASTAL ZONE

Vol. 22 pp. 29-46 2018

ISSN 1643-0115

Original research paper

Received: 17/10/2018
Accepted: 04/12/2018

DECOMPOSITION OF ORGANIC MACROMOLECULAR COMPOUNDS BY HETEROTROPHIC BACTERIA INHABITING SURFACE MICROLAYER, SUBSURFACE WATER AND SEDIMENT-WATER INTERFACE IN THE MARINE HARBOR CHANNEL

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Abstract

Potential capability of heterotrophic bacteria to hydrolytic degradation different organic macromolecules in three water layers in the marine channel were determined. In studied channel hydrolysed proteins and lipids. The heterotrophic microflora decomposition chitin were represented by the least abundant group of these organisms. The highest number of bacteria hydrolyzing tested organic macromolecules isolated from surface microlayer. It was demonstrated that no significant differences in number of bacteria decomposition studied organic compounds existed between different parts of harbour. Bacteria isolated from the water studied channel in different seasons hydrolyzing organic macromolecules with different intensity.

Key words: changes harbour channel, bacteria, organic macromolecules, decomposition

INTRODUCTION

Many water basins can accumulate large quantities of auto and allochthonous organic matter. Heterotrophic bacteria which constitute one of the largest and most important groups of microorganisms are key players in organic matter decomposition in the aquatic ecosystems, mediating their flux of nutrients and energy to higher levels (Kubera and Donderski 2017, Perliński and Mudryk 2017). More than 95% of organic matter accumulated in water basins has various polymeric structure (HMW > 10 kDa)

and the most representative are proteins, polysaccharides, lipids, pectin, cellulose, chitin, nucleic acids or lignin (Celussi and Del Negro 2012, Caruso 2015). For heterotrophic bacteria, those high molecular weight biopolymers constitute a very important source of carbon, nitrogen, and energy, used for biosynthesis and respiratory processes (Joux et al. 2006, Zdanowicz and Mudryk 2017). Many heterotrophic bacteria are known to carry genetic and metabolic potentials to synthesise and control extracellular enzymes, which can degrade and modify a large variety of natural polymers to monomeric compounds (600 Da) such oligomers, dimmers and monomers, which than may be actively assimilated by bacteriocenosis (Perliński and Mudryk 2017). Owing to the dynamic auto induction of their enzymatic systems, those bacteria have the facility of fast metabolic reaction to every chemical compound newly introduced into the water ecosystem (Kirstein 1991, Mudryk 2003). This process is also aided by the participation of different physiologic heterogenic groups of bacteria showing differentiated levels of biochemical activity (Krstulović and Solić 1988, Skórczewski and Mudryk 2005). Hence, during recent years numerous investigations related to ability bacteria to decompose organic matter in freshwater (Walczak 2002), estuarine (Mudryk and Donderski 1997, Skórczewski and Mudryk 2005) and marine environment (Krstulović and Solić 1988, Mudryk et al. 2011) were carried out. However, to our knowledge, no studies are available about characteristic heterotrophic bacteria decomposing macromolecular compounds in a specific water reservoir such as a marine harbour channel. Therefore, the aim of this paper was to determine physiological properties of bacteria isolated from water of marine channel in Ustka, their ability to carry out particular metabolic processes in degradation different organic macromolecular compounds.

MATERIAL AND METHODS

Study area and sampling

This study was carried out in the marine harbour channel, which is the estuarine part of the Słupia River (Poland). The catchment area of the river covers 1,623 km^2 and over 60% of that area is exploited mainly for agricultural purposes (Perliński et al. 2017). This river carries 15.5 $m^3 \cdot s^{-1}$ of water into the Baltic Sea, as well as 200-300 thous. $m^3 \cdot y^{-1}$ 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 studied harbour channel is limited by two breakwaters of about 300 m length, which are also the final part, where the Słupia River enters the sea. According to Perliński (2015) selected chemical and microbiological parameters of the water in the marine harbour channel are presented in Table 1.
The water samples from the channel in Ustka were taken from four sites (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.

