

Lucy Ciera,
*Lynda Beladjal,
**Xavier Almeras,
Tom Gheysens,
***Vincent Nierstrasz,
Lieva Van Langenhove,
*Johan Mertens

Ghent University,
Faculty of Engineering and Architecture,
Department of Textiles,
Technologiepark 907, B-9052 Zwijnaarde, Belgium.
E-mail: lucywanjiru.ciera@ugent.be

*Ghent University,
Faculty of Science,
Department of Biology,
Terrestrial Ecology Unit,
Ledeganckstraat 35, B-9000 Ghent, Belgium.
E-mail: Lynda.Beladjal@UGent.be

**DevanChemicals,
539, Klein Frankrijk, B-9600 Ronse, Belgium.

***The Swedish School of textiles,
University of Borås,
Bryggaregatan 17, SE-501 90 Borås, Sweden

Resistance of *Bacillus Amyloliquefaciens* Spores to Melt Extrusion Process Conditions

Abstract

With the increasing demand for functionalised textile materials, industry is focusing on research that will add novel properties to textiles. Bioactive compounds and their benefits have been and are still considered as a possible source of unique functionalities to be explored. However, incorporating bioactive compounds into textiles and their resistance to textile process parameters has not yet been studied. In this study, we developed a system to study the resistance of *Bacillus amyloliquefaciens* spores against melt extrusion process parameters, like temperature (21, 200, 250, 300 °C), pressure (0.1, 0.6 and 1.0 MPa) and residence time (0, 1 and 10 minutes). The spores were successfully embedded in PET (polyethylene terephthalate) films and fibres through melt extrusion. Afterwards the survival rate of the spores was determined after extrusion and the data was used to develop a quadratic equation that relates the survival rate to the spore concentration.

Key words: *Bacillus amyloliquefaciens*, spores, resistance, melt extrusion.

cal compounds like micro-organisms and their benefits is being explored.

Earlier studies have shown that additives can be introduced to textiles at different production stages like during fibre production, which ensures a strong bond between the polymer and additives. Moreover the compound added to the fibre can migrate slowly to the surface, acting as a slow release mechanism during use [3, 4]. However, for any additive to be successfully incorporated into synthetic textiles during fibre production, they must survive the extreme processing parameters used.

Due to superior mechanical properties, recyclability, low cost and easy processing, PET polymers are extensively used in various textile and technical applications [5, 6]. PET and other thermoplastic polymers are usually processed into fibres through melt extrusion. During extrusion, the polymer is subjected to high temperature, pressures and shear stress for a specific amount of time (residence time) which depend on several factors: the type of polymer, screw profile, die diameter etc. As most biological compounds are unable to withstand such extreme conditions, some bacteria are able to produce spores which are tough non-productive structures that can endure extreme environmental conditions. Therefore these spore forming bacteria are ideal candidates for embedding into fibres during melt extrusion [7 - 11].

Whereas the resistance of *B. amyloliquefaciens* spores to high temperature and pressure has been widely documented, the link between their resistance and the textile fibre extrusion process conditions is missing. Moreover none of the conditions reported matched the extrusion process condition of PET, which includes characteristics of the extruded polymer type like the heat conductivity and shear stress in the extruder, which can influence the survival of spores during extrusion [12 - 15]. Besides this, *Bacillus* species like *B. amyloliquefaciens* have received a lot of attention due to their potential to produce a variety of bioactive compounds, for example biopesticides [16]. In this study, however, the spores of *B. amyloliquefaciens* were used as proof of the concept and therefore we do not aim to add any functionality to the PET fibres at this point.

We first tested the survival rate of *B. amyloliquefaciens* spores against the extrusion parameters used for PET and showed that they can survive extreme extrusion conditions, resulting in successfully embedded spores in textile fibres. This work led to a model system to test the resistance of bioactive compounds for extrusion processing parameters. As new functionalities of bioactive compounds are being explored, this fast, reliable and economically viable technique for testing the resistance of biological compounds will therefore be important. Finally a quadratic equation was derived to relate the survival rate to the concentration of

■ Introduction

Biotechnology is becoming increasingly important in the textile industry, leading to novel multifunctional textile products with unique values and functionalities in contrast to classical textile materials. Some of the new functional properties obtained in textiles and clothing materials via biotechnology include antimicrobial properties and enzymatic modification of textiles e.g. polyester, wool, cotton, etc. [1, 2]. Due to the growing need for multifunctional, eco-friendly and efficient textile products, the use of biologi-

Table 1. Test parameters under which the *Bacillus amyloliquefaciens* spores were tested.

