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Antifungal Activity of Polyhexamethyleneguanidine Derivatives Introduced into Biodegradable Polymers

Maria Swiontek Brzezinska[1] · Maciej Walczak[1] · Aleksandra Burkowska-But[1] · Marta Chylińska[2] ·
Agnieszka Kalwasińska[1] · Joanna Świątczak[1]

Abstract
Experiments were conducted to investigate the antifungal activities of polyhexamethyleneguanidine (PHMG) derivatives introduced into polylactide (PLA), polyhydroxybutyrate (PHB) and polycaprolactone (PCL) against *Aspergillus niger, Penicillium chrysogenum* and *Candida albicans*. All of the PHMG derivatives inhibited the germination of *A. niger* and *P. chrysogenum*. All of the derivatives exerted a much stronger inhibitory effect on the cells of *C. albicans*. PHMG granular polyethylene wax (at the concentration of 1.0%) has a fungicidal effect. The reduction in the number of yeast cells capable of growing on the surface composites PLA, PHB and PCL with PHMG granular polyethylene wax for 24 h was R > 2. PHMG derivatives introduced into PLA decreased hydrolases activity in *A. niger* and *P. chrysogenum*. All of the PHMG derivatives introduced into all investigated polymers inhibited the hydrolases activity in *C. albicans* proportionately to concentration. PHMG granular polyethylene wax at a concentration of 1.0% most strongly inhibited hydrolases activity in yeast. The composites produced from PLA, PHB, PCL and this PHMG derivatives can be used in many areas to reduce the growth of yeast. The studied composites can potentially be used for the production of biomedical or packaging materials.

Graphical Abstract
PHMG derivates introduced into polymer have slightly biocidal properties against molds and strong against yeast. The composites produced from PLA, PHB, PCL and this PHMG derivatives can be used in the many areas to reduce the growth of yeast. The studied composites can potentially be used for the production of biomedical or packing materials.
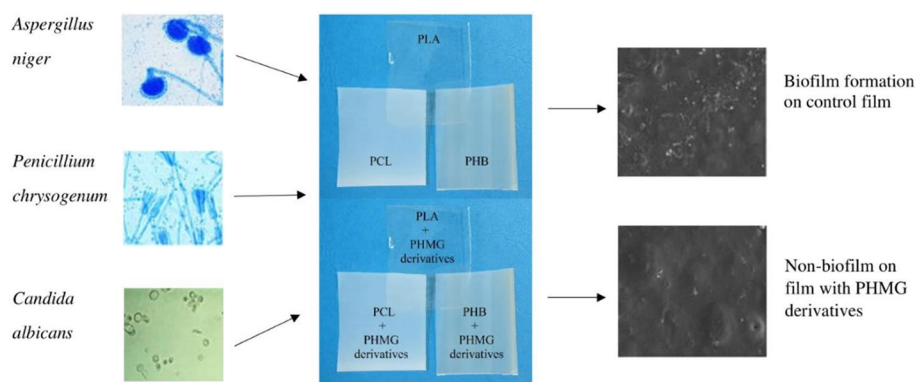
Keywords Biodegradable polymer · PHMG derivatives · Hydrolases

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✉ Maria Swiontek Brzezinska
  swiontek@umk.pl

Extended author information available on the last page of the article

Introduction

For the past few years, intensive research has been carried out in the field of environmentally friendly biodegradable and biocompatible polymers such as polyesters, polyanhydrides, poly(ester amide)s, and biodegradable polyurethanes [1]. Polycaprolactone, polyglycolide, polylactide and polybutylene adipate-co-terephalate find applications in various fields, for example, food packaging [2–4], compost bags, medical sutures, nanoscale or microscale drug delivery vehicles, and temporary scaffolds for tissue regeneration [5–7]. A serious threat to these polymers is the ability to form biofilms. For a few years, the polymer has been modified by introducing different biocidal substances. Introducing antimicrobial substances into polymers by covalent linkages is possible using different methods. According to Jao et al. [8] the antibacterial polymers can be grafted on biodegradable polymers, as shown for grafting of chitosan on polybutyleneadipate-co-terephthalate. Research is being conducted on a number of biocidal substances that potentially can be introduced into polymers. For example, direct contact of *N*-halamine acts as a biocidal on *E. coli* and *Staphylococcus aureus* [9]. Polyhexamethyleneguanidine (PHMG) is a biocidal, cationic polymer based guanidine salt, synthesized from hexamethylenediamine and guanidine HCl [10]. This polymer is stable over a wide range of pH 1–10, non-flammable, and has a high thermal resistance. PHMG is able to eliminate bacteria and some viruses, fungi and unicellular algae. The mechanism of action is that PHMG destabilises the osmotic equilibrium and destructs the cytoplasmic membrane in bacteria or fungi [11]. It was also reported a cooperative binding between guanidine derivatives and DNA, and inhibition the activity of enzyme systems, indicating that antibacterial effect might be also related to other interaction [12, 13]. PHMG and its derivatives are becoming increasingly popular due to its broad range of antibacterial activity and relatively low toxicity. Currently guanidine derivatives with biocidal activity are mainly used as disinfectant in human eye infections and wound care, as water treatment-sand in swimming pools, the disinfection of various solid surfaces, and the impregnation of fabrics [14, 15]. PHMG has been proposed too as a safe and highly effective fungicide and disinfectant in fruits challenged with mould [11, 16]. PHMG derivatives insoluble in water are used as resin and paint coatings or as additives to plastics such as polycarbonate and polyamide [17]. Research into the introduction of PHMG and its derivatives into polymeric materials such as polycaprolactone, polylactide or polyamide has led to the development of the packaging industry. Polyamide 12 film (PA 12) modified polyhexamethylene guanidine dodecylbenzenesulfonate (PHMG-DBS) almost

