Polish J. of Environ. Stud. Vol. 19, No. 3 (2010), 627-633

Original Research

Microbial Degradation of Shrimp Waste in Soil

Maria Swiontek Brzezinska*, Elżbieta Lalke-Porczyk, Maciej Walczak, Wojciech Donderski

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

Received: 2 October 2009
Accepted: 30 December 2009

Abstract

This study presents results of research on a number of microorganisms in soil of the Chełmżyńskie lake watershed and their role in the decomposition of chitin. The authors also examined the level of respiration activity of soil in the presence of shrimp waste. Results demonstrate that the number of microorganisms in soil were variable. During the entire research period, the analyzed groups of microorganisms predominated in ploughland soils with the number of heterotrophic bacteria significantly exceeding that of actinomyces and fungi. The proportion of microorganisms capable of decomposing chitin was greater among actinomycetes and fungi than among heterotrophic bacteria. Chitinolytic bacteria constituted 15-25% of the total number of heterotrophic bacteria and their proportion was higher in sandy soil. Chitinolytic fungi constituted 29-42% and also dominated in sandy soil. Chitinolytic actinomycetes constituted 48-69% and also dominated in sandy soil. The level of respiration activity soil statistically depended on examined factors. Respiratory activity was highest at 24ºC (in summer) and lowest at 6ºC (in spring). During the entire research period, the microorganisms inhabiting sandy soil were more readily able to take advantage of shrimp waste and found shrimp heads the most useful, shells the least useful.

Keywords: soil, OxiTop, shrimp waste

Introduction

Chitin is a very common naturally occurring polymer. The annual production of chitin in the biosphere has been estimated at 10^{10}-10^{11} tons [1]. The presence of chitin in soil is primarily the result of the decay of fungi and insects inhabiting soil permanently or temporarily. The chitin content in mycelium varies from 2.6 to 26.2% of its dry mass, depending on the conditions prevailing in the soil (pH, temperature) and fungus age. Chitin is also present in annelids and some mollusks, and forms the cell wall of diatoms. This polymer also has been found in some algae [2].

Due to its qualities, chitin is a universal substance. As a result of the research conducted on chitin structure and properties, this material has been applied in many fields, including biotechnology, agriculture, medicine, and environmental protection. In the fishing industry, "crab shells" have always been treated as common waste, which, at best, 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 effective due to the fact that the shells are broken down by enzymes, and the acetylglucosamine units of chitin hinder the development of fungi and nematodes in soil [3]. In Taiwan, where seafood is processed on an industrial scale, enormous amounts of waste containing chitin are produced. Utilization of this waste has brought about two benefits: decreased pollution and the production of chito-oligosaccharides, which are useful in biotechnology [4]. Thus, the purpose of this study was to determine the abundances of

*e-mail: swiontek@umk.pl

heterotrophic bacteria, microscopic fungi and actinomycetes in soil and their role in breaking down chitin, as well as to examine the level of respiration activity of soil microorganisms in the presence of shrimp waste.

Materials and Methods

Site

Our research was conducted in the catchment area of Chełmżyńskie Lake. Chełmżyńskie and its catchment area are located in the Kujawsko-Pomorskie voivodeship, in the Chełmińsko-Dobrzyński region. The lake is situated in the southern part of the Pojezierze Chełmińskie region within the Fryba river basin. The landscape is characterized by young glacial features with ice-marginal landforms, eskers, kames, and ribbon lakes typical of this landscape. According to studies conducted by the Provincial Inspectorate of the Environmental Protection in Bydgoszcz, leached soils are the most common in this catchment basin. These soils occur in the southwestern and northern sections of the watershed. Podzoil soils predominate in the southeastern section of the watershed. Silty, peaty, and muck soils occur in areas strongly saturated with underground water and overgrown with peat-forming plants. The areas with these types of soils are used as grassland. Deposits of black earth characterized by considerable thickness of the humic layers (up to 60 cm) and high content of organic carbon sometimes occur among brown soils. Most of the catchment basin area is occupied by ploughland (24.85 km^2). Meadows and pastures occupy 8.875 km^2, while only 0.71 km^2 of the land is forested.