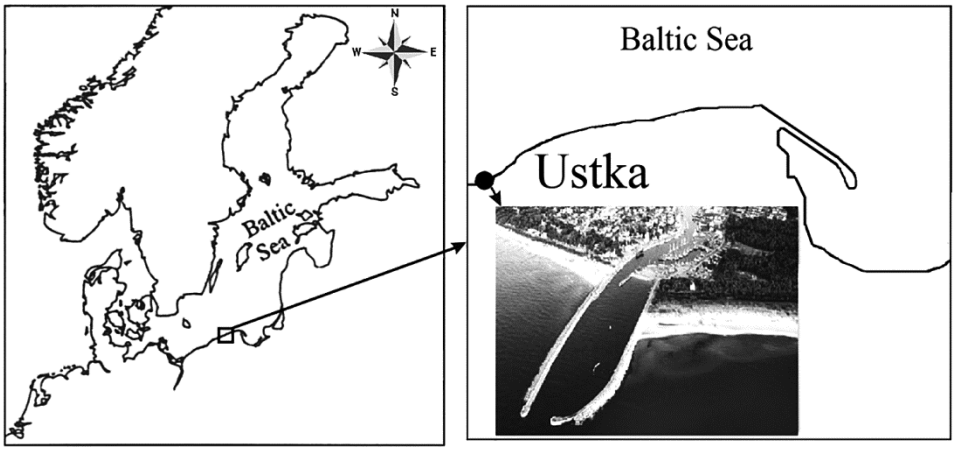

Fig. 1 Map of localization the study marine harbour channel in Ustka

The precise location of each sampling site was taken with a GPS receiver installed on the board of the tugboat. The water samples were collected during autumn in 2012 and winter, spring and summer in 2013. The surface microlayer (SML) samples (thickness 150-250 μm) were collected with a 75x75 cm Garrett net (Garrett 1965) of 0.14 mm mesh size. The water collected in the net was scraped off with the wiper and the sample was collected in a sterile bottle. The subsurface water (SSW) and sediment-water interface (SWI) was collected at used a horizontal van Dorn water sampler (Mudroch and MacKnight 1994). The collected water samples were transferred to sterile bottles using drain valve. Prior to sampling, the Garret net and van Dorn water 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 bacteriological analyses usually did not exceed 3 hours.

Bacterial decomposition various organic macromolecules

For determining number of heterotrophic bacteria (CFU) decomposing selected organic matter, the samples of water were diluted with sterile buffered water (pH 7.2) and inoculated by the spread method, in five replication onto different media containing various organic compounds:

1. The capability to decompose protein, starch, and lipids by bacteria were assayed in Ferrer et al. (1963) medium enriched with either protein (20.0 g per dm^3), starch (5.0 g per dm^3) or lipid (tributyrin) (10 cm^3 per dm^3). For visualization of protein decomposition, Frazier's reagent was poured over the plates (Weyland et al. 1970). Amylolytic bacteria were determined using Lugol's solution (Seiler et al. 1980).

Lipids hydrolysis by bacteria were determined on the basis of clear zone formation around bacterial colonies (Mudryk and Donderski 1997).

2. The ability to decompose deoxyribonucleic acid (DNA) was assayed in Oxoid medium containing 2.0 g DNA per dm^3. Hydrolysis of DNA was disclosed by pouring 1 N HCl.

Table 1

Forecast Values of selected chemical and microbiological parameters in the harbour channel (Perliński 2015)

	st. 1		st. 2	
Parameters	Mean	Range	Mean	Range
$N\text{-}NO_3$ ($mg \cdot dm^{-3}$)	47.6	26.1-103.2	48.0	18.3-136.4
$N\text{-}NH_4$ ($mg \cdot dm^{-3}$)	7.8	1.4-16.0	9.8	3.8-15.7
Cl^- ($mg \cdot dm^{-3}$)	328.5	36.5-751.9	2204.4	94.6-6822.2
O_2 ($mg \cdot dm^{-3}$)	6.7	4.8-10.9	7.5	3.6-11.5
pH	7.3	6.8-7.7	7.3	6.5-7.7
Organic Matter ($mg \cdot dm^{-3}$)	0.4	0.1-0.7	1.3	0.3-3.1
Protein ($\mu g \cdot dm^{-3}$)	23.1	15.1-34.0	21.5	16.3-28.0
Lipids ($\mu g \cdot dm^{-3}$)	110.4	26.2-238.9	117.8	36.3-330.5
Carbohydrates ($\mu g \cdot dm^{-3}$)	72.6	31.3-135.1	76.8	21.7-155.0
Number of heterotrophic bacteria (CFU $10^6 \cdot dm^{-3}$)	31.8	5.5-91.7	30.3	7.3-79.2
Bacterial production ($\mu g\ C \cdot dm^{-3} \cdot h^{-1}$)	49.0	11.2-101.0	25.8	4.4-69.8
	st. 3		st. 4	
Parameters	Mean	Range	Mean	Range
$N\text{-}NO_3$ ($mg \cdot dm^{-3}$)	44.3	22.4-100.1	45.4	19.2-101.9
$N\text{-}NH_4$ ($mg \cdot dm^{-3}$)	13.9	3.9-48.4	14.8	4.2-47.4
Cl^- ($mg \cdot dm^{-3}$)	1246.7	128.9-5944.2	3237.7	210.6-7386.6
O_2 ($mg \cdot dm^{-3}$)	7.1	5.2-9.8	7.3	5.2-9.8
pH	7.4	7.0-7.7	7.5	7.2-7.7
Organic Matter ($mg \cdot dm^{-3}$)	0.7	0.3-1.1	1.6	0.2-3.9
Protein ($\mu g \cdot dm^{-3}$)	21.3	15.3-27.4	23.6	17.3-31.9
Lipids ($\mu g \cdot dm^{-3}$)	95.5	24.4-248.5	369.3	20.1-888.6
Carbohydrates ($\mu g \cdot dm^{-3}$)	60.6	32.0-94.3	50.4	21.8-97.6
Number of heterotrophic bacteria (CFU $10^6 \cdot dm^{-3}$)	34.6	7.8-70.2	26.6	2.7-62.7
Bacterial production ($\mu g\ C \cdot dm^{-3} \cdot h^{-1}$)	26.1	4.3-93.9	14.3	3.6-30.7