Table 1 Extrusion conditions of granulate polymers (data from Institute for Engineering of Polymer Materials and Dyes, Poland)

Extrusion conditions	Polylactide	Polycaprolactone	Polyhydroxybutyrate
Temperature of the first zone (°C)	180	125	140
Temperature of the second, third and fourth zone (°C)	185	130	140
Head temperature (°C)	185	130	150
Screw speed (min⁻¹)	250	250	250
Cooling	Air	Air	Air
Dosing capacity (kg h⁻¹)	4	4	4

completely inhibited the growth of moulds [18]. Yeasts, moulds and mycotoxins are a serious threat because they are, for example, a cause of many food poisonings [19]. The introduction of biocidal substances into packaging materials could protect the food against fungal infections. The fungicidal activity of PHMG is shown by Feng et al. [20]. However, it is not known whether PHMG after incorporation into the polymers also has a biocidal effect. So far, studies have been carried out on the bactericidal properties of PHMG derivatives introduced into polymers. Hence, the purpose of our study was to investigate whether PHMG derivatives introduced in biodegradable polymers have fungicidal properties and the effect of the derivatives on extracellular hydrolytic enzymes.

Materials and Methods

Preparation of Composites

For the production of the tested composites biodegradable Polylactide polymer type 2002D (NatureWorks®, USA) were used with density d = 1.24 g ml⁻¹, Poli (ε-caprolactone) polymer, type CAPA 6800 (Solvay Caprolactones, UK) with density d = 1.1 g ml⁻¹ and t SoGreen®-2001a (Tianjin Green BioMaterial Company, China) polymer mixture containing poly (3,4-hydroxybutyrate), density d = 1.25 g cm⁻³. Extrusion conditions of granulate polymers show Table 1. The three biocidal PHMG derivatives with organic anions: sulfanilic acid salt, stearate, and granular polyethylene wax were used to study. All composites were from the Institute for Engineering of Polymer Materials and Dyes, Poland. The mixture of polymers and additional ingredients were prepared with the use of a co-rotating twin-screw extruder type BTSK 20 (Bühler, Germany) with the screw diameter of 20 mm and L/D = 40, equipped with a segmented plasticizing system used to produce granulated composite. Prior to extrusion of the granulate PLA and PHB materials with moisture removed by placing them in a vacuum thermal chamber (Piovan) at 75 °C and

60 °C respectively for 4 h. Poly (ε-caprolactone) was extruded directly, because it does not require prior drying under appropriate conditions. The composite was cold-granulated with cooling of the pomace in the air at a temperature of 25 ± 3 °C. The granulated product was used to produce flat films using a single-screw extruder type PlastiCorder PLV 151 (Brabender, Germany) equipped with a screw with a diameter of 19.5 mm and $L/D = 25$ equipped with a flat head width of 170 mm die with adjustable gap size and cooperating with triple roll calender rolls having a diameter of 110 mm whose temperature is stabilized by a thermostat. The prepared samples had the following contents of PHMG derivatives: 0.0, 0.2, 0.6, 1.0% (wt). The following symbols of the composites were used in the tests: PLA/PHB/PCL-A (PLA/PHB/PCL with the PHMG salt of sulfanilic acid), PLA/PHB/PCL-W (PLA/PHB/PCL with PHMG granular polyethylene wax), PLA/PHB/PCL-S (PLA/PHB/PCL with PHMG stearate). Control samples were pure PLA, PHB and PCL without PHMG derivatives. The tested PHMG derivatives are compounds of a copolymer produced by PHMG synthesis of an organic carrier according to Patent No: P.388062, 2009 [21]. The biocidal properties of the organic copolymer have been previously investigated. The biocidal effect of the copolymers (sulfanilic acid, granular polyethylene wax, stearate) introduced into PLA/PHB/PCL in concentration 0.2–1.0% was not observed. All composites were from the Institute for Engineering of Polymer Materials and Dyes, Poland.

Determination of Blood Compatibility

The hemolytic activity of PHMG derivatives introduced into the polymers was tested by direct contact methods, according to the modified method Zhou et al. [22]. All derivatives with PHMG concentrations of 0.6 and 1.0% were selected for the study. For this purpose 200 μl anticoagulated sheep blood was added to 10 ml of physiological saline solution containing derivatives PHMG (1 cm^2 area). The positive and negative control was prepared by adding 200 μl of fresh blood to water and physiological saline, respectively. After one hour incubation at 37 °C the suspensions were centrifuged (1000 rpm, 10 min). The absorbance of the supernatant of each sample was measured by microplate reader Multiscan FC (Thermo Fisher Scientific, Waltham, USA) at 540 nm. The study was prepared in triplicate. Hemolysis rate was calculated using this equation:

$$\text{Rate of hemolysis (\%)} = (\text{ODspecimen} - \text{ODnegative})/(\text{ODpositive} - \text{ODnegative}) \times 100$$

Test Microorganisms

The yeast *Candida albicans* ATCC10231 and moulds *Aspergillus niger* ATCC16404 and *Penicillium chrysogenum*

ATCC10106 were used for the study. The yeasts and moulds were maintained on Malt Extract Agar (Biocorp) medium at 4 °C. The *C. albicans* was subcultured on appropriate medium at 37 °C and moulds at 26 °C for 48 h.