Sampling

Samples of sandy and ploughland soils characterized by alkaline and neutral pH were collected for analyses. The samples were collected into sterile jars, which were then placed on ice in an insulated container and transported to the laboratory. Analysis started within 2 hours of sample collection. Samples were collected in the spring (3 April 2007), summer (21 July 2007), and autumn (20 October 2007). The soil reaction was determined using the potentiometric method [5].

Determining Number of Microorganisms in Soil

The number of microorganisms in soil were determined using Koch's plate technique by the surface inoculation on appropriate culture media. Analyzed samples were 10-times diluted, and 0.1 ml samples were placed on the medium surface. Inoculation was carried out in three parallel replicates. The number of heterotrophic bacteria were determined using a medium containing (g/l): peptone (0.1), iron sulfate (0.1), ammonium sulfate (0.1), iron gluconate (0.1) and agar (15). Nystatin (0.1 g/l) was added to the medium to inhibit the growth of fungi. After a 7-day incubation at 22ºC, 50-colony from each sample were inoculated to test tubes with the plate count agar (Merck) medium, and used to determine the chitinolytic properties. Actinomycetes were determined using medium containing (g/l): sodium caseinate (2.0), ferrous sulfate (0.01), asparagine (0.1), magnesium sulfate (0.1), dipotassium phosphate (0.5), and agar (15). Actinomycetes were incubated for 14 days at 28ºC, 50-colony from each sample were inoculated to test tubes with a fresh culture medium with the above composition, and used to determine the chitinolytic properties. On classifying the colony into actinomyces, we considered the following characteristics [6]: the colony edges blurred because of radiating hyphae of substrate mycelium, the colony surface appearance (matt, chalk, down-like, sometimes aerial mycelium noticeable), the colony structure (tough, compact, leather-like), substrate-adherence degree-high, some actinomyces' colonies give off strong soil-like smell.

Microscopic fungi were determined using the Czapek Dox medium containing (g/l): sodium nitrate (3), potassium dihydrogen phosphate (1), saccharose (20), potassium chloride (0.5), magnesium sulfate (0.5), iron sulfate (0.01), and agar (15). Streptomycin (30 mg/l) was added to the culture medium to inhibit the growth of bacteria. Following a 14-day incubation at 25ºC, 50-colonies from each sample were inoculated to test tubes with the Czapek Dox medium, and used to determine the chitinolytic properties. The purity of fungi was examined with macroscopic and microscopic methods by staining the samples with methylene blue in lactophenol.

Determination of Chitinolytic Properties

The chitinolytic properties heterotrophic bacteria, fungi and actinomycetes isolated from soil were determined with the fluorometric method [7, 8].

The microorganisms isolated from soil were cultured in 250 ml Erenmeyer flasks containing 100 ml of a culture medium. Bacteria were cultured in a medium containing (g/l): peptone (0.1), iron sulfate (0.1), ammonium sulfate (0.1), yeast extract (0.1), iron (II) sulfate (0.1), and colloidal chitin (20). Fungi were cultured in a medium containing (g/l): sodium nitrate (3), potassium dihydrogen phosphate (1), saccharose (20), potassium chloride (0.5), magnesium sulfate (0.5), iron sulfate (0.01), and colloidal chitin (20). And finally, actinomycetes were cultured in medium containing (g/l): potassium phosphate dibasic (0.7), potassium phosphate monobasic (0.3), magnesium sulfate (0.5), zinc sulfate (0.001), manganese chloride (0.001), and colloidal chitin (20). The culture medium was inoculated with 1 ml samples of microorganism suspension obtained from a 72-hour incubation on agar slants. Each strain was analyzed in two replicates. Incubation was carried out over 4 days at 25°C, and then the cultures were centrifuged at 10,000 g/min. for 10 min. at + 4ºC.

The activity of exo-chitinases produced by microorganisms was determined using the synthetic fluorogenic substrate 4-methylumbelliferyl N-acetyl-β-D-glucosaminide

Table 1. The number of microorganisms (CFU·10^3·g^{-1} d.w.) in soils of the Chełmżyńskie Lake watershed, ± standard deviation (n = 3), in the bracket – percent chitinolytic microorganisms.