3. Cellulose hydrolysis was determined in medium prepared according Mudryk and Donderski (1997). Colloid cellulose was prepared from Cellulose Powder 11, Whataman, according to Halliwell (1962). Clear zones around the colonies pointed to the cellulose decomposition ability of these bacteria.
4. The ability hydrolyse chitin was estimated in medium prepared according to Helmke and Weyland (1986). Colloidal chitin was prepared using chitin from Windsor–Berkshire (England) according to Lingappa and Lockwood (1962). The appearance of clear zones was accepted as a positive result.

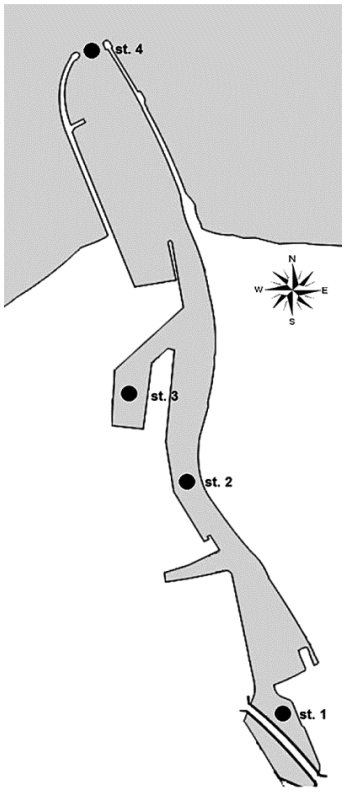

Fig. 2. Location of the sampling sites

All media were adjusted to pH 7.0-7.4 and were sterilized at 117°C for 20 min. Results were read after 6-day incubation at 20°C with the exception of chitin and cellulose, where the decomposition was determined after 21-days incubation, after which the number of bacteria that decomposed organic compounds was counted and results were recalculated per 1 dm^3.

Statistical measures (standard deviation – SD, coefficient of variation – CV, coefficient of dispersion – CD) used in this analysis were based on Velji and Albrihgt (1986). Correlation between bacteria decomposing organic macromolecules was assessed with the Spearman rank order test. The significance of differences in bacteriological parameters among the sites, layers and seasons was assessed by a two-way

ANOVA according to Incera et al. (2003). In order to group the analysed parameters as to their specific properties, the cluster analysis (the Ward method), and Euclidean distance were applied according to StatSoft (2010).

RESULTS

Based on the data presented in Table 2, it was found that heterotrophic bacteria inhabiting water in the harbor channel in Ustka characterized by the ability to hydrolytic decomposition of all studied organic macromolecular compounds. The largest number of bacteria isolated from surface microlayer, subsurface water and sediment-water interface were characterized by organisms that carried out proteins ($16.9 \cdot 10^6$ CFU $\cdot$ dm^{-3}) and lipid ($12.9 \cdot 10^6$ CFU $\cdot$ dm^{-3}) decomposition. There were also relatively many ($8.9 \cdot 10^6$ CFU $\cdot$ dm^{-3}) bacteria characterized by the ability to hydrolyze starch and DNA. On the other hand, the least ($0.9 \cdot 10^6$ CFU $\cdot$ dm^{-3}) a large group of physiological bacteria in the harbor channel in Ustka were organisms depolymerizing chitin.