Antifungal Activity Assay

A common method for determining antimicrobial activity is the diffusion method. However, this method could be not used because PHMG derivatives do not diffuse into the medium. Antifungal properties of PLA, PHB and PCL containing PHMG derivatives were determined according to modified standard ISO 22196 [23]. The analysis was performed in triplicate. Control (PLA/PHB/PCL) and test samples (PLA/PHB/PCL-W, PLA/PHB/PCL-S, PLA/PHB/ PCL-A) covered with the fungal suspensions investigated in the research with a specified number of cells or spores (1×10^5 per ml yeast cells or moulds spores with McFarland's standard) were left for a specified time (0 h—validation of recovery efficiency and 24 h). After this time cells were recovered from the surface and suspended in a solution containing neutralizer. Subsequently, the number of cells capable of growth was determined by the inoculation on PDA (moulds) and Sabouraud agar (yeast). The *C. albicans* was incubated for 48 h at 37 °C and moulds were incubated for 48 h at 26 °C.

Reduction of the number of living and viable cells of tested microorganisms (R) was calculated using the equation:

$$R = \left(U_t - U_0\right) - \left(W - U_0\right)$$

where Uo is the average of the common logarithm of the number of viable fungi recovered from the control samples (PLA/PHB/PCL) immediately after inoculation (validation of recovery efficiency); U_t is the control samples (PLA/PHB/ PCL) after 24 h (controls of survival in time, without PHMG derivatives); W is the average of the common logarithm of the number of viable fungi recovered from the test samples (PLA/PHB/PCL-S, PLA/PHB/PCL-W, PLA/PHB/PCL-A) after 24 h.

Scanning Electron Microscopy (SEM) Observation

PHMG derivatives introduced into polymers showed strong biocidal properties only against *C. albicans*. SEM analysis was performed on films of PHB containing 1.0% PHMG. Fragments 0.5×1.5 cm of the PHB film (control) and PHB-W, PHB-A and PHB-S with 1.0% PHMG (tested sample) were sterilized with ethyl alcohol and placed in a sterile

simax bottle with 10 ml YPD medium (composition g l^{-1}): peptone—20.0, yeast extract—10.0, glucose—20.0). 1 ml suspensions of the yeast with an optical density of 1.0 according to McFarland standards were then transferred to the bottle and incubated at 37 °C for 7 days. After incubation, the films were washed several times with sterile water and dried. The thus prepared films were subjected to SEM analysis. The SEM images of samples were received using the 1430 VP (2001) scanning electron microscope (LEO Electron Microscopy Ltd, England). In order to achieve images of high quality, the samples were sputter-coated with Au–Pd alloy prior to the examination. Images of each sample were taken at ×10,000 magnification.

Determination of Effect of PHMG Derivatives on Hydrolases Activity

Candida albicans was prepared in flasks containing 50 ml of liquid medium (g l^{-1}): casein pepton (20), yeast extract (20), glucose (20). *Aspergillus niger* and *P. chrysogenum* were cultured in a medium containing (g l^{-1}): KH_2PO_4 (0.5) K_2HPO_4 (0.5), $MgSO_4 \cdot 7H_2O$ (0.3), pepton (5), yeast extract (1.5). After 24 h of incubation at 37 °C (yeast) and 26 °C (moulds) the cultures were centrifuged at a speed of 10,000 rpm to separate the cells from the post-culture liquid (+4 °C). The obtained post-culture liquid containing hydrolases were used in further tests. The film (5×5 cm) with 3 ml of the obtained post-culture liquid was placed on sterile Petri plates. The samples were pre-incubated for 4 h at 20 °C. The optimum contact time of the film with the post-culture liquid was previously determined. Then the post-culture liquid was collected from the tested film and put into Eppendorf test tubes. The activity of hydrolases after contact with the film was determined with the use of fluorescein diacetate using the Adam and Duncan method [24]. Concentration of the fluorescein released under the influence of hydrolases within 1 h, at 30 °C was measured using a HITACHI F-2500 spectrofluorimeter at excitation wavelength 480 nm and emission wavelength 505 nm. The control sample was a film without the biocidal substance. The determination was done in triplicate.

