Polish J. of Environ. Stud. Vol. 17, No. 2 (2008), 273-282

Original Research

Utilization of Shrimp Waste as a Respiration Substrate by Planktonic and Benthic Microorganisms

M. Swiontek Brzezinska*, E. Lalke-Porczyk, W. Donderski

Department of Environmental Microbiology and Biotechnology, Institute of Ecology and Environmental Protection, Nicolaus Copernicus University, Gagarina 9, 87-100 Toruń, Poland

Received: April 28, 2007
Accepted: November 27, 2007

Abstract

This study presents results of research on the number of heterotrophic bacteria and fungi in water and bottom sediments of Lake Chełmżyńskie and their role in the decomposition of chitin. The authors also examined the level of respiration activity of water and sediment microorganisms in the presence of shrimp waste. Results demonstrate that the number of heterotrophic bacteria and fungi in water and bottom sediments were variable. The analyzed groups of microorganisms predominated in bottom sediments with the number of heterotrophic bacteria significantly exceeding that of fungi. The proportion of microorganisms capable of decomposing chitin was greater among fungi than among heterotrophic bacteria. In water chitinolytic bacteria constituted 11–19% of the total number of heterotrophic bacteria and in bottom sediments only 3–8%. Chitinolytic fungi constituted 17–67% and dominated in water. In the presence of shrimp waste, the level of respiration activity of microorganisms in water and bottom sediments of Lake Chełmżyńskie clearly depended on examined factors. The temperature, incubation time, and type of respiration substrate had a statistically significant impact on this activity. The highest respiration activity (2.4–90.3 mg $O_2 \cdot dm^{-3}$) of aquatic microorganisms was recorded in the summer, when the water temperature equaled 24°C. In bottom sediments the highest values of respiration activity also were observed in summer (13.4–447.4 mg O_2 g^{-1}dry mass) but alkaline sediments were characterized by higher activity levels. Benthic and planktonic microorganisms were utilizing shrimp heads most effectively and the exoskeletons least effectively.

Keywords: chitinolytic bacteria, chitinolytic fungi, respiration activity, shrimp waste

Introduction

Chitin is a polysaccharide that commonly occurs in the biosphere. This compound is produced in large quantities in aquatic environments [1, 2], primarily by zooplankton. *Euphausia superba* is a dominant zooplankton species in the Arctic Ocean. According to Clarke [3], chitin constitutes from 4 to 10% of krill dry mass. Jerde and Laser [4] estimated that a single population of planktonic *Euphausia pacifica* produces 1.9 x 10^{13} tons of sloughs dry mass annually. Even though chitin is produced in such large quantities in the oceanic environment [5, 6], its content in bottom sediments is relatively low [7].

Due to its wide application, chitin is currently becoming an increasingly common material in biotechnology. Products made of crustacean exoskeletons are used in waste water treatment, skin and hair care, tear-resistant

*e-mail: swiontek@biol.uni.torun.pl

paper, and facilitates the storing of seeds and long-term fruit storage. It is estimated that organisms annually produce from 100 to 200 billion tons of this "shell material". This constitutes an enormous quantity of unused biomass. Only 5000 tons of chitin is salvaged from crabs' exoskeletons from the North Sea and shrimp from Pacific and Greenlandic waters. In the fishing industry, crab shells were always treated as common waste, which, at the most, was considered suitable for livestock feed or was used in agriculture as an inexpensive, natural nitrogen fertilizer.

Today we know that this latter application was very successful due to the fact that the shells are broken down by enzymes, and chitin hinders the development of fungi and nematodes in soil [8]. At times, these undecomposed shells from the drainage basin reach the water column and bottom sediments where they are used by aquatic microorganisms. Chitinolytic microorganisms play a significant role in shrimp exoskeleton degradation. Bacteria and fungi are capable of producing chitinolytic enzymes, which hydrolyze chitin. Thus, the aim of this study was to determine the numbers of heterotrophic bacteria and fungi in lacustrine water and bottom sediments and their contribution to decomposition of chitin as well as to investigate the usage of shrimp waste as a respiration substrate by planktonic and benthic microorganisms in Lake Chełmżyńskie.

Materials and Methods

Object of the Study

The research was conducted in the water and bottom sediments of Lake Chełmżyńskie. This lake and its watershed area are located in the southern section of Pojezierze Chełmińskie, and in part of the Fryba river drainage basin. The catchment area of Lake Chełmżyńskie is situated within the Niecka Brzeżna (coastal basin). The postglacial Chełmżyńskie trough was formed as a result of drainage of continental glacier waters. Lacustrine water fills the deepest section of the trough. Morphometric and trophy characteristic of Lake Chełmżyńskie are presented in Table 1.

Table 1. Morphometric and trophy characteristics of Lake Chełmżyńskie.