Parameters	Values
Temperature, °C	21 (Control), 200, 250 and 300
Pressure, MPa	0.1 (control), 0.6 and 1.0
Residence time, min.	0 (control), 1 and 10

spores during extrusion as a function of the temperature, pressure, residence time, shear stress and polymer used.

Materials and method

Microorganism

Spores of the thermophile bacterial strain *Bacillus amyloliquefaciens* used were obtained from bio-mining in the Moroccan desert and a stock culture was cultivated at Ghent University (Biology department). Spores of *B. amyloliquefaciens* are known to be resistant to high temperatures and pressures [13, 14], making them good candidates for extruding in polymers.

Resistance to temperature, pressure and residence time

To test the resistance of *B. amyloliquefaciens* spores to melt extrusion process parameters like the temperature, pressure and residence time, a special device was designed and developed at Ghent University (Biology Department). The spores were exposed to different test parameters based on a pilot study (Table 1).

Three different conditions were used:

- 1) temperature and atmospheric pressure,
- 2) temperature and pressure,
- 3) room temperature and pressure.

A compressor (HP3) was used to pressurise a known amount of dry spores in the sample holder, and then it was placed in a preheated dry air oven at the testing temperature required (Memmert UFE 500). A probe of thermocouple wire connected to a data logger (Testo 175-T3) was placed in direct contact with the sample in order to follow the actual temperature on the sample. When the temperature required was reached, the sample was left in that temperature for the specified amount of time, after which the sample was removed and left to cool down to room temperature [17].

Then the spores treated were inoculated by dissolving in 1000 µl of sterile physi-

ological saline solution (0.8% NaCl) and diluted up to 10⁻¹⁵ fold. From this dilution, 50 µl was inoculated on a prepared culture medium (Nutrient Agar, Oxoid CM0003). The plates were incubated for 24 h at 40 °C. The survival of spores was determined by counting the colony forming units (CFU), after which a log calculation was made to obtain the results, expressed in log CFU/g dry weight.

The two-sample Kolmogorov-Smirnov statistical test was used to determine the significance of each test parameter on the number of resistant spores.

Extrusion of spores in PET films

Arnite thermoplastic PET pellets (Arnite A02307) from Royal DSM N.V. (Netherlands) were used in extruding both films and fibres. This is a long-chain semi-crystalline thermoplastic polymer with a density of 1.34 g/cm³, water absorption (equilibrium, 23 °C, 50% RH) of 0.30%, viscosity number of 85.0 cm³/g, melt temperature of 255 °C and processing temperature range between 265 - 295 °C.

Prior to extrusion, the PET pellets were dried in an oven for two days at 70 °C, after which pure *B. amyloliquefaciens* spores were homogeneously mixed with the pellets to obtain a 0.5% w/w concentration of spores to PET polymer. Finally this mixture was extruded in a Haake poly-drive extruder (Thermo Electronic Corporation, USA). The three different heating zones of the barrel were set at 265 °C for the feed stock (transport the material), 270 °C for plasticising (compression) and 275 °C for pumping (metering). The die was heated to 275 °C, while the screw speed and take-up velocity were set at 20 r.p.m. and 80 m/min respectively, resulting in a pressure of 0.5 MPa and film thickness of 0.8 mm. The average residence time during the extrusion was approximately one minute. The distribution and abundance of spores

Table 3. Surviving spores in fibres after being sterilised by soaking in sodium hypochlorite (NaClO) solution, Dettol solution (5%) and in ethanol solution (96%) for 10 minutes each or by pasteurizing at 80 °C for 10 minutes (n - number of replicas).

Concentration of spores, %	Amount of samples (n)	Sterilised (Mean Log CFU/g dry fibres)	Pasteurised (Mean Log CFU/g dry fibres)	
0	4	0	0	
2		14 ± 0	14 ± 1	
4			20 ± 1	
6		20 ± 1		19 ± 1
8				
10				

Table 2. Results for two-sample Kolmogorov-Smirnov tests of the effect of temperature, pressure and residence time on number of viable spores (n - number of replica); 1 - Control¹ represents a control temperature of 21 °C, 2 - Control² represents a control pressure of 0.1 MPa, 3 - Control³ represents a control time of 0 minutes.