Results and Discussion

Hemolysis Rate of PHMG Derivatives Introduced into PLA, PHB, PCL

In this paper, the hemolysis test of derivatives PHMG introduced into the polymer was used. All tested composites were safe and had no hemolytic activity. For all composites the hemolysis value tested were not more than 5.0% (Table 2). The hemolysis testing is often used as an indicator of the

Table 2 The toxicity of PHMG derivatives incorporated in the polylactide (PLA), polyhydroxybutyrate (PHB) and polycaprolactone (PCL)

Composites	Concentration of PHMG (%)	Rate of hemolysis (%)
PLA with the PHMG salt of sulfanilic acid	0.6	0
PLA with the PHMG salt of sulfanilic acid	1.0	0
PLA with PHMG granular polyethylene wax	0.6	0
PLA with PHMG granular polyethylene wax	1.0	0.81
PLA with PHMG stearate	0.6	0
PLA with PHMG stearate	1.0	0
PHB with the PHMG salt of sulfanilic acid	0.6	0
PHB with the PHMG salt of sulfanilic acid	1.0	1.0
PHB with PHMG granular polyethylene wax	0.6	0
PHB with PHMG granular polyethylene wax	1.0	0.38
PHB with PHMG stearate	0.6	0
PHB with PHMG stearate	1.0	0
PCL with the PHMG salt of sulfanilic acid	0.6	0
PCL with the PHMG salt of sulfanilic acid	1.0	0.07
PCL with PHMG granular polyethylene wax	0.6	0
PCL with PHMG granular polyethylene wax	1.0	0.11
PCL with PHMG stearate	0.6	0.46
PCL with PHMG stearate	1.0	0.41

injuries to the red blood cell membrane and considered to be a simple and reliable measurement for estimating blood biocompatibility of materials. According to ISO document 10 993-5 1992 hemolysis index is regarded as safe when it is less than 5.0% [22]. Zhang et al. [25] reported excellent biocompatibility of the PHMG-based hydrogels. The low toxicity of PHMG-based polymers during contact exposure show promise not only for surface protection but even for creation of medical materials.

Antifungal Properties of PHMG Derivatives Introduced into PLA, PHB, PCL

The antifungal properties of all the derivatives were positively correlated (R in the range from 0.65 to 0.75) to the concentration of PHMG. All of the PHMG derivatives introduced into PLA, PCL and PHB inhibited the germination of

Fig. 1 Antifungal activity of PHMG introduced into PLA against moulds and yeast. PLA-A (PLA with PHMG salt of sulfanilic acid), PLA-W (PLA with PHMG granular polyethylene wax), PLA-S (PLS/ with PHMG stearate). Values are expressed as mean ± SD (*n* = 3)
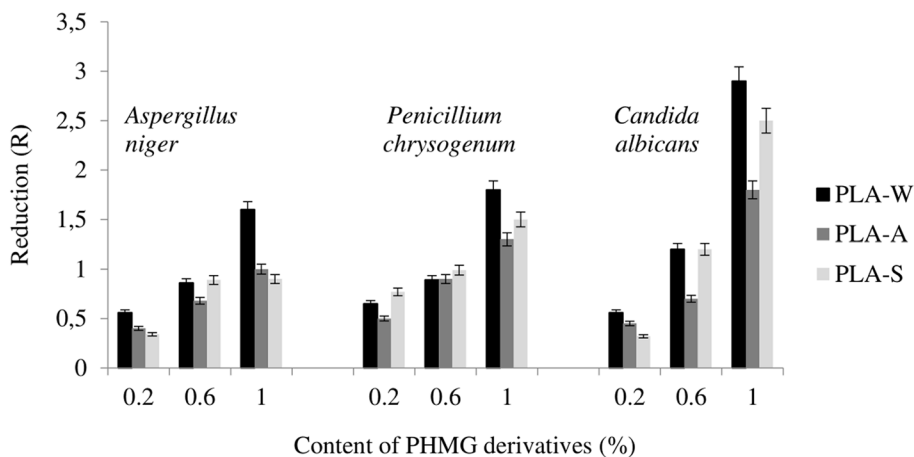