Season	Soil	Heterotrophic bacteria		Microscopic fungi		Actinomycetes	
spring	ploughland	2,500±8.2	(15)	33±5.0	(32)	243±6.0	(48)
	sandy	1,500±3.0	(17)	20 ±2.0	(35)	120±3.0	(60)
summer	ploughland	767±7.0	(13)	56±6.0	(32)	166±2.0	(45)
	sandy	131±2.0	(17)	45±3.0	(40)	90±4.0	(51)
autumn	ploughland	720±1.0	(20)	22±2.0	(32)	130±3.0	(60)
	sandy	500±2.0	(25)	17±3.0	(40)	97±2.0	(69)

(4MU- GlcNac) (Sigma-Aldrich, Poznan, Poland). Methylcellosolve solvent (EGME, C3H8020) (Sigma-Aldrich, Poznań, Poland) was used to prepare a basic 1 mmol L^{-1} solution of 4MU-GlcNac that was then stored at -20ºC. Prior to analysis, a 0.5 mmol L^{-1} working solution was obtained by diluting the basic solution twofold with spectrally pure water. For all assays, duplicates of 4.0 ml acellular post-culturing liquid, 0.5 ml substrate solution (final concentration in a sample was 50 µmol L^{-1}) and 0.5 ml of potassium phosphate buffer (final concentration 10 mmol L^{-1}; pH of the sample) were mixed and incubated for hours in darkness at in situ temperature. The control sample, prior to addition of the substrate, was heated in boiling bath in order to deactivate the enzymes present in the sample. After incubation enzymatic reactions were interrupted thermally. Fluorescence was additionally measured in all analyzed and control samples at the beginning and after incubation. The liberated methylumbelliferone was measured fluorimetrically at 318 nm excitation and 445 nm emission using the Hitachi F 2500 spectrofluorometer. The spectrofluorometer was calibrated with a solution of pure methylumbelliferone (Sigma-Aldrich, Poznań, Poland). One unit of chitinase activity was defined as 1 µmol 4-methylumbelliferone (MU) liberated per hour.

Determination of Soil Dry Weight

In order to determine the dry weight of soil, 10 g soil was dried at 105ºC, and after the result into 1 g dry weight (d.w.)

Determination of Respiration Activity Soil in Presence of Shrimp Waste

The level of respiration activity in soil containing shrimp waste was determined with a respirometric method, measuring system Oxi Top Control 12 [9] based on Platen and Wirtz [10] and the author's own materials. Samples of sandy and ploughland soils (100 g) were put into 500 ml measuring containers. After temperature stabilization, shrimp waste (0.4 g) was added to the samples, and carriers with absorbent CO_2 (0.4 g NaOH) were placed in the containers. The measuring containers were placed in a thermostatic cabinet. Measurements were recorded in the OC 110 control system in the "Pressure p" mode. The activity was measured at the *in situ* temperature for 5 days. In the presence of easily degradable substances, it is used to report BOD_5. The following variants were used during the study: variant I – all parts of the shrimp waste were added to soil, variant II – only head sections of shrimp were added to soil, variant III – chitinous shells were added to soil.

Prior to the addition to soil, shrimp waste was dried at 105ºC, ground, and sterilized in an autoclave for 20 min. at 117ºC. A soil sample without shrimp waste addition was used as a control (endogenous respiration). All samples were analyzed in two replicates. For analysis, the authors used waste produced by the Krymar company 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. According to Sabry [11] shrimp-shell waste is mainly composed of chitin (21.4%), calcium carbonate (40%) and protein 27.9%). Similar results were previously reported for shrimp and crab shell waste [12].

Statistical Analysis

Results were analyzed in STATISTICA 6.0, StatSoft, USA. Aanalysis of Variance (ANOVA) was the primary statistical method used in calculations. This method facilitated comparison of the following independent factors: season, type of soil, incubation time, and type of respiration substrate.

Results

The number of heterotrophic bacteria and fungi and actinomycetes in soil varied and depended on the type of soil and the season. The number of heterotrophic bacteria in the analyzed samples ranged from 131·10^3 to 2,500·10^3 CFU·g^{-1} d.w. The heterotrophic bacteria were the most numerous in the spring (2,500·10^3 CFU·g^{-1} d.w. in ploughland soil and 1,500·10^3 CFU·g^{-1} d.w. in sandy soil). The number of fungi was significantly lower and ranged from

Table 2. ANOVA test comparing the influence of examined factor on respiratory soil activity.

