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MICROORGANISMS POTENTIALLY USEFUL IN THE MANAGEMENT OF POLYURETHANE FOAM WASTE

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Abstract

Polyurethane foams due to their easily regulated domain structure are group of polymers with highly variable properties. They are widely used to provide comfort in many areas of daily life. Because of toxic products polyurethane foam waste cannot be disposed by means of thermal degradation, they also show a high resistance to chemical and physical factors. Those properties combined with their low apparent density and widespread use (about 18 million tons per year) lead to a significant volume of waste that is stored in landfills. In Poland, more than 60% of those landfills, certified as well as illegal, are located in rural areas.

The aim of this study was to determine the enzymatic abilities of microorganisms isolated from foam waste, which can potentially decompose polyurethane (ureases, esterases, proteases, and laccases), and to determine their ability to grow on a medium containing polyurethane foam as the only carbon source.

Most of the tested strains produced ureases while the least produced were laccases and proteases. Four of the tested strains: *Epicoccum nigrum*, *Aspergillus niger, Staphylococcus xylosus* and *Rhodococcus* spp. showed significant growth on the medium with polyurethane foam as sole carbon source.

Keywords: polyurethane foam, biodegradation, PUR

INTRODUCTION

Plastic materials are used since mid-ninetieth century. Their usage has been increasing since 1950 for average 8.7% per year till it reached 26 million in 2014 (PlasticsEurope reports 2013 and 2015). Unfortunately, with the increase of the consumption of these materials there is observed growth in amount of wastes that are polluting environment. At present, more than 30% of plastics produced in Europe end their lives on landfill (Wirpsza, 1992).

Data of Polish Central Statistical Office indicate that in 2015 in Poland 394 controlled municipal waste landfills operated with a total area of 1 927.0 hectares. 284 of these landfills are located in rural areas occupying 1 258.3 hectares. This means that the problem of the accumulation of plastic waste is largely transferred to the rural areas. Another concern is about so-called "wild landfills". It is estimated that in Poland more than 10 000 of them is functioning and about 60% of that amount occurs in rural areas. Furniture, mattresses, and other everyday objects, which include polyurethane foams, are found in large quantities in certified landfills as well as illegal dumps.

Production of polyurethane (PUR) on an industrial scale started in the fifties of the twentieth century and is still growing, which is associated with the use of new technological solutions that allow to reduce production costs and increase the ability to model their properties (Wirpsza, 1992; Cregut et al., 2013). At present annual global production of PUR reaches about 20 million tons.

PUR is a group of polymers with widely differing properties and broad application. The polymer consists of alternately arranged rigid segments (made from isocyanates and extending compounds) and flexible ones (usually polyether or polyester chains), between which there are hydrogen bonds in significant number (Stepien 2010; Howard, 2002). This permits combining flexibility of the product with wear resistance, mechanical strength and hardness (Wirpsza, 1992).

Properties of polyurethanes are a result of chemical and spatial structure of their molecules. These polymers do not have a defined structure of sequencing monomers, but the basic building block for most of them are polyisocyanates. Their cyanate group reacts with hydroxyl group of polyols what leads to creation of urethane bond that is the characteristic bond of all PURs. Type of isocyanate, polyol mixture composition and their relative amounts can be changed depending on the desired characteristics of the product (Wirpsza, 1992).

The PUR foam materials stand for approximately 85-90% of polyurethane products. They are used in many areas of life in order to ensure the comfort (mattresses, upholstered furniture, clothes) and safety, as a means of thermal isolation, acoustic isolation, sport safety mattresses etc. There are three groups of PUR foams: flexible, rigid and semi-rigid foams, with the highest application rate for flexible foam (about 66%) (Wirpsza, 1992; Howard, 2002).

Polyols most commonly used for polyurethanes synthesis are polyethers and polyesters. Polyether foams have a larger market share due to the much lower price of polyether polyols and their greater resistance to hydrolysis and chemical agents. Polyester plastics are used in products that require a higher mechanical strength (Gomez et al., 2014).

Polyurethane waste can be classified as production waste, resulting from the typical production processes, and postconsumer waste, produced by final customers. Amount of the second type of waste is estimated for 30 million tons per year (Cregut et al., 2013). Management of those waste can be divided in three routes: collecting on landfills, incineration and recycling. Due to the formation of toxic products during combustion and the high appliance cost, thermal utilization is the least frequently used method. Since polyurethanes are not soluble in water or conventional organic solvents (such as ethanol, methanol, isopropanol or acetone), and their chemical structure provides a resistance to pH over a wide range or temperature changes, the degradation by chemical agents is not effective enough (Wirpsza, 1992).