Fig. 2 Antifungal activity of PHMG derivates introduced into PCL against moulds and yeast. PCL-A (PCL with PHMG salt of sulfanilic acid), PCL-W (PCL with PHMG granular polyethylene wax), PCL-S (PCL with PHMG stearate). Values are expressed as mean ± SD (*n* = 3)
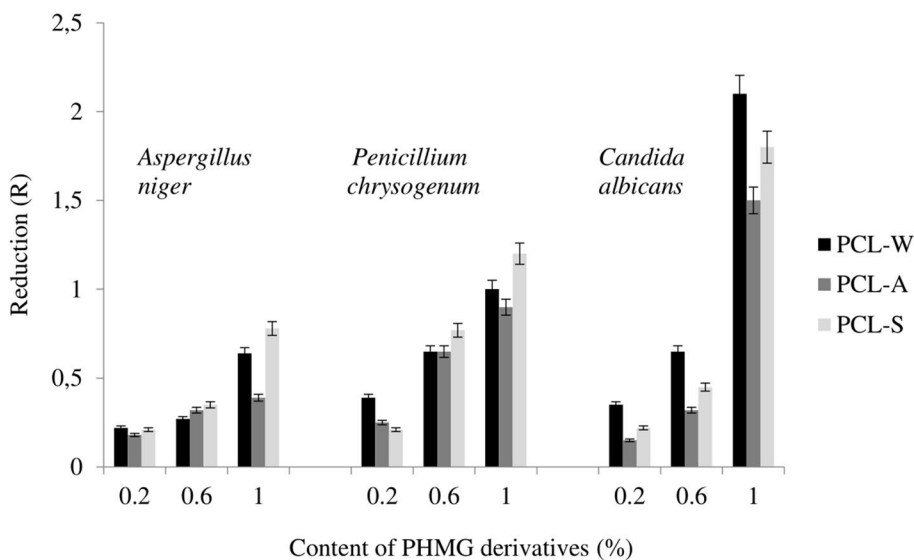
Fig. 3 Antifungal activity of PHMG introduced into PHB against moulds and yeast. PHB-A (PHB with PHMG salt of sulfanilic acid), PHB-W (PHB with PHMG granular polyethylene wax), PHB-S (PHB with PHMG stearate). Values are expressed as mean ± SD (*n* = 3)
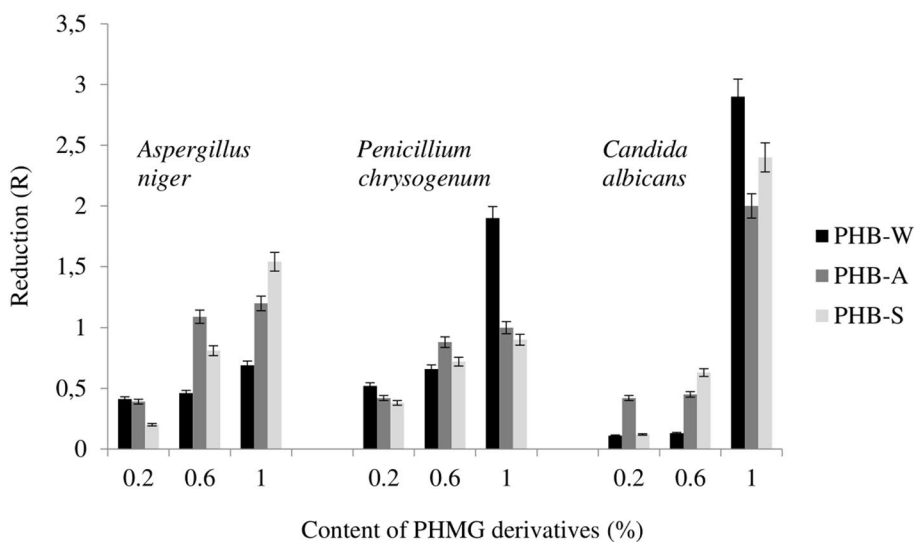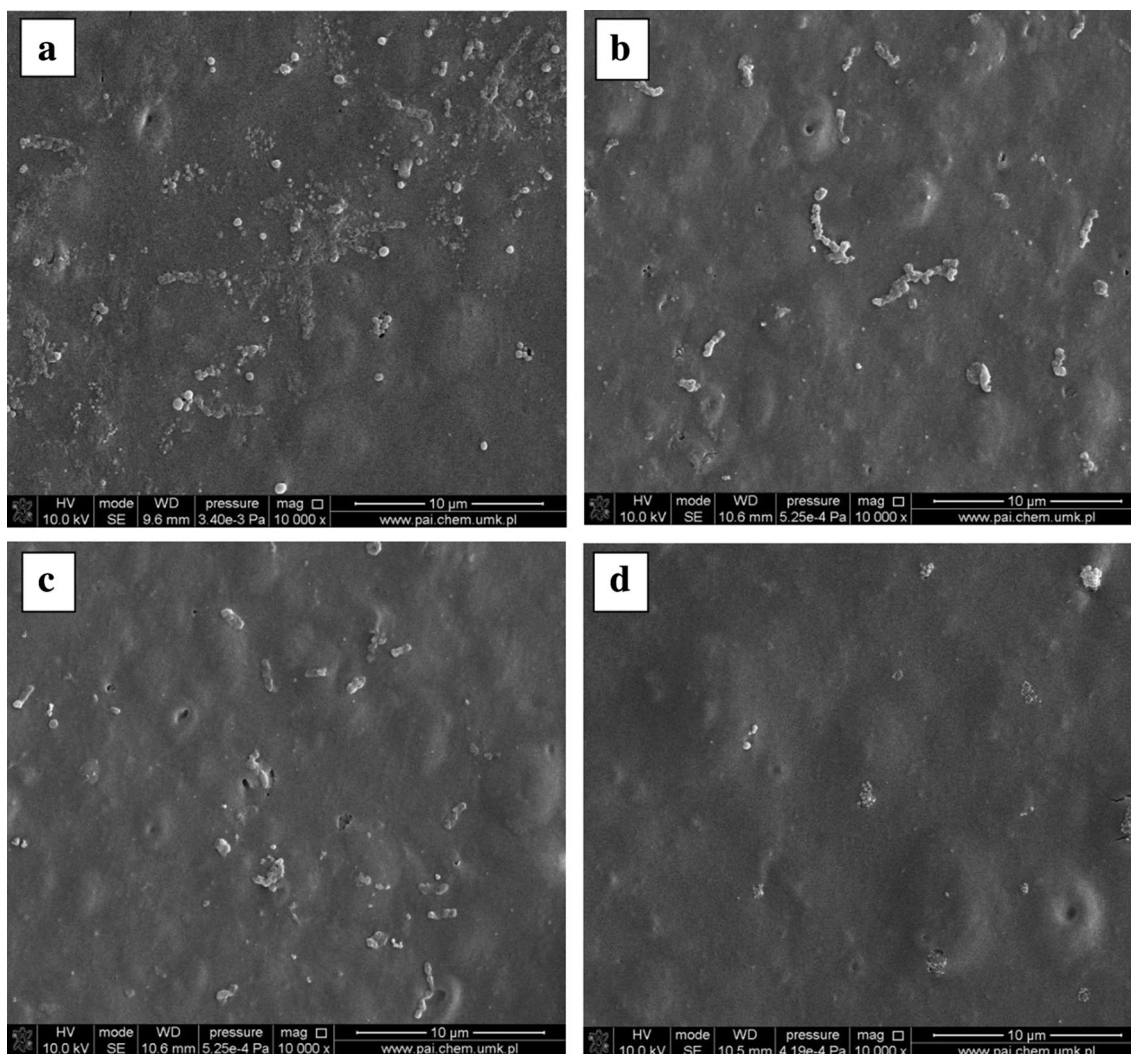
Fig. 4 Scanning electron micrographs of biofilm of *C. albicans* on surface of films containing 1% PHMG. **a** PHB without PHMG, **b** PHB with PHMG salt of sulfanilic acid, **c** PHB with PHMG stearate, **d** PHB with PHMG granular polyethylene wax