Table 2

Occurrence of bacteria able to decompose selected macromolecules in water studied channel

Macromolecule compounds	Statistical parameters				
	Mean (10^6 CFU·dm^{-3})	Range (10^6 CFU·dm^{-3})	SD	CV [%]	CD
protein	16.9	0.5-81.7	19.0	112.5	213.68
lipids	12.9	1.0-62.3	15.8	122.1	192.49
DNA	8.9	0.3-35.0	8.8	99.0	87.48
starch	8.9	0.2-30.0	8.8	99.2	87.41
cellulose	6.0	0.2-40.0	9.8	164.1	160.57
chitin	0.9	0.2-5.0	1.1	116.1	12.22

Figure 3 shows data on the presence of bacteria characterized by the ability to hydrolyze organic macromolecular compounds in three studied water layers. In the vertical profile the largest number of all bacteria groups with ability to decompose different macromolecular compounds were found in surface microlayer. The least numerous tested physiological groups of bacteria isolated from subsurface water.

Data showing the horizontal distribution of heterotrophic bacteria capable of degrading the tested organic macromolecular compounds is shown in Figure 4.

No regular pattern in horizontal distribution of studied physiological group were established. In all parts of investigated channel the abundance of neustonic and planktonic bacteria with ability to decompose organic macromolecules compounds showed similar level. Only proteolytic bacteria showed maximum density at the border of the Słupia River mouth and the studied channel (st. 1) and lipolytic bacteria in coal basin (st. 3). In horizontal profile the most actively transformed among tested organic macromolecules by heterotrophic bacteria in all sites studied channel was proteins and the most slowly was chitin.