Factor	df	variation	F ratio	p - level
Temperature	2	40,891.0	53.1564	< 0.000000
Soil type	1	4,667.6	6.0677	< 0.015530
Incubation time	5	27,552.1	35.8165	< 0.000000
Respiration substrate	2	20,727.2	26.9444	< 0.000000
Inergroup variability	97	769.3		

df – number of independent variables, F ratio: among – group variance to within – group variance, p – significant level

$17 \cdot 10^3$ to $56 \cdot 10^3$ CFU·g^{-1} d.w. and their maximum was observed in the summer ($56 \cdot 10^3$ CFU·g^{-1} d.w. in ploughland soil and $45 \cdot 10^3$ CFU·g^{-1} d.w. in sandy soil) The number of actinomycetes in the analyzed samples ranged from $90 \cdot 10^3$ to $246 \cdot 10^3$ CFU·g^{-1} d.w. The actinomycetes were the most numerous in the spring ($243 \cdot 10^3$ CFU·g^{-1} d.w. in ploughland soil and $120 \cdot 10^3$ CFU·g^{-1} d.w. in sandy soil) (Table 1).

The proportion of microorganisms capable of breaking down chitin was greater among actinomycetes and fungi than among heterotrophic bacteria. Chitinolytic bacteria constituted 15-25% of the total number of heterotrophic bacteria and they were more common in sandy soil. Chitinolytic fungi constituted 29-42% and also dominated in sandy soil. Until 45-69%, actinomycetes were capable of breaking down chitin. They were also more common in sandy soil (Table 1).

The level of respiration activity soil statistically depended on examined factors (Table 2). The temperature and duration of incubation ($p<0.00$), the type of soil ($p<0.015$), and the type of respiration substrate ($p<0.00$) had a statistically significant impact on the activity. Respiration activity in present shrimp waste was proportional to incubation duration (Table 3, Fig. 1). The highest activities were recorded in summer (in ploughland soil: all parts – 140 mg $O_2 \cdot g^{-1}$ d.w shrimp, heads – 210 mg $O_2 \cdot g^{-1}$ d.w. shrimp and shells – 90 mg $O_2 \cdot g^{-1}$ d.w. shrimp. In sandy soil: all parts – 220 mg $O_2 \cdot g^{-1}$ d.w. shrimp, heads – 260 mg $O_2 \cdot g^{-1}$ d.w. shrimp and shells – 90 mg $O_2 \cdot g^{-1}$ d.w. shrimp). The lowest respiratory activites were in spring (Table 3, Fig. 2). During the entire research period, the microorganisms inhabiting sandy soil were more readily able to utilize shrimp waste and found shrimp heads the most useful; shells, the least (Table 2, Fig. 3).

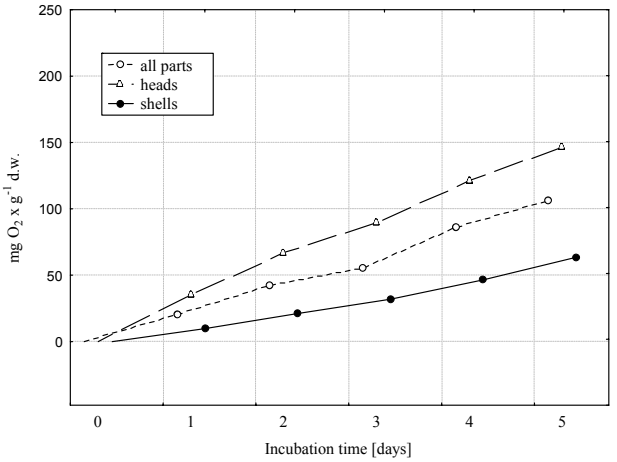

Fig. 1. Biological oxygen demand in soil in the presence of shrimp waste (average).

Discussion

Chitin is an important building material and occurs in particular living organisms. This polymer is essential in supporting the external skeleton of invertebrates; it is also an important structural component of exoskeletons of crustaceans, insects, and arachnids. Chitin also occurs in annelids, some mollusks, diatoms, and some algae [2]. In the soil environment, chitin is continuously synthesized by fungi. It is believed that decomposition of fungi and insects permanently or temporarily inhabiting the soil is the main source for such large quantities of this polymer in soil [2].