Most of the polyurethane foams recycling methods are only applicable to the waste from production processes, not solving the problem of those from the users of finished products. Another difficulty in the recycling process is the heterogeneity of products containing polyurethane foam, which prevents the use of standard methods of conduct based on current technology. Because of these difficulties, the main way of those wastes management is their storage, which combined with their low apparent density leads to the accumulation of large volumes of waste in landfills (Cregut et al., 2013).

Both environmental concerns and legislation (Directive of the European Parliament and of the Council 2008/98/EC) are the reason that the problem of polyurethanes accumulation in environment needs to be diligently addressed on the large scale. Accordingly, there is a need to develop more efficient and safe methods of distribution of polyurethane foams that are used at present. The greatest hope is in biological methods. Reports in the literature indicate that there are groups of microorganisms capable of biodegradation of some types of foams. Among these are bacteria of the genera *Acinetobacter*, *Bacillus*, *Corynebacterium*, *Pseudomonas*, *Rhodococcus*, *Staphylococcus*, or *Actinomycetes* and fungi like *Aspergillus*, *Aureobasidium*, *Chaetomium*, *Cladosporium*, *Penicillium*, *Trichoderma* (Howard 2002; Cregut et al., 2013).

To comprehend the process of biodegradation, understanding of the interaction between the material and microorganism and the biochemical processes involved in the degradation is required. When the polyurethane foams are taken into consideration, physical and mechanical part of the degradation process is very important. Due to the spatial structure of polymer cells the first stage of biodegradation is usually a breach of their structure. Therefore, a significant share of the microorganisms giving promising results in the tests are the molds that by

growing through the walls of foam cells contribute to hydrogen bonds network rupture what is increasing the availability of covalent bonds in the molecules for the extracellular enzyme (Howard 2002, Russel et al., 2011).

In most polyetherurethane foam types urethane, ether, and urea bonds can be found. They present a potential target for the enzymes that are secreted by microorganisms during the degradation processes (Loredo-Trevino, 2011). In terms of chemical and spatial structure urethane bond is similar to urea bond and peptide bond. Therefore, it can be target for proteases of lower specificity and for urease. During foaming of the polyurethane mass with water, due to a reaction of an isocyanate group with a molecule of water, there is amino group created, which then reacts with another isocyanate group leading to the formation of the urea bond, which is the second type of bond that can be targeted by urease. Polyetherurethane foams are less susceptible to biodegradation than polyesterurethane because the flexible segments contain ether bonds which are resistant to hydrolysis. These chains are however, somewhat more susceptible to oxidation than the polyester chains (Cregut et al., 2013). Among the enzymes that catalyze oxidation reactions laccases appear highly promising. They are the group of enzymes oxidizing many organic compounds. Due to the low substrate specificity of the active site of these enzymes they are likely to be able to oxidize also polyether chains (Polak, Jarosz-Wilkołazka, 2007). Results of previous studies demonstrate that the esterases are enzymes with potential of polyurethane degradation therefore the tested microorganisms were also examined for presence of those enzymes (Gomez et al., 2014).

The purpose of this study is characterization of microorganisms isolated from polyurethane foam waste in terms of the production of enzymes potentially useful in the breakdown of bonds present in the polyurethane foams (urease, esterases, proteases, and laccases) and the possibility of usage of polyurethane foam as an only source of carbon by these microorganisms. Due to the much greater use of polyetherurethane foams and their greater resistance to biodegradation studies presented in this paper are focused on foams of this type.

MATERIALS AND METHODS

Microbiological material

Microorganisms were isolated from the surface of polyetherurethane foam waste, which for a period of five years have been subjected to environmental factors in the landfill. Swabs were taken from places where the material surface showed signs of biological corrosion (discoloration, pitting, cracking). The isolation of pure cultures of bacteria and fungi was carried out on TSA media (30°C, 48h) and MEA (28°C, 5 days), respectively. In addition in this research collection strains were included: *Aspergillus niger* ATCC 16404 and *Hormoconis res-*

inae ATCC 38834 from the American Type Culture Collection and *Chaetomium globosum* ŁOCK 0476, from the Pure Culture Collection of Industrial Microorganisms of Institute of Fermentation Technology and Microbiology ŁOCK 105 (Darby and Kaplan, 1968).