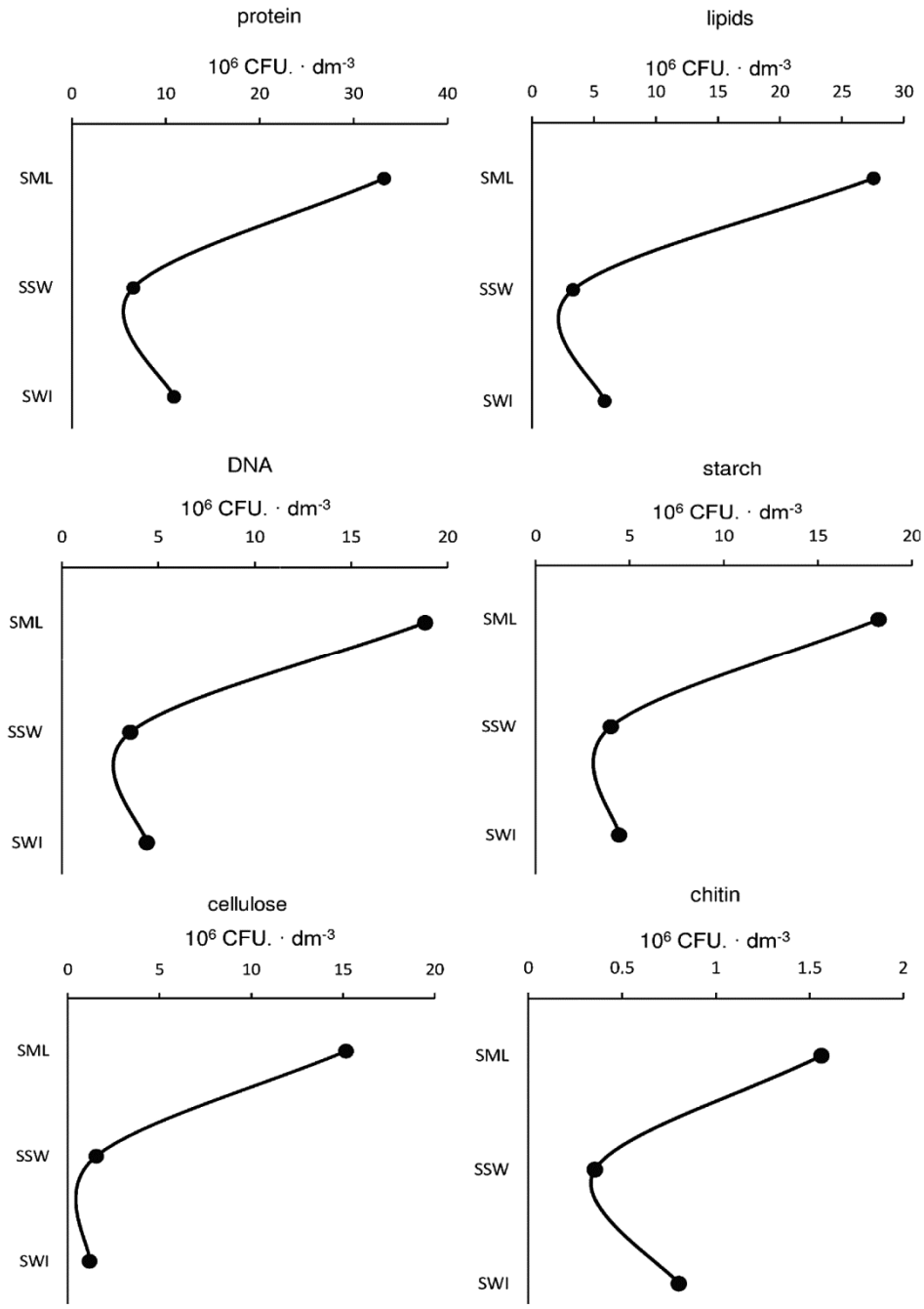

Fig. 3. Vertical variations of number of bacteria capable of degrading the tested organic macromolecules in three water layers in studied channel (average from the pooled data of all sites and seasons), n = 16

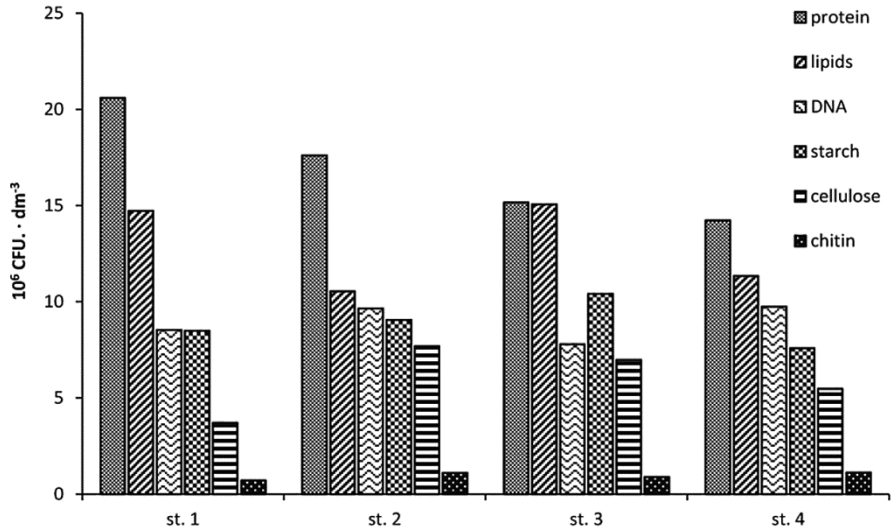

Fig. 4. Spatial distribution of the studied bacterial physiological groups (average from the pooled data of all water layers and seasons), n = 16

The data presented in Figure 5 shows that the seasonal dynamics the number of bacteria carrying out the process of macromolecular compound degradation were subject to significant oscillations.

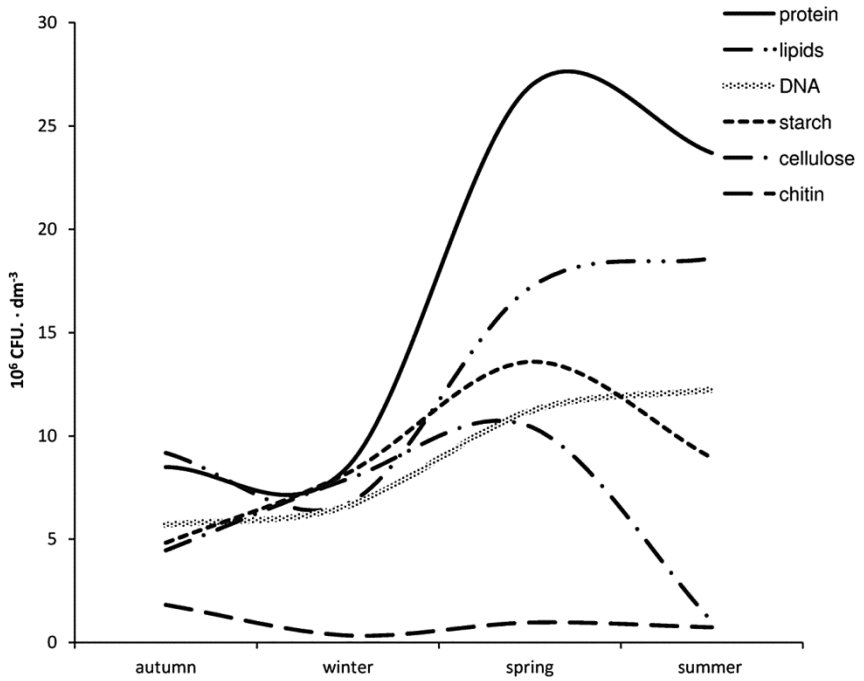

Fig. 5. Seasonal fluctuations bacteria decomposition organic macromolecules in water channel in Ustka (average from the pooled data of layers and sites), n = 16