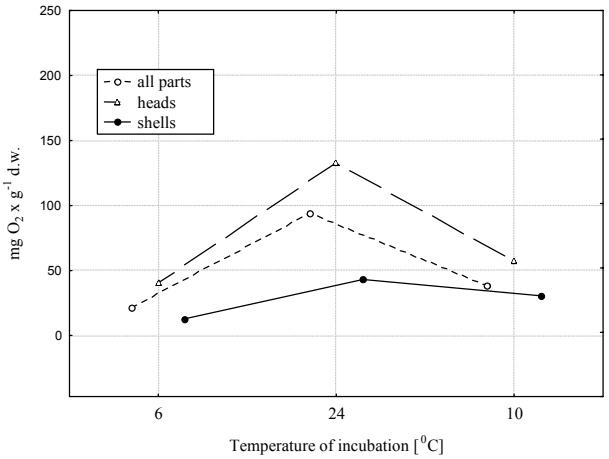

Fig. 2. Impact of temperature on respiration activity in soil (average).

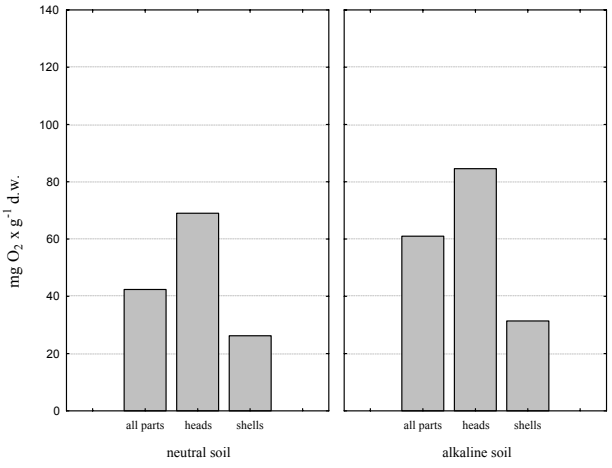

Fig. 3. Respiratory activity depending on the soil type.

Table 3. Respiration activity (mg $O_2 \cdot g^{-1}$ d. w.) soil in present shrimp waste. ± standard deviation (n = 3).

Season	Soil	Temperature of incubation	Respiration substrate	Incubation time [days]				
				1	2	3	4	5
spring	ploughland	6ºC	all parts	5±1.0	10±1.0	18±1.0	30±1.1	50±2.1
			heads	10±1.1	25±1.1	35±1.1	65±1.0	87±2.2
			shells	0±0	5±2.1	8±1.2	19±2.2	28±2.0
	sandy	6ºC	all parts	8±1.2	16±2.0	22±2.1	35±2.1	69±1.2
			heads	25±1.0	35±1.2	48±2.0	68±2.2	89±1.2
			shells	0±0	10±2.2	15±1.1	25±1.0	45±2.3
summer	ploughland	24ºC	all parts	33±2.1	80±2.3	90±2.2	120±1.1	140±1.0
			heads	55±1.2	120±1.0	160±2.0	180±1.2	210±2.6
			shells	17±1.3	45±2.1	60±1.2	80±2.2	90±2.4
	sandy	24ºC	all parts	51±1.3	90±1.0	120±1.1	190±2.3	220±3.3
			heads	68±2.1	140±1.1	180±1.3	220±2.1	260±3.1
			shells	10±3.1	20±1.0	45±1.2	60±2.3	90±2.4
autumn	ploughland	10ºC	all parts	8±2.2	19±2.1	25±1.0	65±1.1	70±1.0
			heads	15±2.0	33±2.0	52±2.0	85±1.2	110±2.3
			shells	5±2.1	10±2.2	22±2.2	35±2.2	48±2.4
	sandy	10ºC	all parts	20±1.4	37±1.0	55±2.1	76±2.3	89±1.1
			heads	40±1.0	50±2.3	65±2.0	110±2.1	125±2.0
			shells	27±1.2	37±2.1	42±2.0	60±1.0	80±3.1