A. niger and *P. chrysogenum* (Figs. 1, 2, 3), but reduction of live cells (R) for both moulds incubated on films containing a 1.0% PHMG was a smaller than 2. According to standard ISO 2219616 [23], the reduction of the number of cells capable of growth by two orders of magnitude (R ≥ 2) is interpreted as a biocidal effect of the investigated composite. All of the derivatives (at the concentration of 1.0%) have a much stronger inhibitory effect on the cells of *C. albicans* (Figs. 1, 2, 3). The reduction of yeast cells capable of growth (R) on the surface composites PLA, PHB and PCL with PHMG granular polyethylene wax (at the concentration of 1.0%) for 24 h was R > 2. Therefore, the composites PLA-W, PHB-W and PCL-W containing a 1.0% PHMG can be considered biocidal against yeast.

SEM analysis also shows a significant reduction in the number of yeast cells. On the surface of the PHB film without PHMG was observed a lot of yeast cells. However, on

PHB film containing PHMG salt of sulfanilic acid and stearate granular yeast of cells was less. On surface PHB with polyethylene wax at a concentration of 1.0% was observed in single yeast cells (Fig. 4).

The study of fungicidal properties of PHMG introduced biodegradable polymers is fragmented. The studies mainly focused on the inhibitory effects of PHMB or PHMG on bacteria growth. PHMB exhibits bacteriostatic properties at low concentrations (typically 1–10 mg l^{-1}) but has bactericidal characteristics at higher concentrations [26, 27]. Feng et al. [20] have demonstrated the fungicidal activity of polyhexamethylene biguanide (PHMB) and polyhexamethylene guanidine (PHMG) against *Geotrichum citri-aurantii*. According to authors, PHMG and PHMB treatments significantly inhibited arthroconidia germination and mycelial growth of *G. citri-aurantii* in vitro. PHMG and PHMB at 5 mg l^{-1} inhibited 97.8% and 95.8% of the germination

Fig. 5 Effect of PHMG introduced into PLA on hydrolase moulds and yeast. PLA-A (PLA with PHMG salt of sulfanilic acid), PLA-W (PLA with PHMG granular polyethylene wax), PLA-S (PLA with PHMG stearate). Values are expressed as mean ± SD ($n = 3$)
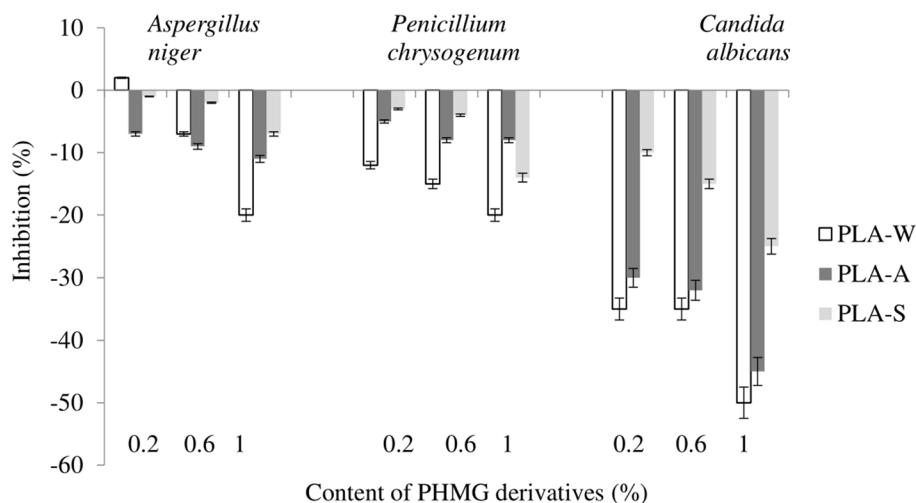
of *G. citri-aurantii* arthroconidia, respectively. Razzaghi-Abyaneh et al. [28] reported that PHMG inhibited fungal growth of *Aspergillus parasiticus* in a dose-dependent manner, and can inhibit completely the growth of *A. parasiticus* when a concentration of 2 mg l^{-1} is used. Choi et al. [29] showed that the antifungal activity of PHMGH was greater than or equal to that of amphotericin B. In particular, PHMGH (1.25 µg ml^{-1}) was more potent than amphotericin B (2.5 µg ml^{-1}) against *Candida* species.