Characteristic	Value
Area (ha) [(1)]	271.1
Maximal deph (m) [(1)]	27.1
Mean deph (m) [(1)]	6.1
Maximal length (m) [(1)]	6125
Maximal spread (m) [(1)]	550
Length of shore line (m) [(1)]	20985
Total phosphorus (mg · dm^{-3}) [(2)]	0.04 – 0.1
Total nitrogen (mg · dm^{-3}) [(2)]	1.1 – 1.79
Electrolytic conductivity (μS · cm^{-1}) [(2)]	601 – 703

[(1)] data supplied by The Provincial Inspectorate of the Environmental Protection in Bydgoszcz

[(2)] data supplied by Department of Environmental Microbiology and Biotechnology, Nicolaus Copernicus Universisty (data for 0.5 m depth, spring, summer, autumn 2006)

Sampling

The water samples characterized by neutral and alkaline were collected at depths of up to 5 cm with an automatic pipettor PipeBoy (De Wille Biotechnology), while the bottom sediment samples (neutral and alkaline) were collected with a tube scoop with 5 cm diameter and 0.75 m length. All samples were aseptically transported in sterile jars and temporarily stored on ice in an insulated container at ±7°C. They were analyzed immediately after transport to the laboratory. The time between sample collection and microbiological analyses did not exceed 3 hours. All samples were collected from the littoral zone, seasonally in the spring (April 8, 2006), summer (August 15, 2006), and autumn (October 20, 2006). The bottom sediments reaction was determined with the potentiometric method [9].

Determining Numbers of Heterotrophic Bacteria

The numbers of heterotrophic bacteria in water and bottom sediments were determined using a plate technique by applying surface inoculation on nutrient agar. After a 7-day incubation at 20°C, the colonies that grew on this medium were enumerated and the results were expressed per 1 cm^3 of water and 1 g dry mass of sediments. Subsequently, 50 colonies were isolated from each sample and used for determination of chitinolytic activity by the fluorometric method [10, 11].

Determining Numbers of Fungi

The numbers of fungi in water and bottom sediments was determined using a plate technique by applying surface inoculation on Czapek Dox agar. After a 14-day incubation at 25°C, the colonies that grew on this medium were enumerated and results were expressed per 1 cm^3 of water and 1 g dry mass of sediments. Subsequently, 50 colonies were isolated from each sample and used for determination of chitinolytic activity by the fluorometric method [10, 11].

Respiration Activity Water and Bottom Sediments of Lake Chełmżyńskie in Presence of Shrimp Waste

Manometric methods are respirometric methods based on pressure change following oxygen consumption in a her-

metically closed bottle containing the sample. The apparatus used was an OxiTop® – Control developed by WTW. This apparatus measures oxygen consumption almost continuously over the incubation period. The measurrment of BOD with OxiTop® – Control in water was carried out according to the operating instructions provide by supplier [12, 13]. The respiration activity levels bottom sediments were determined according to the operating instructions provide by Platen and Wirtz [14] and the author's own materials. The following variants were used during the study:

variant I – all parts of the shrimp were added to the water and bottom sediments,

variant II – only shrimp heads were added to the water and bottom sediments,

variant III – chitin exoskeletons were added to water and bottom sediments.

Prior to the addition to water and bottom sediments, shrimp waste was dried at 105°C, ground, and sterilized in an autoclave for 20 min. at 117°C. Water and bottom sediment samples without shrimp waste additions were used as a control (endogenous respiration). All samples were analyzed in two replicates. For analyses, the authors used waste produced by the company Krymar in Iłów during shrimp processing. Krymar has been involved in processing seafood, primarily the shrimp *Pandalus borealis,* since 1991. Shrimp are caught in the ocean (mainly the North Sea), frozen and transported to Poland on special pallets. In the production facility in Iłów, employees, working in aseptic conditions, separate the meat from the shells.

Determination of Respiration Activity of Water Microorganisms

Water with alkaline and neutral pH was used for analyses. The 500 ml measuring containers were filled with 250 ml of lacustrine water. Subsequently, a magnetic stirrer was placed inside and a solution of nitrification inhibitor NT 600 was added (5 drops). Shrimp waste (0.04g) was added after the temperature in the containers had stabilized. Next, a rubber carrier containing absorbent CO_2 (0.4 g NaOH) was placed in each measuring container and the OxiTop measuring heads were tightly screwed on. The measuring containers were placed on a mixing platform and put in a thermostatic cabinet. The measured values were recorded in the OC 110 control system in the "Special BOD" mode. The incubation was carried out at *in situ* temperature for 5 days.

Determination of Respiration Activity of Sediment Microorganisms

100 g samples of bottom sediments with neutral and alkaline pH were placed in 500 ml measuring containers. After temperature stabilization, shrimp waste (0.04 g) was added to the samples, and carriers with absorber CO_2 (0.4 g NaOH) were placed in the containers. The measuring containers were placed in a thermostatic cabinet. The measured values were recorded in the OC 110 control system in the "Pressure p" mode. Incubation was carried out at *in situ* temperature for 5 days.

Statistical

Some results were analyzed in STATISTICA 6.0. Aanalysis of Variance (ANOVA) was the primary statistical method used in calculations. This method facilitated comparison of the following independent factors: temperature, environmental pH, incubation time, and respiration substrate.

Results

The numbers of heterotrophic bacteria and fungi in water and bottom sediments were variable and depended on environmental factors and the sampling season. The number of heterotrophic bacteria in bottom sediments equaled $2.6–90 \cdot 10^4$ cells$\cdot g^{-1}$ dry mass, while the quantity of fungi was much lower: 154–2100 cells$\cdot g^{-1}$ dry mass. In surface water these numbers were considerably lower: 300–800 bacterial cells$\cdot cm^{-3}$ and 20–250 fungi cells cm^{-3}. The highest numbers of both types of microorganisms were observed in the summer (Tables 2, 3).

The proportion of microorganisms capable of decomposing chitin was greater among planktonic than among benthic microorganisms. Chitinolytic planktonic bacteria constituted 11–19% of total heterotrophic bacteria, but

Table 2. Microbiological analysis of water of Lake Chełmżyńskie.