Test parameters	Amount of samples (n)	p-value
Temperature, °C		
200 vs. control ¹	12	0.913
250 vs. control ¹		0.256
300 vs. control ¹		0.517
Pressure, MPa		
0.6 vs. control ²	12	0.056
1.0 vs. control ²		0.781
Time, min.		
1 vs. control ³	12	0.726
10 vs. control ³		1.000

in the films were determined by Scanning Electron Microscopy (Joel Quanta 200 F FE-SEM). Samples for the SEM were prepared by placing them on a stub, where they were gold coated using a sputter coater afterwards (Balzers Union SKD 030).

Extrusion of spores in PET fibres

After drying the PET pellets as explained earlier, pure *B. amyloliquefaciens* spores were added by gravimetric dosing during spinning to obtain a 0 (control), 2, 4, 6, 8 and 10% w/w concentration of spores to PET polymer. This mixture was extruded into multi-filament fibres by a single screw extruder (General Extrusion Technology, China). The three different heating zones of the barrel were set at 280 ± 10 °C, 290 ± 5 °C and 295 ± 2 °C. The die was heated at 295 °C and the pressure was around 6.0 ± 0.2 MPa, while the average residence time was approximately 5 ± 0.5 minutes.

Resistance of extruded spores in films and fibres

The resistance of the extruded spores in the PET films and fibres was determined by growth/germination biological assay

[18], which means that *B. amyloliquefaciens* spores that survived the extrusion processing conditions will form a colony after inoculation and be detected as viable. Before inoculation, the samples had to be sterilised to remove contamination due to the extrusion. Several sterilisation techniques like pasteurisation and sterilisation with chemicals can be used [18]. In order to test the most effective sterilisation method to use, two techniques were tested: pasteurisation and sterilisation with chemicals.

The samples were cut into small pieces to end up with 48 samples, each weighing about 85 mg. Half of them were then sterilised by soaking in Sodium hypochlorite (12% NaClO), in Dettol solution (5%) and in ethanol (96%) for 10 minutes each. The other half of the samples was sterilised by pasteurising in a water bath (Grant JB aqua 12) at 80 °C for 10 minutes. After each sterilisation process, the samples were dissolved in 1.0 ml

of sterile physiological saline solution, then diluted and inoculated on nutrient agar plates and incubated at 40 °C for 24 hours. Finally the plates were visually checked for colony forming spots.

To generate a statistical equation that explains the relationship between the survival rate and concentration of extruded *B. amyloliquefaciens* spores, the experimental results from the 'sterilised' column were used (Table 3).

Results and discussion

Effect of temperature

The effect of temperature on the heat resistance of *B. amyloliquefaciens* spores is given in Figure 1.a, showing a decrease in the number of viable spores with increasing temperature. The decrease in surviving spores means spores were killed during the heat treatment, increasing with a rise in temperature. Extreme dry heat can kill spores by damaging and

mutating the DNA [19 - 21]. DNA damage is mostly due to loss of base through depurination [10, 22 - 24].

Effect of pressure

The effect of pressure on the viability of *B. amyloliquefaciens* spores is shown in Figure 1.b. For the pressure range tested, from 0.1 to 1.0 MPa, pressure seemed to have no effect on the number of surviving spores. An explanation for the resistance of spores to pressure could be provided by DNA-binding proteins protecting the spore's DNA, the repair of DNA damage during spore germination, and by the impermeability of the spore's coat to high pressure [8, 9]. Additionally *B. amyloliquefaciens* spores were reported to resist pressures up to 1,700 MPa [26 - 28], which is much higher than the highest pressure tested (1.0 MPa). The pressure range tested was too low to kill the spores and therefore is not an important parameter in the extrusion of spores/PET films and fibres.