The quantitative and qualitative composition of microorganisms in soil depends on the soil type, its structure, humidity, and oxygenation level. In the analyzed soils, the abundance of heterotrophic bacteria was much higher than that of fungi and actinomycetes. In general, microorganisms were more common in ploughland (neutral soils) than in sandy soils (alkaline soils). The neutral pH and greater access to organic matter in ploughland soils had a significant impact on the growth of microorganisms. The type and abundance of microorganisms in soils were also largely affected by agricultural operations and the type of crops. Numerous researchers [2, 13, 14] maintain that agrotechnological treatment and fertilization had a significant impact on abundances of microorganisms and selection of entire groups in soil. Having analyzed the microbiological activity of soil of a mountain meadow, Barabasz et al. [15] reported that mineral fertilization of soil, and especially nitrogen fertilization, had a positive impact on the microbiological activity of the soil. The authors observed the greatest increase in bacterial abundance in the analyzed soil, while changes in the abundances of fungi and actinomycetes were insignificant.

Chitin, in spite of its complex structure, constitutes an important source of carbon and nitrogen for heterotrophic microorganisms [16], and its breakdown may be catalyzed by chitinases synthesized primarily by bacteria, fungi, and actinomycetes. One gram of ploughed soil contains up to 10^6 organisms capable of decomposing chitin [17]. According to Marszewska-Ziemięcka et al. [2] chitin is most effectively decomposed by actinomycetes in soil. Paul and Clark [18] suggests that as much as 90% of soil actinomycetes are capable of breaking down this polysaccharide. In contrast, El-Fiky et al. [19] demonstrated that only 7% actinomycetes from the rhizosphere of tomato and green pepper were capable of decomposing chitin. Active actinomycetes participate in chitin remineralization; however, it is certain that bacteria and fungi also significantly contribute to the decomposition of this polysaccharide in soil [2, 17, 18]. According to the results of this study, heterotrophic bacteria play an important role in breaking down chitin, but the role of fungi is even greater. Podgórska [20], who examined microflora of sandy beach ecotones, found low abundances of chitinolytic bacteria in beach sand in Sopot. However, the author does not specify to what extent fungi contributed to the decomposition of chitin. Gray and Baxby [21] and Sarathchandra et al. [22] observed that soil pH and humidity had a significant impact on the type of microorganisms that break down chitin in soil. In acidic soils, decomposition of chitin is dominated by fungi. On the other hand, bacteria are the most important microorganisms

capable of chitin decomposition in neutral or alkaline soils with high humidity [23]. De Boer et al. [24] came to similar conclusions after examining chitinolytic organisms of dune soil. Based on our research, it can be concluded that the abundance of both chitinolytic bacteria and fungi were higher in alkaline soils. In this case, the content of organic matter in soil, and not its pH, could have been the determining factor. Sandy soils are poorer in organic matter; as a result, microorganisms utilize all available sources of carbon and nitrogen even if the compounds are hard to break down. We previously observed a similar relationship in water and bottom sediments of lakes with different nutrient contents. Between 5.4 and 11.4% of heterotrophic bacteria present in water of eutrophic lake Jeziorak were capable of breaking down chitin, while in oligomesotrophic lake Jasne, this value ranged from as much as 10.2 to 18.7%. In bottom sediments of lake Jeziorak, there was only 2.7-4.4% of chitinolytic bacteria, and in lake Jasne 4.9-7.3% [25].

Oxygen plays an important role in chemical and biological processes in both aquatic and soil ecosystems. 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 biotests of water analysis. This test is widely applied to define organic water pollution and to control the performance of wastewater treatment plants [26]. Vähäoja et al. [27], BOD OxiTop method used to biodegradation of different types of forestry oils-hydraulic, motor and chain oils-in a groundwater environment and in forest soil. Our study was conducted in environmental conditions that play an important role in the decomposition of organic matter. In the presence of shrimp waste, the respiration activity of microorganisms in analyzed samples was significantly correlated with temperature and incubation duration. A temperature of 24ºC had the highest oxygen absorption by microorganisms, while at 6ºC this process was much slower. The incubation duration 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. In addition to chitin, shrimp waste contains large quantities of protein and lipid. It is most probable that microorganisms used the protein and lipids first, followed by chitin. Shrimp shells that contain chitin, which is difficult to break down, were used by microorganisms at the slowest rate. The results of numerous studies [28, 29] have 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 is considered a difficult-to-assimilate compound whose oxidation requires a large amount of energy. Therefore, in order to investigate the respiration activity of microorganisms, the authors used a method that facilitates the recording of data 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 low and disproportionate to the activity that occurs in situ. Massardier-Nageotte et al. [30] investigated the aerobic biodegradability of four different polymers by microbial inoculum extracted from the soil using an OxiTop respirometer. Among the 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 were tested. The positive degradation reference compound selected was cellulose, and polyethylene served as a negative reference (as a "non-biodegradable polymer"). All tested polymers were degraded the most after 28 days. Vähäoja et al. [27] report that biodegradation of different chain oils (mineral, tall and rapessed oils) in groundwater environment was proportional to the incubation time. The oils were degraded the most after 28 days.