Season	pH water	Numbers of heterotrophic bacteria (CFU · cm^{-3})	Numbers of fungi (CFU · cm^{-3})	Participation of chitinolytic bacteria (%)	Participation of chitinolytic fungi (%)
spring	7 8.5	300 300	120 90	17 16	50 67
summer	7.2 8.6	500 800	250 20	15 11	29 25
autumn	7.3 8.5	500 600	100 70	19 11	17 50

Table 3. Microbiological analysis of bottom sediments of Lake Chełmżyńskie.

Season	pH bottom sediments	The number of heterotrophic bacteria (CFU · g^{-1}dry mass)	The number of fungi (CFU · g^{-1}dry mass)	Participation of chitinolytic bacteria (%)	Participation of chitinolytic fungi (%)
spring	7 8.9	$26 \cdot 10^3$ $26 \cdot 10^3$	2100 400	3 7	29 25
summer	7.2 9.0	$67 \cdot 10^4$ $90 \cdot 10^4$	300 160	4 3	7 6
autumn	7 8.9	$36 \cdot 10^4$ $45 \cdot 10^4$	290 154	4 8	40 14

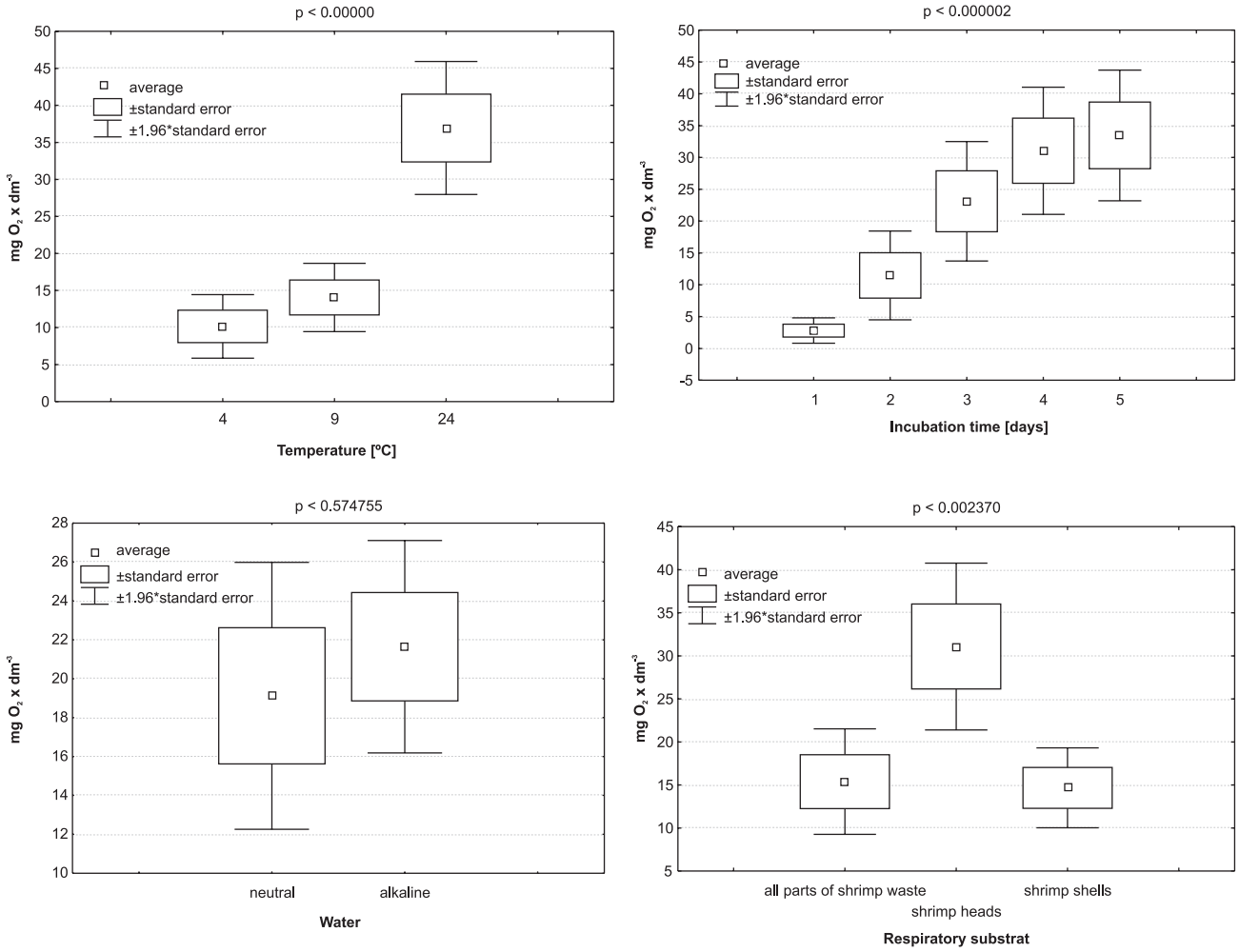

Fig. 1. Impact of examined factors on microorganism respiration activity in water of Lake Chełmżyńskie.

only 3–8% of benthic bacteria. Chitinolytic fungi isolated from water constituted no less than 17–67%, while in bottom sediments they constituted 6–40% (Tables 2, 3).

Analyses of respiration activity of lacustrine microorganisms in the presence of shrimp waste demonstrated that the differences were considerable. The temperature and incubation time, as well as type of shrimp waste had a statistically significant impact on respiration activity within the water. However, no significant differences in respiration activity were observed in water with neutral and slightly alkaline pH (Fig. 1). The highest respiration activity (2.4–90.3 mg O_2 dm^{-3}) of aquatic microorganisms was recorded in the summer, when the water temperature equaled 24°C. In the spring and autumn, these values were similar (Table 4).