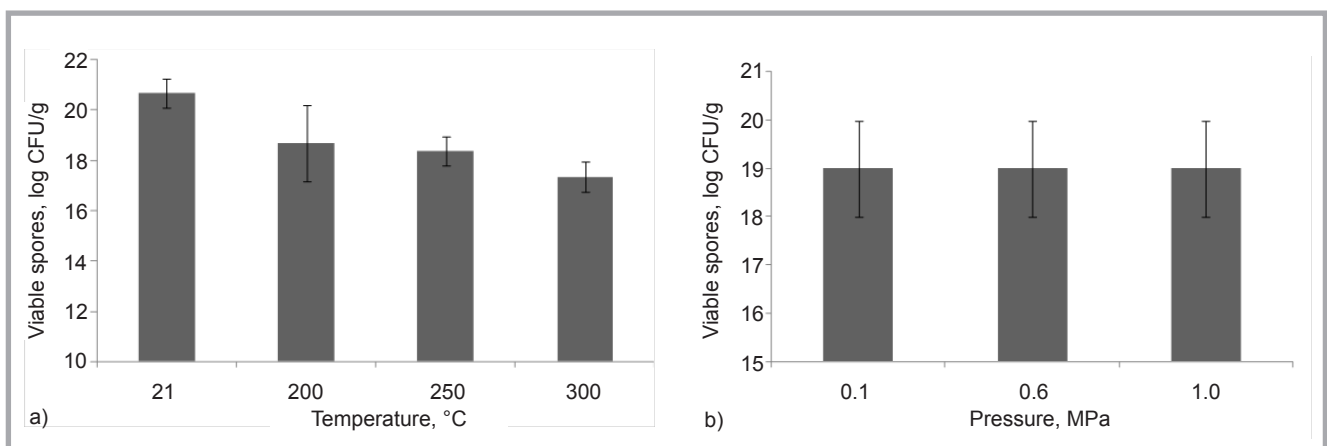


Figure 1. The effect of temperature (a) and pressure (b) on the viability of *Bacillus amyloliquefaciens* spores (four replicas each).

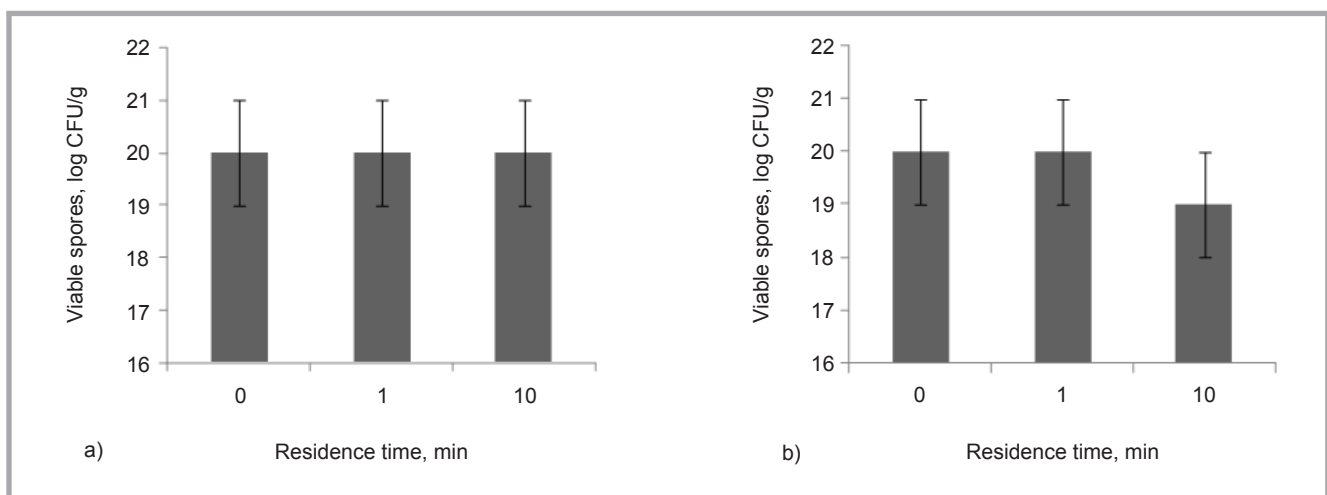


Figure 2. The effect of residence time on the viability of *Bacillus amyloliquefaciens* spores; a) under pressure (1.0 MPa) and b) under high temperature (300 °C) (four replicas each).



Figure 3. Photograph of PET film embedded with spores showing visible spots of spores on the film surface (indicated by arrows).