Our study demonstrated that the microorganisms were able to better utilize shrimp waste in an alkaline environment (sandy soil). The total number of analyzed microorganisms was higher in neutral soils (ploughland), but alkaline soils (sandy soil) had more chitinolytic bacteria, fungi and actinomycetes. These microorganisms are more adapted to assimilate chitin compounds due to their chitinolytic properties. Furthermore, sandy soils are poor in nutrients and this forces microorganisms to use compounds that are difficult to assimilate. On the other hand, it could be assumed that bacteria assimilate a larger fraction of shrimp waste than fungi. Heterotrophic bacteria develop better in an alkaline environment than fungi and because of that, they can be more active metabolically. Furthermore, this study demonstrated that chitinolytic activity of fungi is lower than that of bacteria. Bacteria produce chitinases, which dissolve cell walls of fungi, and thus limit or even halt their development [31]. Our earlier study [32] demonstrated that respiratory activity was highest at 24ºC (in summer) and lowest at 6ºC (in spring). During the entire research period, the microorganisms inhabiting sandy soil were more readily able to take advantage of shrimp waste and found shrimp heads the most useful; shells the least. Respiration activity depends on the temperature, substrate and type of soil, but the changes of respiration activity are not always correlated with the growth and development of microorganisms.

References

1. GOODAY G.W. Physiology of microbial degradation of chitin and chitosan. Biodegradation **1**, 177, **1990**.
2. MARSZEWSKA-ZIEMIĘCKA J., MALISZEWSKA W., MYŚKÓW W., STRZELCZYK E. Microbiology soil and manure organic. PWRiL, Warszawa, **1974**.
3. JASZKOWSKI K. Chitin could heal. Focus **8**, (71), 25, **2001**.
4. HUANG J.H., CHEN C.J., SU Y.C. Production of chitinolytic enzymes from a novel species of *Aeromonas*. J. Ind. Microbiol. **17**, 89, **1996**.