In the presence of shrimp waste, the level of respiration activity of microorganisms in bottom sediments of Lake Chełmżyńskie was clearly related to examined factors. Temperature, incubation time, bottom sediment reaction, and type of respiration substrate had a statistically

Table 4. Microorganism respiration activity in water of Lake Chełmżyńskie.

Season	pH water	Temperature	Respiratory substrate	Incubation time [days]				
				1	2	3	4	5
spring	7	4°C	all parts of shrimp waste	* 0	0	3.3	11.0	12.0
			shrimp heads	0	0	9.0	34.9	36.0
			shrimp shells	0	0	2.9	9.0	10.5
	8.5	4°C	all parts of shrimp waste	0	1.5	8.4	15.8	19.2
			shrimp heads	0	2.5	15.3	33.6	39.8
			shrimp shells	0	2.3	6.6	12.6	18.3
summer	7.2	24°C	all parts of shrimp waste	2.4	26.3	40.8	50.2	55.3
			shrimp heads	14.4	52.1	77.1	88.3	90.3
			shrimp shells	2.4	13.7	24.1	30.6	33.4
	8.6	24°C	all parts of shrimp waste	2.7	23.2	36.7	41.2	46.2
			shrimp heads	11.2	39.2	59.6	66.7	70.1
			shrimp shells	1.6	15.2	29.0	32.6	34.1
autumn	7.1	9°C	all parts of shrimp waste	2.3	2.7	17.6	21.9	22.0
			shrimp heads	8.3	8.5	12.6	15.0	15.9
			shrimp shells	0	0	1.9	2.0	2.3
	8.5	9°C	all parts of shrimp waste	2.0	4.3	15.7	18.5	19.0
			shrimp heads	5.8	14.9	28.7	40.8	42.0
			shrimp shells	0	0	16.7	24.4	26.0

Explanation: * respiration activity in mg of $O_2 \cdot dm^{-3}$

significant impact on this activity (Fig. 2). As was found for water samples, the highest values of respiration activity were observed in summer (13.4 – 447.4 mg O_2 g^{-1}dry mass). However, alkaline sediments were characterized by higher activity levels.

Regarding the type of respiration substrate added to water and bottom sediments, it was concluded that microorganisms were utilizing shrimp heads most effectively and exoskeletons least effectively.

Discussion

Chitin is one of the most common polysaccharides on Earth, and it serves as a supporting substance of the exoskeleton of arthropods [15]. The main source of chitin is crustacean wastes. It also occurs in fungi and insects [16]. Shrimp and crab processing waste containing chitin, protein, and calcium carbonate are generally pre-treated by the processes if size reduction, deproteination, and demineralization to obtain a chitin suitable for bioconversion or other uses [17, 18]. Chitin and its derivatives have high economic value owing to their versatile biological activities and agrochemical applications [19].

The numbers of heterotrophic bacteria and fungi differ, and depend on the environment of their origin. In water bodies, the numbers of bacteria and fungi vary considerably in different seasons and even over different times of day. In shallow eutrophic and mesotrophic lakes, where the water is being nearly continuously mixed throughout the water column, the maximal numbers of bacteria occur in the water column usually in summer or at the end of summer, with the minimum in winter. In contrast, in deep oligotrophic bodies of water, significant fluctuations in bacterial numbers occur only in the surface layer [20].

Our study found that the number of heterotrophic bacteria and fungi was by far higher in bottom sediments than in surface water. This phenomenon is commonly observed. However, when examining numbers of specific groups of (e.g. chitinolytic) microorganisms, this phenomenon is not so apparent. Numerous studies report that chitinolytic microorganisms are more common in environments that are poor in organic matter. In lacustrine bottom sediments and also in eutrophic lakes rich in organic matter, the fraction of chitinolytic bacteria is much smaller than in lacustrine water. The presence of organic matter hinders the usage of chitin by microorganisms. According to Swiontek Brzezinska [21], on average 5.4–11.4% of het-