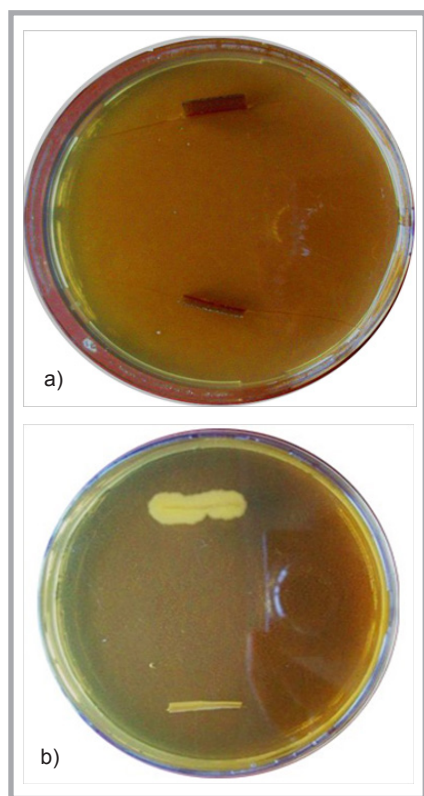


Figure 4. Viability tests (PET Films inoculated in nutrient medium and incubated for 24 h at 40 °C. a) control PET film with no spores showing no growth, b) PET films extruded with 0.5% of spores showing bacterial growth.

Effect of residence time

The influence of the residence time on the viability of *B. amyloliquefaciens* spores under pressure (1,0 MPa) and at high temperature (300 °C) was examined. Whereas the residence time under pressure showed no effect (**Figure 2.A**), that at high temperature showed a decrease in the number of surviving spores after 10 minutes (**Figure 2.B**). Since

B. amyloliquefaciens spores are well known to be resistant to high pressures [27 - 29], a pressure of 1,0 MPa is too low to cause any decrease in the survival of spores, even after 10 minutes of exposure. In contrast, the effect of the residence time at high temperatures is a linear decrease in the amount of surviving spores with increasing temperature (**Figure 1.a**). This is in accordance with previous results, in that fewer spores of *B. amyloliquefaciens* survive with increasing temperature [11, 12], which means that any change made in the residence time or temperature will have a great effect on the number of spores that survive the process. The longer the spores are exposed to high temperatures, the more likely it becomes that the spores will not survive the processing.

Statistical analysis

The statistical significance of the temperature, pressure and residence time on the number of viable spores was determined using the two-sample Kolmogorov-Smirnov test, the results of which are given in in **Table 2**. This test compared the distribution of values for each parameter against the control values. The analysis showed that none of the parameters tested saw a significant difference in the viable spores (**Table 2**, $p > 0.05$), meaning that neither temperature, pressure, nor time had a significant influence on the number of spores that survived the process. This lack of significance may be attributed to the low operating pressure levels and short residence time, which were discussed earlier. Additionally high temperature treatment has been shown to increase the relative hydrophobicity of the spore surfaces due to the denaturing of spore coat proteins, resulting in an agglomeration of the spores, which increases their survival chances against extreme temperatures [20, 30 - 33].

Extrusion of spores in PET films

PET films of 0.8 mm thickness incorporated with *B. amyloliquefaciens* spores were successfully extruded in a stable process. The surface of the PET films was very characteristic, in that many spots were visible due to the inclusion of the spores in the film, as can be seen in **Figure 3**. This is a first indication of the feasibility to successfully incorporate bacteria spores in extruded polymer films.

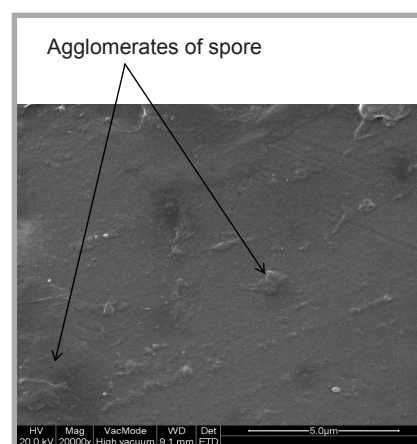


Figure 5. SEM micrograph of a PET film embedded with spores. The surface is characterised by spore aggregations (indicated by arrows).

The viability of the spores in the spore/PET films was tested by inoculation in nutrient medium and incubating for 24 h at 40 °C. This tests the survival of spores through germination/growth under favorable conditions [18]. The viability test results of the PET films are shown in **Figure 4**. The control sample, being pure PET film with no spores (**Figure 4.A**), did not show any growth, whereas the sample extruded with 0.5% spores showed bacterial growth all around the polymer film (**Figure 4.B**). The growth observed in **Figure 4.B** confirms that *B. amyloliquefaciens* spores survived the extrusion parameters very well.

As discussed earlier, the resistance of *B. amyloliquefaciens* spores to PET film extrusion can be explained by three factors, namely the low operating pressures, the short residence time and the formation of spore agglomerates during extrusion. Several factors can cause the spores to agglomerate in the extruder: the hydrophobic nature of the spores, heat treatment and shear stress [34, 35]. An example of spore aggregation in the PET film is given in the SEM picture shown in **Figure 5**.