Identification of isolated microorganisms

Fungi identification was based on macroscopic and microscopic morphological features according to the diagnostic keys (Domsch et al., 1993; Samson et al., 2006) after cultivation on Czapek-Dox for 7 days at 25°C.

Identification of yeast was carried out using standard methods taking into account the macroscopic characteristics and the microscopic morphology of the colony, type of propagation and biochemical abilities showed in tests API 20C AUX (bioMerieux).

Identification of the bacteria was carried out using standard methods based on morphological characteristics, gram stain, the presence of oxidase (Bactident® Oxidase, Merck), catalase, and evaluation of biochemical abilities using tests API Staph, API Coryne and API 20E (bioMerieux).

Enzymatic abilities of tested microorganisms

Ability to produce ureases was tested on Christensen urea agar (peptone 0.1%; dextrose 0.1%; NaCl 0.5%; KH_2PO_4 0.2%; urea 2.0%; phenol red 0.0012%; agar 1.5%; pH 7.2). Microbial strains were plated on medium, using single colony collected by microbiological loop as an inoculum and incubated at 25°C, results were checked after 24 and 48 hours. Samples for which there was no significant change during this time were left to further investigation after total incubation time of 7 days, in order to exclude the possibility of a slower hydrolysis of urea. Sterile medium is yellow or bright orange. The result of decomposition of urea by microorganisms is the alkalization followed by change of the color to pink (Loredo-Trevino et al., 2011).

Laccases production was evaluated on medium with ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) as a substrate (glucose 0.4%; K_2HPO_4 0.2%; KH_2PO_4 0.7%; NH_4NO_3 0.1%; $MgSO_4 \times 7H_2O$ 0.01%; NaCl 0.06%; agar 1.5%; 1μM ABTS 1ml; 1μM $CaSO_4 \times 5H_2O$ 1ml). Strains were plated on medium, with single colony collected by microbiological loop as an inoculum and incubated for 8 days at 25°C. Staining of medium for turquoise to navy blue means that the tested organisms produce laccase (Loredo-Trevino et al., 2011).

The ability to produce protease was tested on a casein agar (skim milk powder 5%; casein hydrolysate 0.5%; yeast extract 0.25%; glucose 0.1%; agar 1.25%). Microbial strains were plated on medium, using single colony collected by microbiological loop as an inoculum and incubated at 25°C, checking the

results after 24 and 48 hours. The presence of a clear zone around the colony proves proteolytic abilities of studied microorganisms.

In order to determine whether the tested microorganisms produce esterases they were plated on medium with an addition of Tween 80 (peptone 1.0%; NaCl 0.5%; $CaCl_2$ 0.08%; agar 1.5%; Tween 80 10.0 ml/L), with single colony collected by microbiological loop as an inoculum. Plates were incubated for 48 hours at 25°C for yeasts and molds and at 30°C for bacteria. The turbidity zone has been observed around the colony when esterases were present (Loredo-Trevino et al., 2011).

Microorganisms ability to use polyurethane foam as a sole carbon source

In order to test the ability of microorganisms to use polyurethane foam as the sole carbon source nutrient solution was prepared (KH_2PO_4 0.2%; K_2HPO_4 0.7%; NH_4NO_3 0.1%; $MgSO_{4*}7H_2O$ 0.01%; $ZnSO_{4*}7H_2O$ 0.0001%; $CuSO_{4*}7H_2O$ 0.00001%; $FeSO_{4*}7H_2O$ 0.01%, $MNSO_{4*}7H_2O$ 0.0002%, agar 2%). Polyurethane foam (basic type, density 25kg/m^3) was added to this medium. Final concentration of foam was 10g/L, and before the introduction into the media it was triturated in a mortar to obtain a homogeneous medium. Tested microorganisms were plated on this medium, using single colony collected by microbiological loop as an inoculum and their ability to grow was observed in 25°C. (Urgun-Demirtas et al., 2007).

RESULTS AND DISCUSSION

Polyurethane foams, which are the subject of the first phase of the study, were stored under natural conditions for a period of 5 years. Macroscopic changes on the surface testified to the ongoing process of decomposition. Surface was colonized by different groups of organisms that found there the best conditions for their growth. It can be assumed that this type of waste will be a good source to search for microorganisms that can be potentially useful for the foams biodegradation. From swabs taken from the polyurethane foams wastes seventeen strains of microorganisms were isolated, including 8 species of fungi, 3 of yeasts and of 6 bacteria (Table 1). The natural environment of those microorganisms is the soil, and they were also isolated from plant material.