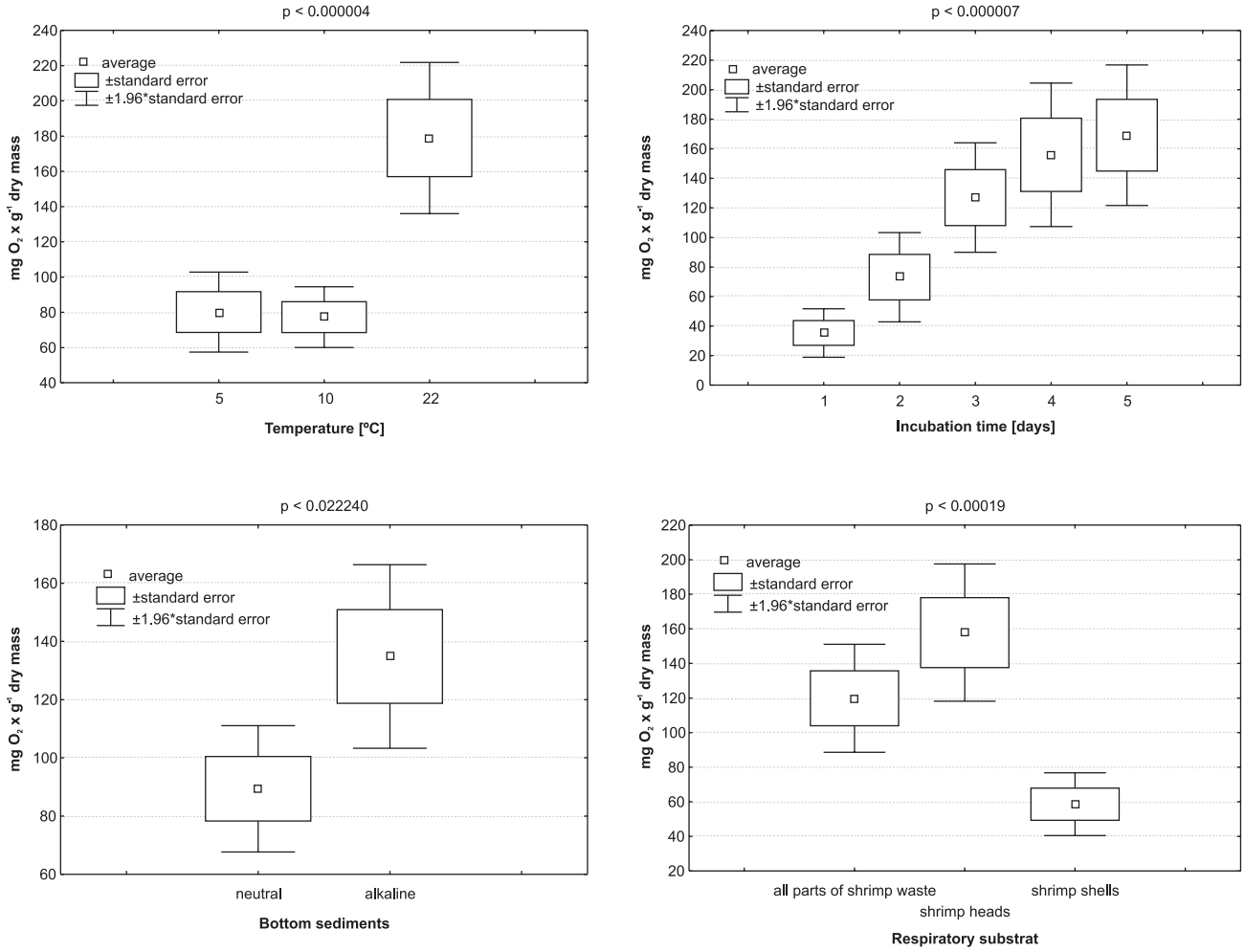

Fig. 2. Impact of examined factors on microorganism respiration activity in bottom sediments of Lake Chełmżyńskie.

erotrophic bacteria present in the water of eutrophic Lake Jeziorak were capable of breaking down chitin, while in oligo-mesotrophic Lake Jasne, this value ranged from as much as 10.2 to 18.7%. In bottom sediments of Lake Jeziorak, the author observed only 2.7–4.4% of chitinolytic bacteria and in Lake Jasne, 4.9–7.3%. Donderski [22] obtained similar results when enumerating chitinolytic bacteria in water and bottom sediments of lakes Jasne and Jeziorak. In bottom sediments of Lake Jeziorak, this author observed only 7.5% of chitinolytic bacteria, while in Lake Jasne, 16%. These bacteria were far more abundant in the water of the examined lakes (29.5% in Lake Jasne and 15% in Lake Jeziorak). Mudryk [23] reports that 10.6% of bacteria found in the surface waters of estuarine Lake Gardno break down chitin, while in bottom sediments, only 5%. Podgórska [24] found only 0-6% of chitinolytic bacteria in sea water and beach sand in Sopot, while Skórczewski [25] observed 7.2% of these bacteria in lake Gardno. Sugita et al. [26] obtained similar results in the ocean environment.

Oxygen plays an important role in aquatic ecosystems because it is used in the majority of chemical and biological processes. Heterotrophic microorganisms, which re-mineralize organic matter, are the most active users of oxygen. The analysis method of respiration activity used in this study is known. The BOD OxiTop method is one of the simplest biotest of water analysis. This test is widely applied to define organic water pollution and to control the performance of wastewater treatment plants [27]. Vähäoja et al. [28] found good methods of simulating the biodegradations of different types of forestry oils-hydraulic, motor and chain oils-in a groundwater environment and in forest soil. The aim of their work was to evaluate the applicability of the respirometric BOD OxiTop method for monitoring oil biodegradation in groundwater, using different chain oils as model compounds. The BOD OxiTop method is a highly reliable method for determining the BOD_s of chemicals. The BOD OxiTop methods can be used to study aerobic biodegradation. However, published information on use of the BOD Oxitop method in environmental studies is still scarce [29]. Therefore, it is impossible to compare our results with data from other publications. However, the obtained results are an important source of information about the role of microorganisms in oxidation of hard-to-decompose compounds, such as chitin. The data regarding the microorganism respiration activ-

Table 5. Microorganism respiration activity in bottom sediments of Lake Chełmżyńskie.