Extrusion of spores in PET fibres

The concentration of spores successfully incorporated in PET fibres was from 0 to 10% w/w. Concentrations higher than 10% w/w blocked the spinnerets and therefore extrusion could not take place.

The two sterilising techniques studied proved to be equally efficient for fibres before inoculation (**Table 3**). PET fibres extruded with a 2% spore concentration showed the smallest survival of spores,

while concentrations of 4, 6, 8 and 10% of spores showed almost the same survival rate. These results further confirm the resistance of *B. amyloliquefaciens* spores to melt extrusion process parameters.

■ Quadratic equation

The equation developed to relate the survival rate of spores to the concentration of extruded spores was

$$y = 1.714 + 5.82x - 0.42x^2$$

where, y is the number of viable spores, x the concentration of compounded PET pellets, and x^2 is the squared term for the concentration of compounded PET pellets. The fitted curve is represented in **Figure 6** ($R^2 = 0.91$).

This quadratic equation can be used to relate the survival rate of *B. amyloliquefaciens* spores with concentration of spores extruded in PET fibres at 265 - 300 °C temperature, 6.0 ± 0.2 MPa pressure and 5 ± 0.5 minutes residence time.

■ Conclusion

In this study, we have demonstrated a suitable technique for testing the resistance of spores to extrusion process parameters (temperature, pressure and residence time). Results showed that none of the parameters tested had a significant effect on the survival of *Bacillus amyloliquefaciens* spores. Therefore the technique demonstrated proved to be cheap, easy and fast, hence it can be used as a model system to study the biological response of spores in relation to high temperature, pressure and residence time. It was also shown that spores can be successfully incorporated directly into PET polymer matrix during extrusion to develop textile bio-aggregates.

Acknowledgement

We would like to thank Jürgen Verstraeten, Biology Department, Ghent University for technical help. This work was funded by the European Commission, Grant agreement NMP2-SE-2009-228639.

References

- Gomes I, Sarkar P, Rahman S, Rahim M, Gomes D. Production of cellulose from *Talaromyces emersonii* and evaluation of its application in eco-friendly functional finishing of jute-based fabrics. *Bangladesh Journal of Microbiology* 2007; 24: 109-114.

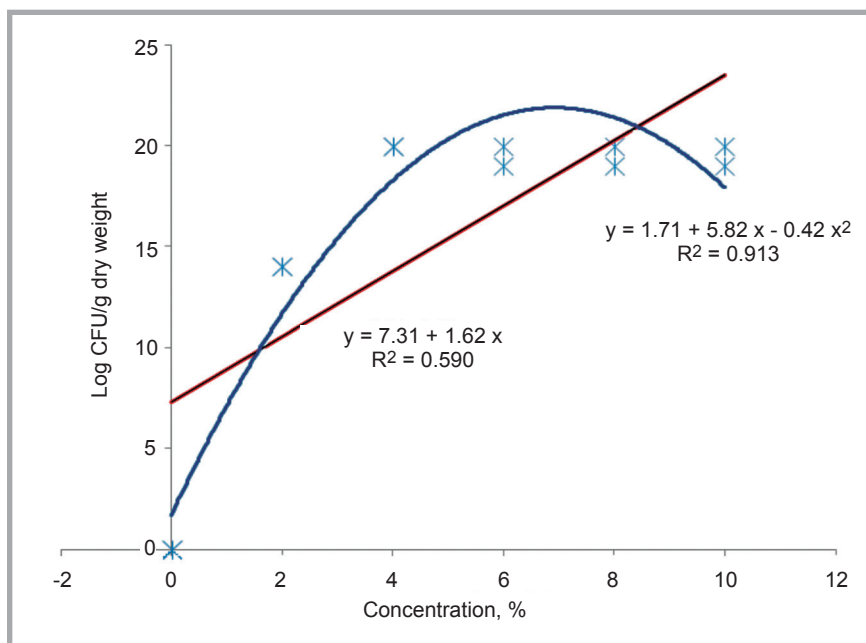


Figure 6. Quadratic curve relating the survival rate of spores to their concentration during extrusion.