The most rapid development of bacteria hydrolysing proteins, starch and cellulose occurred in spring. The organisms capable of performing the processes of lipids and DNA hydrolysis dominated in water studied channel in spring and summer and chitin in autumn. All studied physiological groups with the exception chitinolytic bacteria the least numerous were noted in autumn and winter.

The relationships abundance of analysed physiological bacterial groups decomposed organic macromolecules are given as the correlation matrix in Table 3. In water studied channel all bacteriological parameters show positive correlations between each other. The highest ($r = 0.81$-0.83, $p < 0.001$) positive level correlations were noted between bacteria hydrolyse protein and lipid and DNA and also between lipids and DNA.

Table 3

Correlation coefficient between studied physiological bacterial groups

Nonparametric Spearman's correlation coefficients in dataset						
	protein	lipids	DNA	starch	cellulose	chitin
protein						
lipids	0.81***					
DNA	0.83***	0.82***				
starch	0.64***	0.63***	0.71***			
cellulose	0.42**	0.56***	0.49***	0.51***		
chitin	0.35*	0.53***	0.38**	0.19	0.33*	

n = 48 in all cases. Significance (p) is indicated by asterisks: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

By grouping the results by the sites, layers and seasons the factorial ANOVA test was carried out to detect significant differences between the number of bacteria capable to decomposition of organic compounds (Table 4). All studied bacteriological parameters differed significantly between the studied layers and between layers and season. Non-significant differences in studied physiological bacterial groups between the sites were noted.

Table 4

Analyses of the Kruskal–Wallis test in the abundance of the major heterotrophic bacterial groups due to season, site and layer.

Source of variation	protein		lipids		DNA		starch		cellulose		chitin	
	H	p	H	p	H	p	H	p	H	p	H	p
site	2.71	ns	0.92	ns	0.75	ns	2.52	ns	0.51	ns	0.26	ns
layer	19.03	***	26.75	***	23.18	***	23.18	***	22.69	***	15.72	***
season	12.75	**	3.70	ns	4.67	ns	4.67	ns	9.76	*	16.35	***
site × layer	21.53	*	29.46	**	30.08	**	26.66	**	26.01	**	17.18	ns
site × season	19.61	ns	11.55	ns	10.90	ns	15.98	ns	11.71	ns	21.89	ns
layer × season	34.52	***	32.74	**	37.56	***	29.77	**	35.24	***	37.15	***

Explanations: H – the Kruskal–Wallis test
p – significance level
ns – non-significant
Significance (p) is indicated by asterisks: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

The results of hierarchical dendrogram analysis of the studied phylogenetic groups showed two distinct clusters (Fig. 6). The first cluster (A) comprised bacteria decomposed DNA, starch, cellulose and chitin, while the second cluster (B) comprised proteolytic and lipolytic bacteria.

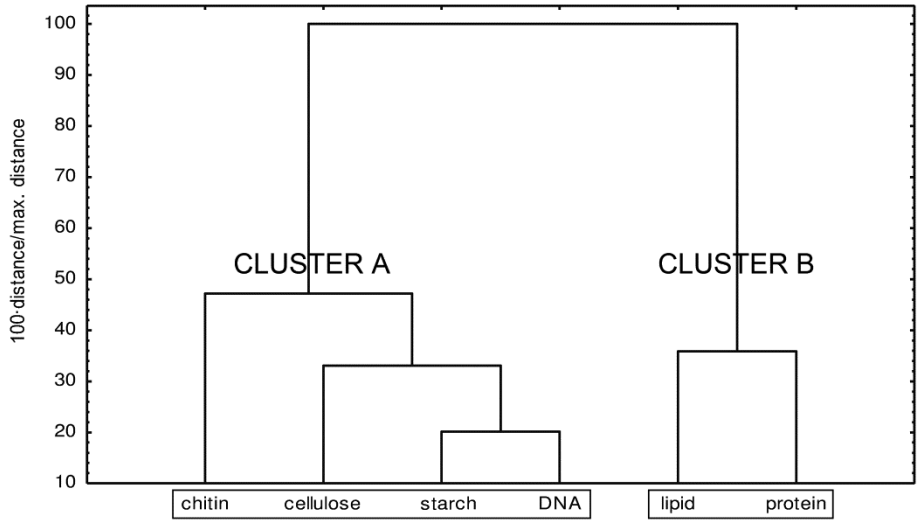

Fig. 6. Cluster for all physiological groups bacteria (parameters applied: Euclidean distance, full binding)