Season	pH bottom sediments	Temperature	Respiratory substrate	Incubation time [days]				
				1	2	3	4	5
spring	7	5°C	all parts of shrimp waste	*7.2	43.4	101.3	123.0	130.0
			shrimp heads	36.2	101.3	130.3	144.7	150.0
			shrimp shells	7.2	7.2	21.7	21.7	26.3
	8.9	5°C	all parts of shrimp waste	37.2	59.5	126.4	156.1	160.0
			shrimp heads	74.3	96.6	163.5	193.2	200.0
			shrimp shells	7.4	14.9	14.9	22.3	25.0
summer	7.2	22°C	all parts of shrimp waste	12.9	116.2	206.6	232.4	240.8
			shrimp heads	71.0	135.6	187.2	271.1	291.1
			shrimp shells	25.8	51.6	71.0	90.4	101.4
	9.0	22°C	all parts of shrimp waste	60.4	181.2	255.0	308.7	316.1
			shrimp heads	46.0	255.0	335.5	442.9	447.4
			shrimp shells	13.4	40.3	127.5	161.1	171.3
autumn	7.0	10°C	all parts of shrimp waste	13.9	20.9	55.8	69.7	100.6
			shrimp heads	13.9	20.9	83.7	83.7	134.2
			shrimp shells	7.0	13.9	19.7	20.5	50.0
	8.9	10°C	all parts of shrimp waste	21.5	57.3	114.6	128.9	137.8
			shrimp heads	43.0	50.1	114.6	143.2	174.3
			shrimp shells	35.8	50.1	107.4	136.1	137.8

Explanation: * respiration activity in mg of $O_2 \cdot g^{-1}$dry mass

ity in water and bottom sediments of Lake Chełmżyński demonstrate that the utilization of shrimp waste as a respiration substrate was greater in sediments than in surface water. Most probably, this discrepancy is associated with the lower abundance of microorganisms in water than in bottom sediments. However, Donderski [30], Strzelczyk et al. [31], and Podgórska [24], who investigated respiration activity of isolated bacterial strains, reported an opposite pattern. These authors found that in the presence of various substrates, respiration activity of planktonic bacteria was higher in comparison to the activity of benthic bacteria. The differences between the data obtained in this study and the results cited above could be associated with the specificity of the research itself. This study focuses on a relationship between the mixture of microorganisms inhabiting water and bottom sediments and environmental conditions. Also, the authors of this paper used a different method of measuring oxygen utilization by microorganisms. Populations of particular physiological groups of bacteria develop in different environments. These groups are optimally adapted to utilization of specific respiration substrates. Shrimp waste is definitely a very specific substrate and is utilized by numerous groups of microorganisms. In addition to chitin, shrimp waste contains large quantities of protein and lipid. Massardier-Nageotte et al. [32] investigated the aerobic biodegradability of four different polymers by microbial inoculum extracted from the soil using OxiTop respirometer. Among four tested polymers that are degraded the most is MB (blend starch + polycaprolactone). Eastar bio (poly(butadiene) adipate-*co*-terephthalate) is degraded much less and polylactic acid is almost not degraded. In order to check the activity of the microbial inoculum, one or more compounds that meet the criteria for ready biodegradability author's tested. The positive degradation reference compound selected was cellulose, and polyethylene served as a negative reference (as a "non-biodegradable polymer").

This study takes into account the environmental factors (temperature, pH) that play an important role in natural environments. Respiration activity of microorganisms was significantly correlated with temperature in both water and bottom sediments. The highest oxygen absorption by microorganisms occurred at a temperature of 22-24°C, while temperatures of 4-5 and 9-10°C slowed down this rate process. The time of incubation samples had a similarly significant impact on the ability to use shrimp waste. During the first 24 hours of the experiment, the amount of oxygen utilized by the microorganisms was minimal

and then increased significantly in the following 24 hours. Most probably, this phenomenon is related to the adaptation of the organisms to this substrate. Microorganisms used the protein and lipids first, followed by the chitin. Shrimp shells, which primarily consist of chitin, were used by microorganisms at the slowest rate, while shrimp heads (without shells), containing large quantities of protein and lipids, were the most useful respiration substrate. This relationship is particularly well pronounced in neutral bottom sediments. In the exclusive presence of shrimp shells, the quantity of oxygen absorbed by microorganisms was relatively small. Swiontek Brzezinska et al. [2006, unpub. data] report that in soil the level of respiration activity of microorganisms of the Chełmżyńskie lake watershed in the presence of shrimp waste was clearly related to different factors. Microorganism respiration activity was proportional to the incubation time and ranged from 0 to 550 mg O_2 g^{-1}dry soil mass. The highest activities were recorded in summer (6–550 mg O_2 g^{-1}dry soil mass), when the temperature was highest (24°C), and the lowest, in spring. At that time, the temperature did not exceed 6°C. The results of numerous studies [33, 34, 35] demonstrated that glucose and casein hydrolysate, due to their simple structure, are respiration substances actively assimilated by bacteria. In contrast, chitin has a complex structure and, therefore, is considered a difficult-to-assimilate compound, whose oxidation requires a large amount of energy. Thus, in order to investigate the respiration activity of microorganisms, the authors used a method that facilitates data recording at 24-hour intervals. It is true that the commonly used Clark's electrode facilitates measurements of respiration activities over short time periods, but when microorganisms require more time to initiate the respiration processes, the respiration activity recorded by such respirometers can be very low and disproportionate to the activity that occurs *in situ*. Vähäoja et al. [27] report that biodegradation of different chain oil (mineral, tall and rapessed oils) in groundwater environment was proportional to the incubation time. The oils were degraded the most after 28 days. Massardier-Nageotte et al. [32] obtained similar results. All tested polymers were degraded the most after 28 days.