- Kumar V, Meenakshisundaram S, Selvakuma N. Conservation of cellulose enzyme in bio-polishing application of cotton fabrics. *Journal of the Textile Institute* 2008; 99, 339-34.
- Hong K, Park J, Sul I, Youk J, Kang T. Preparation of antimicrobial Poly (vinyl alcohol) nanofibers containing silver nanoparticles. *Journal of polymer science Part B: Polymer Physics* 2006; 44: 2468-2474.
- Voort M, García D, Moezelaar R, Abee T. Germinant receptor diversity and germination responses of four strains of the *Bacillus cereus* group. *International Journal of Food Microbiology* 2010; 139: 108-15.
- Broda J, Gawlowski A, Fabia J, Slusarczyk C. Super-molecular structure of polypropylene fibers modified by additives. *Fibers and textiles in Eastern Europe* 2007; 15: 30-33.
- Gashti M, Moradian S. Effect of Nanoclay Type on dyeability of polyethylene Terephthalate/clay nanocomposites. *Journal of Applied Polymer Science* 2012; 5, 125: 4109-4120.
- Alderton G, Snell N. Chemical states of bacterial spores: dry heat resistance. *Applied Microbiology* 2012; 17: 745-749.
- Setlow P. Spores of *Bacillus subtilis*: their resistance to and killing by radiation, heat and chemicals. *The Society for Applied Microbiology, Journal of Applied Microbiology* 2006; 101: 514-525.
- Setlow P. Bacterial spore resistance. In: *Bacterial stress responses*. American society for microbiology. Storz G & Henge-Aronis R. (ed.), Washington, D.C. 1999: 217-230.
- Setlow P, Gisela S, Regine H. *Resistance of bacterial spores*. ASM press (American society for microbiology) 1752 N street NW Washington, 2000, DC 20036-2804.
- Beladjal L, Mertens J, Clegg JS. Biochemical and biophysical aspects of the tolerance of anhydrobiotic crustacean embryos to very high temperatures. *Journal of Thermal Biology* 2008; 33: 117-127.
- Anthierens T, Ragaert P, Verbrugge S, Ouchchen A, De Geest B, Noseda B, Mertens J, Beladjal L, Cuyper D, Dierickx W, Prez F, Devlieghere F. Use of endospore-forming bacteria as an active oxygen scavenger in plastic packaging materials. *Innovative Food Science and Emerging Technologies* 2011; 12, 594-59.
- Juhee A, Balasubramaniam V. Physiological Responses of *Bacillus amyloliquefaciens* Spores to High Pressure. *Journal of Microbiology and Biotechnology* 2007; 17: 524-529.
- Margosch D, Gänzle M, Ehrmann M, Vogel R. Pressure inactivation of *Bacillus* endospores. *Applied and Environmental Microbiology* 2004; 70: 7321-7328.
- Mertens J, Beladjal L, Devlieghere F, Verbrugge S, Sas B, Du Prez F, Anthierens T, Ouchchen A. Incorporation of thermo-resistant and/or pressure-resistant organisms in materials. *Resilux* 2010, WO2010/034776A1.
- Nagórska K, Bikowski M, Obuchowski M. Multicellular behavior and production of a wide variety of toxic substances support usage of *Bacillus subtilis* as a powerful biocontrol agent. Review. *Acta Biochemica Polonica* 2007; 54: 495-508.
- Mertens J, Beladjal L, Alcantara A, Fougnyes L, Van Der Straeten D, Clegg JS. Survival of dried eukaryotes (anhydrobiotes) after exposure to very high temperatures. *Biological Journal of the Linnean Society* 2008; 93: 15-22.
- Yang W, Ponce A. Rapid endospore viability assay of *Clostridium sporogenes*