DISCUSSION

Microorganisms mainly heterotophic bacteria inhabiting aquatic ecosystems are able to decompose a wide spectrum of organic compounds ranging in molecular size from monomers to polymers (Chróst 1991, Quemeneur and Marty 1992, Mudryk and Podgórska 2006). The intensity of decomposition of organic molecules in water basins depends on the number of bacteria capable of carrying out those processes, on the activity of their extracellular hydrolytic enzymes and on the quantity and quality of the organic matter (Mudryk et al. 2011).

The results of the present study showed that among the heterotrophic bacteria inhabiting marine harbor channel in Ustka organisms hydrolyzing proteins were the most numerous physiological group. In other water bodies, proteolytic bacteria were also a predominant group among heterotrophic bacteriocenosis (Krstulović and Solić 1988, Mudryk and Donderski 1997, Worm et al. 2000, Mudryk 2003). According to Mallet and Debroas (1999) and Worm et al. (2000) proteolytic bacteria have been shown to play a key role in the processes of modification and transformation of protein compounds accumulated in water basins. Degradation of proteins in natural waters is initially mediated by bacterial extracellular proteolytic enzymes mainly aminopeptidases, which hydrolyze residues containing peptide bonds (Ainsworth

and Goulder 2000, Thompson and Sinsabaugh 2000). In opinion Patel et al. (2000), high concentration of proteins, polypeptides, peptides, and amino acids which in water bodies is constitute 35-55% of dry mass of organic matter is the major factor causing intensive growth of proteolytic bacteria. Proteins, mainly proteoglycans and glycoprotein are the most important, easily utilizable carbon, nitrogen and energy sources for aquatic heterotrophic bacteria (Wehr et al. 1999, Kiersztyn et al. 2002). Live as well as dead phytoplankton, zooplankton, meiobentos, macrobentos and detritus are the main sources of proteins in the water basins (Billen and Fontigny 1987, Romani and Sabater 2000).

Besides proteolytic bacteria, one the most abundant physiological group bacteria in studied channel were bacteria capable of hydrolyzing lipids. According to Martinez et al. (1996), lipolytic bacteria have been shown to play a key role in the processes of modification and transformation of lipid compounds in water basins. Lipids are actively assimilated by bacteria and used in respiratory processes or components of bacterial membranes (MacCarthy et al. 1998). Lipolytic bacteria synthesized esterases and lipases which are capable of attacking emulsified mono-, di- and triglycerides, and of splitting them with the yield of glycerol and fatty acid residues (Gajewski et al. 1997). According to Gajewski and Chróst (1995) and Mudryk and Skórczewski (2004) lipases belong to the group of the most active enzymes produced by aquatic bacteria. In water ecosystems concentration of lipids in dissolved and particulate organic matter ranges from 10 to 500 $\mu g \cdot dm^{-3}$ (Gajewski et al. 1997). Lipids and fatty acids are integral components of all living organisms and are widely distributed in aquatic environments (Reemtsma et al. 1990). In aquatic ecosystems, lipids compounds constitute 3-55% of all accumulated organic matter (Gajewski and Chróst 1995, Marty et al. 1996). Lipids are a very heterogeneous group of compounds; the most important classes include: triglycerides, diglycerides, fatty acids, sterols, phospholipids, glycolipids, sulpholipids, wax esters and aliphatic hydrocarbons (Parrish 1988, Jaffe et al. 1995). Similarly as proteins phytoplankton, zooplankton, meiobentos, macrobentos and detritus are the main sources of lipids in the water basins (Albers et al. 1996).

In the water of the studied channel bacteria capable of chitin hydrolysis were usually rare. These data corresponded with results obtained by Sugita et al. (1987), Mudryk and Donderski (1997) and Mudryk et al. (2011) in estuarine and marine environment. This is probably related to its containing very diverse food substance, many of which be metabolized more easily and more readily than chitin. At the same time Münster and Chróst (1990) draw attention on fact that microbial depolymerization of chitin requires synergistic activity of many hydrolytic enzymes which are produced mainly by fungi and actinomycetes which could activity secret different chitinases.