The antimicrobial activity of PHMB could be attributed to its ability to cause phospholipid phase separation and loss of membrane function in bacteria and yeast [26, 30, 31]. Zhou et al. [27] reported that PHMG damaged membrane and intracellular structures of *E. coli*, resulting in subsequent leakage of intracellular components and cellular inactivation. According to Feng et al. [20] PHMG and PHMB markedly damaged hyphal plasma membranes of *G. citri-aurantii*, possibly by a similar mechanism to that of *E. coli*. Similarly, Schindler and Hauser [32] reported that PHMG diffuses through the cellular membrane and binds to the cytoplasmic membrane forming a complex with the phospholipid molecules of the lipid bilayer, destabilizes the osmotic equilibrium and destroys cytoplasmic membrane, causing leakage of the cell content. According to Choi et al. [29] the antifungal mechanism assay, using *C. albicans* as a model, suggests that PHMGH exerts its antifungal activity by forming pores in the plasma membrane with the majority of pore size being between 2.3 and 3.3 nm, causing an ion loss.

There is no doubt that PHMG has biocidal properties [10, 11]. However, the strength of its performance may change after modification. The incorporation of PHMG into polymers can reduce the power, therefore a PHMG concentration with a biocidal effect must be established. In addition, the sensitivity of various microorganisms to PHMG is different. Our studies showed inhibition of germination of *A. niger* and

P. chrysogenum, but it was lower than two orders of magnitude (R < 2). Therefore according to standard ISO 2219616 [23] the investigated composite have not a biocidal effect against moulds. On the other hand, our study showed that after incubation of *C. albicans* on PLA, PHB and PCL films containing granular polyethylene wax at a concentration of 1.0% PHMG for 24 h, there was a reduction of live yeast cells R > 2. According to standard ISO 2219616 [23] these composites showed a biocidal effect against yeast. It means that, mould spores can be more resistant to PHMG than yeasts. Walczak et al. [33] determined bactericidal properties of polylactide (PLA) films containing three different PHMG derivatives. According to the authors only PLA-W (at a concentration of at least 0.6%) has a bactericidal effect. For *E. coli* and *Staphylococcus aureus* strains incubated on PLA films containing PLA-W at a concentration of 0.6 and 1.0% for 24 h, there was a reduction of live and viable bacterial cells R ≥ 2. Kondratyuk et al. [18] tested the fungicidal properties of polyamide foils (PA-12). They were coated with polyhexamethylene guanidine dodecylbenzenesulfonate (PHMG-DBS) at a concentration of 3–10%. Tests were carried out against test mixtures culture: *Aspergillus terreus, A. niger, Penicillium funiculosum, P. ochrochloron* and *Trichoderma viride*. PA-12 films were found to have pronounced antifungal properties when modified with 5 wt% of polymeric biocide PHMG-DBS. The absence of colonies of fungal test-cultures on the PA-12 film surface was observed under the experimental conditions.

Effect of PHMG Derivatives on Fungal Hydrolase Activity

PHMG derivatives introduced into PLA decreased hydrolases activity in *A. niger* and *P. chrysogenum*. PLA with the PHMG salt of sulfanilic acid and granular polyethylene wax strongly inhibited activity of *C. albicans* hydrolases,