- spores. *International Journal of Food Microbiology* 2009; 133: 213-216.
19. Marquis R, Sim J, Shin S. Molecular mechanism of resistance to heat and oxidative damage. *Journal of Applied Bacteriology* 1994; 76: 40S-48S.
 20. Furukawa S, Narisawa N, Watanabe T, Kawarai T, Myozen K, Okazaki S, Ogihara H, Yamasaki M. Formation of the spore clumps during heat treatment increases the heat resistances of bacterial spores. *International Journal of Food Science and Technology* 2005; 102: 107-111.
 21. Furukawa S, Noma S, Shimoda M, Hayakawa I. Effect of initial concentration of bacterial suspensions on their inactivation by high hydrostatic pressure. *International Journal of Food Science and Technology* 2002; 37: 573-577.
 22. Beaman T, Pankratz H, Gerhardt P. Heat Shock Affects Permeability and Resistance of *Bacillus stearothermophilus* Spores. *Applied and Environmental Microbiology* 1988; 54: 2515-2520.
 23. Setlow B, Setlow P. Heat Inactivation of *Bacillus subtilis* Spores Lacking Small, Acid-Soluble Spore Proteins Is Accompanied by Generation of a basic Sites in Spore DNA. *Journal of Bacteriology* 1994; 176, 7: 2111-2113.
 24. Lee S, Sim S. Increased heat resistance of *Geobacillus stearothermophilus* spores heat-shocked during sporulation. *Journal of Microbiology and Biotechnology* 2006; 16: 633-636.
 25. Setlow P. Mechanisms for the prevention of damage to the DNA in spores of *Bacillus* species. *Annual Review of Microbiology* 1995; 49: 29-54.
 26. Gould G. History of science – Spores Lewis B Perry Memorial Lecture 2005. *Journal of Applied Microbiology* 2006; 101: 507-513.
 27. Minh H, Dantigny P, Perrier-Cornet J, Gervais P. Germination and inactivation of *Bacillus Subtilis* spores induced by moderate hydrostatic pressure. *Biotechnology And Bioengineering* 2010; 107: 876-883.
 28. Reineke K, Mathys A, Knorr D. The Impact of High Pressure and Temperature on Bacterial Spores: Inactivation Mechanisms of *Bacillus subtilis* above 500 MPa. *Journal of Food Science* 2011; 76: M189-M197.
 29. Nicholson W, Munakata N, Horneck G, Melosh H, Setlow P. Resistance of *Bacillus* endospores to extreme terrestrial and extraterrestrial environments. *Microbiology and Molecular Biology Reviews* 2000; 64: 548-572.
 30. Li-Chan S, Nakai D, Wood F. Hydrophobicity and solubility of meat proteins and their relationship to emulsifying proteins. *Journal of food science* 1984; 49: 345-350. In: Matin M, Casas C, Cambero M, San B. Study of the effect of heat (treatments) on meat protein denaturation as determined by ELISA. *Food Chemistry* 1992; 43: 147-150.
 31. Wiencek K, Klapes N, Forgrding P. Hydrophobicity of *Bacillus* and *Clostridium* spores. *Applied Environmental Microbiology* 1990; 56: 2600-2605.
 32. Pflug I, Holcomb R, Gomez M. Principles of the thermal destruction of microorganisms, 2001. In: Furukawa S, Narisawa N, Watanabe T, Kawarai T, Myozen K, Okazaki S, Ogihara H, Yamasaki M. Formation of the spore clumps during heat treatment increases the heat resistances of bacterial spores. *International Journal of Food Science and Technology* 2005; 102: 107-111.
 33. Myozen K, Okazaki S, Ogihara H, Yamasaki M. Formation of spore clumps during heat treatment increases the heat resistance of bacterial spores. *International Journal of Food Microbiology* 2005; 102: 107-111.
 34. Senior A, Moir A. The *Bacillus cereus*-GerN and GerT protein homologs have distinct roles in spore germination and outgrowth, respectively. *Journal of Bacteriology* 2008; 190: 6148-6152.
 35. Margosch D, Ehrmann M, Buckow R, Heinz V, Vogel R, Gañzle M. High-Pressure-Mediated Survival of *Clostridium Botulinum* and *Bacillus amyloliquefaciens* endospores at high temperature. *Applied and Environmental Microbiology* 2006; 72: 3476-3481.

Received 25.06.2013 Reviewed 17.12.2013

XX Workshop on 'New Aspects of the Chemistry and Applications of Chitin and its Derivatives'



INVITATION

On behalf of the Board of the Polish Chitin Society I have both a pleasure and an honour to invite you to participate in the **XVII Seminar and Workshop on "New Aspects of the Chemistry and Applications of Chitin and its Derivatives"** which will be held in **Łódź, Poland, September 24th – 26th, 2014**.

The aim of the conference is to present the results of recent research, development and applications of chitin and chitosan.

It is also our intention to give the conference participants working in different fields an opportunity to meet and exchange their experiences in a relaxing environment.

Best regards
Malgorzata M. Jaworska Ph.D., D.Sc., Eng.

For more information please contact:

CONFERENCE SECRETARY
M. Skłodowskiej-Curie 19/27, 90-570 Łódź, Poland
tel. (+48) 42 638 03 339, fax (+ 48) 42 637 62 14,
e-mail: ptchit@ibwch.lodz.pl www.ptchit.lodz.pl