Environmental pH is an important factor that affects enzymatic activity. However, the results of our study did not demonstrate significant differences in the rate of oxygen absorption by microorganisms in water with different pH. During the entire research period, the pH of water was neutral (pH = 7.0) or slightly alkaline (pH = 8.1–8.5). Clearly, such a difference in pH has no impact on microorganism respiration activity. However, the observations in bottom sediments were different due to the fact that, throughout the year, pH was neutral and evidently alkaline. The rate of oxygen absorption by microorganisms was much higher in alkaline sediments. Swiontek Brzezinska et. al [2006, unpub. data], who investigated respiration activity of soil microflora, reported that the microorganisms inhabiting alkaline soil were more readily able to utilize shrimp waste and found shrimp heads the most useful and shells the least. It could be assumed that bacteria assimilate a larger fraction of shrimp shells than fungi. Admittedly, numbers of chitinolytic fungi in the investigated environments are higher than bacteria capable of decomposing chitin, but it is possible that fungi are less active than bacteria. Besides, bacteria produce chitinases, which dissolve cell walls of fungi, and thus limit or even halt their development [36]. Chitin itself has anti-fungal properties, and therefore has been applied in agriculture. Recently, Wang and Huang [19], Wang et al. [37] investigated the bioconversion of shrimp and crab shell power (SCSP) from marine waste for bio-fungicide production. Wang et al. [38] demonstrated that *Pseudomonas aeruginosa* K-187 was a chitinase-producing strain in a SCSP medium and that *P. aeruginosa* K-187 was an antifungal strains in the SCSP medium displaying broad antagonism towards fungal phytopathogenes. Chang et. al [39] report that *Bacillus cereus* QQ308 produced antifungal hydrolytic enzymes, comprising chitinase, chitosanase and protease, when grown in a medium containing shrimp and crab shell power (SCSP) produced from marine waste.

Conclusions

1. The numbers of heterotrophic bacteria and fungi in water and bottom sediments were variable and depended on environmental pH and the sampling season.
2. The proportion of microorganisms capable of decomposing chitin was greater among planktonic than among benthic microorganisms. Chitinolytic bacteria constituted 3–19% of total heterotrophic bacteria. Chitinolytic fungi constituted no less than 6–67%.
3. The highest respiration activity of aquatic and benthic microorganisms was recorded in the summer, when water and bottom sediment temperature equaled 24–22°C.
4. The bottom sediment reaction had a statistically significant impact on this activity. However, no significant differences in respiration activity were observed in water with neutral and slightly alkaline pH.
5. Planktonic and benthic microorganisms were utilizing shrimp heads most effectively and the exoskeletons least effectively.

Acknowledgements

This study was supported by State Committee Scientific Research project 2PO4G 063 29.

Reference

1. HERWIG R. P., PELLERIN N. B., IRGGENS R. L., MAKI J. S., STALEY J. T. Chitinolytic bacteria and chitin mineralization in the marine waters and sediments along the Antarctic Peninsula. FEMS Microbial. Ecol. **53**, 101, **1988.**