Among the isolated fungi only three (*Phoma, Epicoccum* and *Acremonium*) do not belong to the types identified in the literature as a potential microorganisms degrading polyurethanes however, representatives of these types are among the factors of biodeterioration of different technical materials. Other isolated species are known as organisms with versatile enzymatic abilities, which are involved in the biological corrosion of various materials, including polyurethane foams, glass fiber composites, epoxy resins, polyamide, polyethylene etc.

(Falkiewicz-Dulik et al., 2015). Among them, fungi of the genera *Aspergillus*, *Penicillium* and *Trichoderma* were isolated. Incubation of *A. flawus* on a PUR foam for 30 days at 28°C resulted in 60% of the weight loss of the polymer (Mathur and Prasad 2011).

Table 1. Strains isolated from the surface of the polyurethane waste

Fungi	Yeasts	Bacteria
Acremonium strictum *Aspergillus fumigatus* *Aspergillus niger* *Epicoccum nigrum* *Penicillium spp.* *Phoma sp.* *Trichoderma spp.* *Trichoderma harzianum*	*Candida krusei* *Cryptococcus albidus* *Rhodotorula mucilaginosa*	*Staphylococcus xylosus* *Pantoea spp* *Staphylococcus warneri* *Rhodococcus spp* *Arthrobacter spp.* *Microbacterium spp.*

Among the microorganisms studied by Loredo-Trevino in 2011 genus *Trichoderma* showed the best growth after 96 hours incubation on mineral medium with the addition of PUR (3g/L). Also, the strain of *Penicillium* showed ability to grow on this medium.

Yeasts do not appear in the literature among the microorganisms potentially degrading polyurethanes, however, isolated in this studies species of the genera *Rhodotorula*, *Candida* and *Cryptococcus* have known abilities of degradation of petroleum materials, which makes them microorganisms, worth considering where polyurethanes are being tested.

The isolated species of bacteria include organisms, which had been reported in the literature as probable participants in the breakdown of polyurethanes.

Including *Rhodococcus* and isolated by Shah et al., in 2008: *Micrococcus*, *Arthrobacter* and *Staphylococcus* that formed clearance on mineral medium with 0.5% PUR after incubation at 37°C for 48 hours. The representative of the last identified type *Pantoea agglomerans*, participates in the distribution of many petroleum compounds including alkylbenzene sulfonates and ethylene-propylene copolymers (Falkiewicz-Dulik et al., 2015; Khleifat 2006).

Enzymes producing abilities

Isolated microorganisms were tested for selected enzymes production. In the screening tests the ability to produce urease, proteases, laccases and esterases was rated. In addition to the strains coming from the environment selected species from the collections were analyzed. They were chosen according to the literature data that indicated that they might show ability to degrade polyurethanes (Nakajima-Kambe et al., 1999).

Table 2. Enzymatic abilities of tested strains; (+) abilities reported; (-) tested abilities not reported

Organism	Origin	Genus/species	Urease	Proteases	Esterases	Laccases
Fungi	Environmental strains	*Acremonium strictum*	+	-	+	+
		Aspergillus fumigatus	+	-	-	-
		Aspergillus niger	-	+	-	+
		Epicoccum nigrum	+	-	-	-
		Penicillium spp.	+	+	-	-
		Phoma ssp.	+	+	-	+
		Trichoderma spp.	+	-	+	+
		Trichoderma harzianum	+	-	+	-
	Strains from collections	*Aspergillus niger*	-	+	-	+
		Chaetomium globosum	+	-	-	+
		Hormoconis resinae	+	-	+	-
Yeasts	Environmental strains	*Candida krusei*	+	-	-	-
		Cryptococcus albidus	+	-	+	-
		Rhodotorula mucilaginosa	-	-	+	-
Bacteria		*Arthrobacter* spp.	+	-	+	-
		Microbacterium spp.	+	+	+	-
		Pantoea spp.	-	+	+	-
		Rhodococcus spp.	+	-	+	-
		Staphylococcus warneri	+	-	+	-
		Staphylococcus xylosus	+	+	+	-

About 80% of the tested strains produced urease. This feature have not been presented by one genus of fungi: *Aspergillus niger* neither environmental strain nor the collection strain, one strain of yeasts: *Rhodotorula* and one strain of bacteria: *Pantoea* (Deletoile 2016, Cregut et al., 2013).