5. BEDNAREK R., DZIADOWIEC H., POKOJSKA U., PRUSINKIEWICZ Z. Ecology and Soil Science Research. PWN Warszawa pp. 344, **2004**.
6. KÄMPFER P. The Family *Streptomycetaceae*, Part I: Taxonomy. Prokaryotes **3**, 538, **2006**.
7. HOPPE H.G. Use of fluorogenic model substrates for extracellular enzyme activity (EEA) measurements of bacteria. [In:] Handbook of methods in aquatic microbial. Ecology [red] Kemp PF, Sherr BF, Cole JJ, Lewis London pp. 509, **1993**.
8. 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 Microbiol. Ecol. **10**, 223, **1996**.
9. BOD measurement with OxiTop Control system. WTW, **1997**.
10. PLATEN H., WIRTZ A. The measurement of respiration activity soil with a respirometric method (measuring system Oxi Top Control). WTW/OxiTop®/Appliance, **1999**.
11. SABRY S.A. Microbial degradation of shrimp-shell waste. J. Basic Microbiol. **32**, (2), 107, **1992**.
12. REVAH-MOISEEV S., CORROAD P. A. Conversion of the enzymatic hydrolysate of shell fish waste chitin to single-cell protein. Biotechnol. Bioeng. **23**, 1067, **1981**.
13. BARABASZ W., FILIPEK-MAZUR B., BABRASZ J. The preliminary estimation of soil biological activity under the conditions of long – term fertilizing experiments. Proceedinds of the International Symposium "Long – term static fertilizer experiments". Warsaw-Cracow, pp. 267, **1993**.
14. SMYK B., RÓŻYCKI E., KOPEĆ S. Influence of mineral fertilise on microbiological activity of soils and production of meadow mountain ecosytems. Zeszyty Problemowe Postępów Nauk Rolniczych **162**, 203, **1975**.
15. BARABASZ W., FILIPEK-MAZUR B., CHMIEL M.J., GRZYB J., FRĄCZEK K. Microbiological activity of soil after 30 years of statistical dune experiment in Czarny Poto near Krynica. Zeszyty Problemowe Postępów Nauk Rolniczych **465**, 647, **1999**.
16. MC CREATH K.J., GOODAY G.W. A rapid and sensitive microassay for determination of chitynolytic activity. J. Microbiol. Meth. **14**, 229, **1991**.
17. SCHLEGEL G.H. General Microbiology. PWN. Warszawa, **2003**.
18. PAUL E.A., CLARK F.E. Microbiology and biochemistry of soil. Wydawnictwo Uniwersytetu Marii Curie – Skłodowskiej Lublin, **2000**.
19. EL-FIKY Z.A., MANSOUR S.R., EL-ZAWHARY Y., ISMAIL S. DNA – fingerprints and phylogenetic studies of some chitinolytic actinomycete isolates. Biotechnology, **2**, 131, **2003**.
20. 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**.
21. GRAY T.R.G., BAXBY P. Chitin decomposition in soil. The ecology of chitinoclastic microorganisms in forest soil. Trans .Br. Soc. **51**, 293, **1968**.
22. SARATHCHANDRA S.U., WATSON R.N., COX N.R., MENNA M.E. BROWN J.A., BURCH G., NEVILLE F.J. Effects of chitin-amendment of soil on microorganisms, nematodes and growth of white clover (*Trifolium repens* L.) and perennial ryegrass (*Lolium perenne* L.). Biol. Fertil. Soils **22**, 221, **1996**.
23. VELDKAMP H. A study of the aerobic decomposition of chitin by microorganisms. Meded Landbouwhogesch Wageningen **55**, 127, **1955**.
24. DE BOER W., GERARDS S., GUNNEWIEK P.J.A., MODDERMAN R. Response of the chitynolytic microbial community to chitin amendments of dune soil. Biol. Fertil. Soils **29**, 170, **1999**.
25. SWIONTEK BRZEZINSKA M. Occurrence and activity chitynolytic of bacteria in lakes of different trophy. Ph. Doctor's Thesis. Institute of Ecology and Environmental Protection (Nicolaus Copernicus University) Toruń, **2004**.
26. HUFSCHMID A., BECKER-VAN SLOOTEN K., STRAWCZYNSKI A., VIOGET P., PARRA S., PÉRINGER P., PULGARIN C. BOD_5 measurements of water presenting inhibitory Cu^{2+}. Implications in using of BOD to evaluate biodegradability of industrial wastewaters. Chemosphere **50**, 171, **2003**.
27. VÄHÄOJA P., KUOKKANEM T., VÄLIMÄKI I., VUOTI S., PERÄMÄKI P. Biodegradabilities of some chain oils in groundwater as determined by the respirometric BOD OxiTop method. Anal. Bioanal. Chem. **381**, 445, **2005**.
28. DONDERSKI W., STRZELCZYK E. Manometric studies with bottom sediments of three lakes. Acta Microbiol. Pol. **29**, 21, **1980**.
29. GODLEWSKA-LIPOWA W. O_2 – consumption as an indicator of heterotrophic actinity of bacteria in lakes of different trophc conditions. Arch. Hydrobiol. Beih. **12**, 11, **1979**.
30. MASSARDIER-NAGEOTTE V., PESTRE C., CRUARD-PRADET T., BAYARD R. Aerobic and anaerobic biodegradability of polymer films and physico – chemical characterization. Polym. Degrad. Stabil. **91**, 620, **2006**.
31. EL-TARABILY K.A., SOLIMAN M.H., NASSAR A.H., AL-HASSANI H.A., SIVASITHAMPARAM K., MCKENNA F., HARDY G.E. ST. J. Biological control of *Sclerotinia minor* using a chitinolytic bacterium and actinomycetes. Plant Pathology **49**, 573, **2000**.
32. SWIONTEK BRZEZINSKA M., LALKE-PORCZYK E., DONDERSKI W. Shrimp waste as a respiration substrate for soil mikroflora of the Chełmżyńskie Lake watershed. P. J. Natur Sc. **23**, (1), 164, **2008**.