2. BRENDT A., HÜLLER H., KAMMEL U., HELMKE E., SCHWEDER T. Cloning, expression and characterization of a chitinase gene from the Antarctic psychrotolerant bacterium Vibrio sp. Strain Fi:7. Extremophiles, **5**, 119, **2001.**
3. CLARKE A. The biochemical composition of krill, Euphausia superba, from south Geeorgia. J. Exp. Mar. Biol. Ecol. **43**, 221, **1980.**
4. JERDE C. W., LASKER R. Molting of Euphausia shrimp: shipboard observations. Limnol. Oceanography. **11**, 120, **1996.**
5. YU C. LEE A. M., BASSLER B. L., ROSEMAN S. Chitin utilization by marine bacteria. A physiological function for bacterial adhesion to immobilized carbohydrates. J. Biol. Chem. **266**, 24260, **1991.**
6. BOYER J. N. Aerobic and anaerobic degradation and mineralization of C-chitin by water column and sediment inocula of the York River estuary, Virginia. Appl. Environ. Microbial. **60**, 174, **1994.**
7. POULICEK M., JEUNIAUX C. Chitin biodegradation in marine environments: an experimental approach.Biochem. System. Ecol. **19**, 385, **1991.**
8. JASZKOWSKI K. Chitin could heal. Focus **8** (71), **2001.**
9. BEDNAREK R., DZIADOWIEC H., POKOJSKA U., PRUSINKIEWICZ Z. Ecology and soil science related research. Publishing House Science and Research PWN. **2004.**
10. HOPPE H.G. Use of fluorogenic model substrates for extracellular enzyme activity (EEA) measurements of bacteria, p. 509-512. [In:] Handbook of methods in aquatic microbial. Ecology. [Ed.] Kemp P.F., Sherr B. F., Cole J.J., Lewis, London. **1993.**
11. MARTINEZ J., SMITH D. C., STEWARD G. F., AZAM F. Variability in ectohygrolytic enzyme activities of pelagic marine bacteria and its significance for substrate processing in the sea. Aquatic Microbial Ecology. **10**, 223, **1996.**
12. WTW. Bedienungsanleitung System OxiTop® Control. Wissenschaftlich-technische Werkstätten (WTW), Weilheim. **1997.**
13. WTW. Applikationsbericht BSB 997 230: Respirometrische BSB_5-Bestimmung von häuslichem Abwasser mit dem OxiTop® Control – oder OxiTop®-Messystem. Wissenschaftlich-technische Werkstätten (WTW), Weilheim. **1998.**
14. PLATEN H, WIRTZ A. The measurement of respiration activity soil with a respirometric method (measuring system Oxi Top Control). WTW/OxiTop ®/Appliance. **1999.**
15. KOPEČNÝ J., HODRAOVÁ B., STEWART C. S. The isolation and characterization of a rumen chitinolytic bacterium. App. Microbial., **23**, 195, **1996.**
16. NICOL S. Life after death for empty shells. New Sci. **129**, 46, **1991.**
17. AYE K. N., KARUPPUSWAMY R., AHAMED T., STEVENS W. T. Peripheral enzymatic deacetylation of chitinand reprecipitated chitin particles. Biores. Technol. **97**, 557, **2006.**
18. HE H., CHEN X., SUN C., ZHANG Y., GAO P. Preparation and functional evaluation of oligopeptide-enriched hydrolysate from shrimp (*Acetes chinensis*)treated with crude protease from *Bacillus sp.* Sm98011. Biores. Technol. **97**, 385, **2006.**
19. WANG S. L., HUANG J. R. Microbial reclamation of shellfish waste fort he production of chitinases. Enzyme Microb. Technol. **28**, 376, **2001.**
20. PALUCH J. NIEWOLAK S. Microbiology of water. Warszawa, **1973.** [In Polish]
21. SWIONTEK BRZEZINSKA M. Occurrence and activity of chitinolytic bacteria of different trophy. Doctoral thesis, Department of Environmental Microbiology and Biotechnology, UMK, Torun, **2004.** [In Polish]
22. DONDERSKI W. Chitinolytic bacteria in water and bottom sediments of two lake of different trophy. Acta Microbial. Pol. **2**, 163, **1984.**
23. MUDRYK Z. The role of heterotrophic bacteria in the decomposition processes of some macromolecular compounds in the estuarine Gardno Lake, Pol. Arch. Hydrobiol. **38**, 153, **1991.**
24. PODGÓRSKA B. The role of bacteria in transformation of organic matter of sandy beach ecotones of the Baltic sea. Doctoral thesis. Institute of Oceanography PAN (Polish Academy of Science), Sopot; **2002.** [In Polish]
25. SKÓRCZEWSKI P. The role of bacterioneuston and bacterioplankton in transformation of organic matter in estuarine lake Gardno. Doctoral thesis. PAP, Słupsk, **2003.** [In Polish]
26. SUGITA H., OSHIMA K., FUSHION T., DEGUCHIO Y. Substrate specify of heterotrophic bacteria in the water and sediment of carp culture pond. Aquaculture, **64**, 39, **1987.**
27. HUFSCHMID A., BECKER-VAN SLOOTEN K., STRAWCZYNSKI A., VIOGET P., PARRA S., PÃRINGER P., PULGARIN C. BOD_5 measurements of water presenting inhibitory Cu^{2+}. Implications in using of BOD to evaluate biodegradability of industrial wastwwaters. Chemosphere **50,** 171, **2003.**
28. VÄHÄOJA P., KUOKKANEM T., VÄLIMÄKI I., VUOTI S., PERÄMÄKI. Biodegradabilities of some chain oils in groundwater as determined by the respirometric BOD OxiTop method. Anal Bioanal Chem. **381,** 225, **2005.**
29. REUSCHENBACH P**.,** PAGGA U., STROTMANN U. Water Res **37**, 1571, **2003.**
30. DONDERSKI W. Aerobic heterotrophic bacteria lakes of different trophy. Wyd. UMK Torun, **1983.** [In Polish]
31. STRZELCZYK E., STOPIŃSKI M., MYZYK G. Studies on metabolic activity of single and mixed cultures of planktonic and benthic bacteria of two lakes different trophy. AUNC Torun Limnol. Papers **16**, 3, **1988.**
32. MASSARDIER-NAGEOTTE V., PESTRE C., CRUARD-PRADET T., BAYARD R. Aerobic and anaerobic biodegradability of polymer films and physico-chemical characterization. polymer Degradation and Stability **91,** 620, **2006.**
33. GODLEWSKA – LIPOWA W. O_2 – consumption as an indicator of heterotrophic activity of bacteria in lakes of different trophic conditions. Arch. Hydrobiol. Beih., **12**, 11, **1979.**
34. DONDERSKI W., STRZELCZYK E. Manometric studies with bottom sediments of three lakes. Act Microbial. Pol. **29**, 21, **1980.**
35. RHEINHEIMER G. Microbiology of water. PWRiL, Warszawa, **1987.** [In Polish]
36. EL – TARABILY K. A., SOLIMAN M. H., NASSAR A. H., AL – HASSANI H. A., SIVASITHAMPARAM K, MCK-

ENNA F., HARDY G. E. ST. J. Biological control of Sclerotinia minor using a chitinolytic bacterium and actinomycetes. Plant Pathology **49**, 573**, 2000.**

37. WANG S. L., SHIH I. L., LIANG T. W., WANG C. H. purification and characterization of two antifungal chitinases extracellularly produced by *Bacillus amyloliquefaciens* V 656 in a shrimp and crab shell powder medium. J. Agric. Food Chem. **50**, 2241, **2002.**
38. WANG S. L., YIEH T. C., SHIH I. L. Purification and characterization of a new antifungal compound produced by *Pseudomonas aeruginosa* K-187 in a shrimp and crab shell power medium. Enzyme Microb. Technol. **25,** 439, **1999.**
39. CHANG W-T., CHEN Y-CH., JAO CH-L. Antifungal activity and enhancement of plant growth by *Bacillus cereus* grown on shellfish chitin wastes. Bioresource Technol. **98**, 1224, **2007.**