Next in the group of most commonly produced enzymes are esterases (60%). Proteases and laccases are present in similarly small number of strains, respectively, 30% and 35% (Table. 2)

Among tested fungi almost all (except for *Aspergillus niger*) produced urease, while only four genera produced protease (*Aspergillus niger*, *Penicillium* spp., and *Phoma* spp.) as well as esterases (*Acremonium strictum*, *Trichoderma* spp., *Trichoderma harzianum*, *Hormoconis resinae*). All microorganisms, which were producing laccase, belonged to the fungi (Howard, 2002; Nakajima-Kambe, 1999).

Candida krusei and *Cryptococcus albidus* produce urease. Esterases were produced by *Cryptococcus albidus* and *Rhodotorula mucilaginosa*. The tested yeast did not show the ability to produce protease or laccase. All tested bacterial strains produced esterase, but none were positive for laccases. The only strain that did not give a positive response in the urease test was *Pantoea* spp (Falkiewicz-Dulik et al., 2015).

Microorganisms ability to use polyurethane foam as a sole carbon source

The growth of strains on the plates with PUR medium was observed every 24 hours to check for positive answer to this test and compared to one that were plated on MEA (fungi) or TSA (bacteria) to confirm if the growth was strong enough to form colonies similar to one on the rich medium.

Table 3. The ability of microorganisms to grow with PUR foam as a sole carbon source; (-) no growth; (+) slight growth; (++) good growth

Organism	Origin	Genus/species	Growth on the PUR medium
Fungi	Environmental strains	*Acremonium strictum*	-
		Aspergillus fumigatus	-
		Aspergillus niger	++
		Epicoccum nigrum	++
		Penicillium spp.	+
		Phoma ssp.	-
		Trichoderma spp.	+
		Trichoderma harzianum	+
	Strains from collections	*Aspergillus niger*	++
		Chaetomium globosum	-
		Hormoconis resinae	+
Yeasts	Environmental strains	*Candida krusei*	-
		Cryptococcus albidus	+
		Rhodotorula mucilaginosa	-
Bacteria		*Arthrobacter* spp.	+
		Microbacterium spp.	-
		Pantoea spp.	-
		Rhodococcus spp.	++
		Staphylococcus warneri	-
		Staphylococcus xylosus	++

Ability to grow on a medium containing PUR foam as a sole carbon source could be observed for eight strains of the mold: *Aspergillus niger* environmental strain and from the collection, *Epicoccum nigrum*, *Penicillium* spp., *Trichoderma* spp., *Trichoderma harzianum* and *Hormoconis resianae*. Among yeast only *Cryptococcus albidus* could grow on a PUR medium. In the case of bacterial strains half were able to grow on the polyurethane foam: *Arthrobacter* spp., *Rhodococcus* spp., *Staphylococcus xylosus*. Among all growing microorganisms only five: *Epicoccum nigrum*, *Aspergillus niger* from the collection and environmental, *Staphylococcus xylosus* and *Rhodococcus* spp. had the colonies, that were significant in size and their morphology was similar to those growing on the media that contain easily available carbon source.

After comparison of eleven strains growing on the medium with polyurethane foam with their enzymatic abilities can be observed that *Aspergillus niger* is the only one producing laccase, while it has no urease activity. Seven strains (except *A. niger*, *E. nigrum*, *Penicillium spp*.) produce esterases. Only four of those eleven strains produced proteases.

CONCLUSION

Of the seventeen strains isolated from foam waste nine were mentioned in the literature as microorganisms with possible ability of polyurethanes degradation. Rest of them have been noted to degrade petroleum materials, which means that they could also be effective in the degradation processes of polyurethanes, the synthesis of which is based on petroleum compounds.

Among the enzymes, which are presented as the potential degradation factors for bonds present in the molecules of the polyurethane foam, most commonly produced by tested microorganisms was urease. The least produced enzymes were laccases and proteases.

Eleven strains indicated growth capability on a medium with PUR foam as a sole carbon source. Nine of them are urease-producing microorganisms. This could mean that the possible mechanism of degradation of polyurethanes is based on the decomposition of urea bonds (or possibly urethane bond) by the urease, which leads to the release of smaller molecules, which can be the substrates for further microbial processes. In the case of *Aspergillus niger*, which was the only species that did not produce urease, but gave a positive response in the test for laccase, it can be assumed that the degradation is based on a different mechanism, which probably starts with the oxidation of ether linkages in the polyether chain or breakage of urethane chain by proteases.

This mechanism of degradation suggests that microorganisms reported in the literature and in these studies as capable of growth on PUR begin the process of decomposition, while other microorganisms degrade newly created compounds.

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