Fig. 6 Effect of PHMG introduced into PCL on hydrolase moulds and yeast. PCL-A (PCL with PHMG salt of sulfanilic acid), PCL-W (PCL with PHMG granular polyethylene wax), PCL-S (PCL with PHMG stearate). Values are expressed as mean $\pm$ SD ($n = 3$)
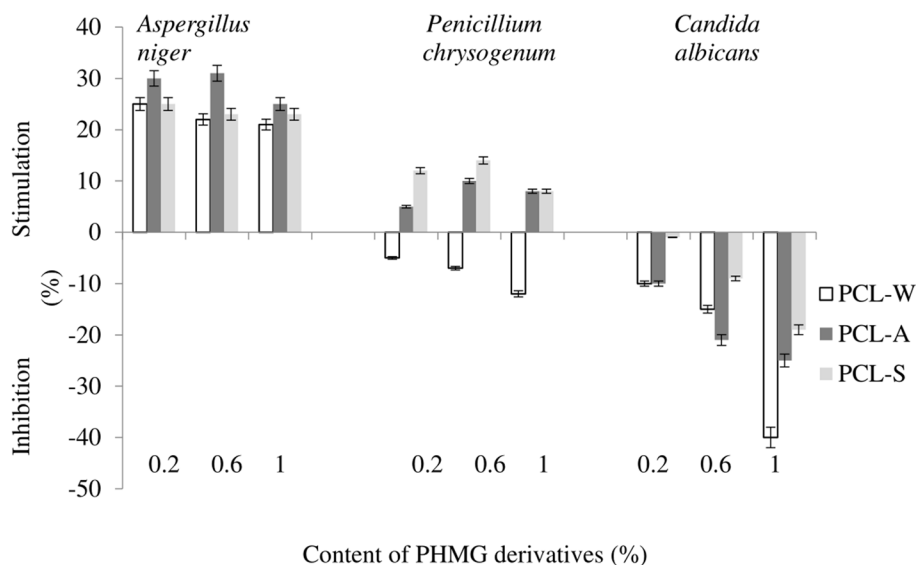
Fig. 7 Effect of PHMG introduced into PHB on hydrolase moulds and yeast. PHB-A (PHB with PHMG salt of sulfanilic acid), PHB-W (PHB with PHMG granular polyethylene wax), PHB-S (PHB with PHMG stearate). Values are expressed as mean $\pm$ SD ($n = 3$)
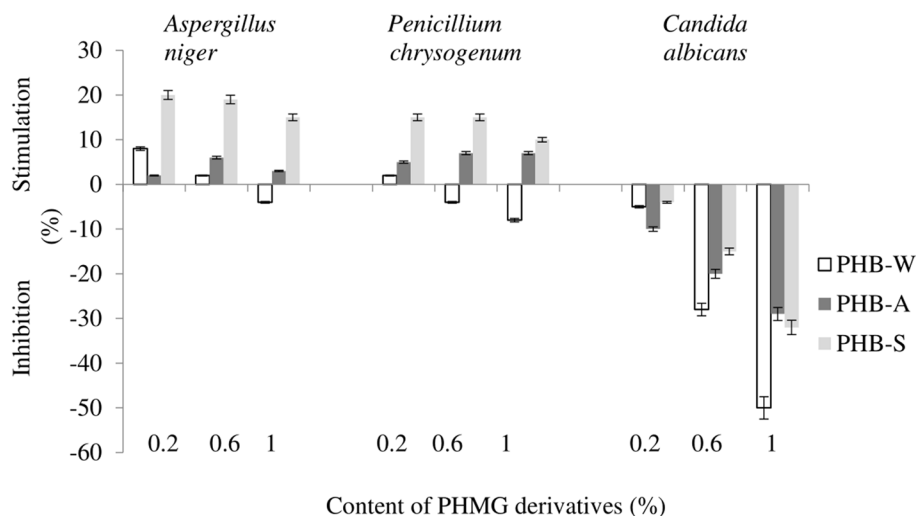
proportionately to the concentration (Fig. 5). Some PHMG derivatives introduced into PCL and PHB stimulated hydrolase activity in moulds. However PCL and PHB films with the PHMG granular polyethylene wax at a concentration 1.0% most strongly inhibited a hydrolases activity in yeast and state ranges from 40 to 52% depending on the type of film (Figs. 6, 7).

PHMG not only affects the permeability of the cell membrane of microorganisms but it can also inhibit the activity of enzyme systems such as hydrolysis responsible for degradation of polymers. Our research shows that PHMG significantly inhibited only the activity of *C. albicans* hydrolases. Walczak et al. [13] and Swiontek Brzezinska et al. [34, 35] studied the effect of PHMG derivatives introduced into the PLA, PHB and PCL on the activity of the bacterial hydrolases. Their research shows that the tested composites do not have a significant

impact on reducing the activity of hydrolases. They only observed inhibition of intercellular dehydrogenase activity. The effect of PHMG enzymatic activity inhibition of fungi is not well known. Undoubtedly, damage to the enzyme system will adversely affect the degradation of polymers in the environment. PLA is a polymer which is totally biodegradable by proteases, mainly serine proteases and esterases. Biodegradation, depending on the conditions under which it is carried out, may be slow or fast. Rapid degradation is conducive to high temperature and humidity [36]. PHB is degraded with esterases. *Penicillium funiculosum* and *Aspergillus fumigatus* are capable of producing these enzymes [37, 38]. Similarly, PCL degradation occurs with lipase and esterase [39]. *Aspergillus* sp. *Penicillium* sp., some filamentous fungi and yeast are capable of PCL hydrolysis [40]. Our research has shown that PHMG derivatives do not adversely affect the activity

of fungal hydrolases, so that the degradation of the tested composites in the environment will not be disturbed.

Conclusions

All of the PHMG derivatives inhibited the germination of *A. niger* and *P. chrysogenum*. All of the PHMG derivatives had a much stronger biocidal effect on the cells of *C. albicans*. All of the PHMG derivatives inhibited the germination of *A. niger* and *P. chrysogenum*. All of the PHMG derivatives had a much stronger biocidal effect on the cells of *C. albicans*. The composites produced from PLA, PHB, PCL and PHMG granular polyethylene wax derivative in concentration 1.0% can be used in many areas to reduce the growth of yeast (e.g. the production of biomedical or packaging materials). At the same time, PHMG derivatives introduced into biodegradable polymer do not adversely affect the hydrolase in *A. niger* and *P. chrysogenum* and therefore biodegradation of composites in the environment can proceed correctly.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval Research did not involve Human Participants and/or Animals.

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Affiliations

Maria Swiontek Brzezinska[1] · **Maciej Walczak**[1] · **Aleksandra Burkowska-But**[1] · **Marta Chylińska**[2] ·
Agnieszka Kalwasińska[1] · **Joanna Świątczak**[1]

[1] Department of Environmental Microbiology
and Biotechnology, Faculty Biology and Environmental
Protection, Nicolaus Copernicus University in Torun,
Lwowska 1, 87-100 Torun, Poland

[2] Faculty of Chemistry, Department of Chemistry
and Photochemistry of Polymers, Nicolaus Copernicus
University in Torun, Gagarina 7, 87-100 Torun, Poland