Many authors (Skórczewski 2003, Zdanowicz 2009, Mudryk at al. 2011) draw attention to the considerable variability in abundance heterotrophic bacteria decomposed organic macromolecules in the vertical profiles of water basins. The results of the present study also indicate clearly that there were differences in number of bacteria characterized by the ability to hydrolyze organic macromolecular compounds between three studied water layers. The largest number of all bacteria groups with ability to decompose different macromolecular compounds were found in microlayer surface. In this ecotone exist many mechanisms, such as: adsorption, diffusion, flotation, convection, precipitation, turbulent mixing, rising bubbles, atmospheric deposition, scav-

enging, primary production, which causes an accumulation large amounts of various organic matter (Santos et al. 2013, Chen at al. 2016). Dissolved and particulate organic matter can be enriched up to 1000 times in the SML compared to subsurface waters (Aller et al. 2005). Higher in surface microlayer than deep water subsurface water enrichment organic matter generate in this biotop the optimal conditions for the development and accumulation different physiological bacterioneuston groups ability to decomposing organic macromolecules (Aller et al. 2005, Kuznetsova et al. 2005). At the same time, the large accumulation of bacteria in the surface microlayer stems from the fact that these organisms characterised by the presence in the external cell structures of mucopolisaccharides, glycoproteides, phosphatcholine and lecithin polymers which, being hydrophobic, show properties of active attachment to the surface microlayer (Walczak and Donderski 2005).

According to Coelho et al. (2010) and Nikrad et al. (2014) differences composition of microbial populations in water basins may reflect the variation in concentration and composition, availability and degradability of organic matter, as well as changes in the level of bacterial metabolic activity. In our study, there were no significant differences in the abundance of neustonic and planktonic bacteria with ability to decompose organic macromolecules compounds among the study sites of the channel in Ustka. These results may suggest homogeneity in the composition of organic matter and lack of differences in their concentrations along the horizontal profile of the studied water basin that corresponds well with the results of earlier studies (Mudryk and Podgórska 2006, Walczak and Swiontek-Brzezinska 2010).

Based on our research, it was found that the number of bacteria characterized ability of decomposition organic macromolecules in the port channel in Ustka showed seasonal variability. The maximum determined bacterial physiological groups in the studied water basin were generally observed in the spring and summer seasons. These maxima correspond with the results obtained in other estuaries, marine waters (Takenaka et al. 2007, Cunliffe et al. 2009) and freshwater reservoirs (Antonowicz et al. 2015, Zdanowicz and Mudryk 2017). According to these authors, most probably the main stimulus for the growth of bacteria in spring and summer is the intensive development of phytoplankton, which releases assimilates that are immediately used by bacteria as food or energy substrate. Summer maxima of the heterotrophic of bacteria in the studied channel may also be the effect of relatively high temperatures at this time of year **(**Mudryk and Skórczewski 2007, Zdanowicz and Mudryk 2017). According to Cottrell and Kirchman (2000) and Takenaka et al. (2007) temperature is one of the most important abiotic environmental factor directly governing bacterial activity, resulting in high bacterial abundance.

In conclusion, we hope that the results of the present study could be important information on the potential role of enzymes in the processes of organic matter transformation and self-purification of water basins.

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DEPOLIMERYZACJA ORGANICZNYCH MAKROMOLEKUŁ PRZEZ BAKTERIE HETEROTROFICZNE ZASIEDLAJĄCE BŁONĘ POWIERZCHNIOWĄ, WODĘ PODPOWIERZCHNIOWĄ I PRZYDENNĄ W MORSKIM KANALE PORTOWYM

Streszczenie

Badania bakteriologiczne przeprowadzono w estuariowym odcinku rzeki Słupi będącym morskim kanałem portowym Ustce. Wodę z czterech stanowisk badawczych pobierano z błony powierzchniowej oraz z warstwy wody podpowierzchniowej i przydennej. W badanych próbach przy użyciu metod hodowlanych oznaczono liczebność bakterii heterotroficznych zdolnych do depolimeryzacji sześciu wielkocząsteczkowych związków organicznych. Uzyskane wyniki badań wykazały, że wśród bakterii neustonowych i planktonowych najliczniejsze były organizmy

proteolityczne i lipolityczne, a najmniej licznie występowały organizmy chitynolityczne. Wykazano liczbowe zróżnicowanie występowania badanych grup fizjologicznych bakterii w profilu wertykalnym, a homogenne ich występowanie w profilu horyzontalnym. Badane organizmy charakteryzowały się dużą dynamiką zmian sezonowych.