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Nanotribological investigation of the poly (3-hydroxybutyrate) films manufactured from the storage polyesters produced by *Halomonas elongata* DSM 2581

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Abstract:

This study aims to the evaluation of the tribological behavior of different polyester biofilms fabricated via the solvent casting method. Three polyester films were designed and investigated in this study each containing 1% w/v constituents including a **PHBh** film prepared out of the PHB extracted from the extremely halotolerant bacteria *Halomonas elongata* DSM2581^{T}, a **PHBc** film fabricated using a commercially available PHB, and a **PHBVc** film generated using the commercial poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV). The spectroscopy-in-point of AFM was used for adhesion force measurements and the AFM lateral mode was applied for friction analysis. The tribological investigations of PHBh film revealed a biodegradable material with low roughness as well as small adhesion and friction forces. The wear behavior was analyzed by considering three scratching forces (10 μN, 20 μN, 30 μN), 40μm the scratching length and 5 minutes the scratching time for all investigated materials. After, by using the scanning mode of AFM, the removed scratched material was estimated. The obtained experimental results indicate a good tribological behavior of the new developed PHBh film compared with the biofilms obtained from commercially raw material.

Keywords: Poly(3-hydroxybutyrate) films, AFM, Adhesion, Friction, Wear

Contents:

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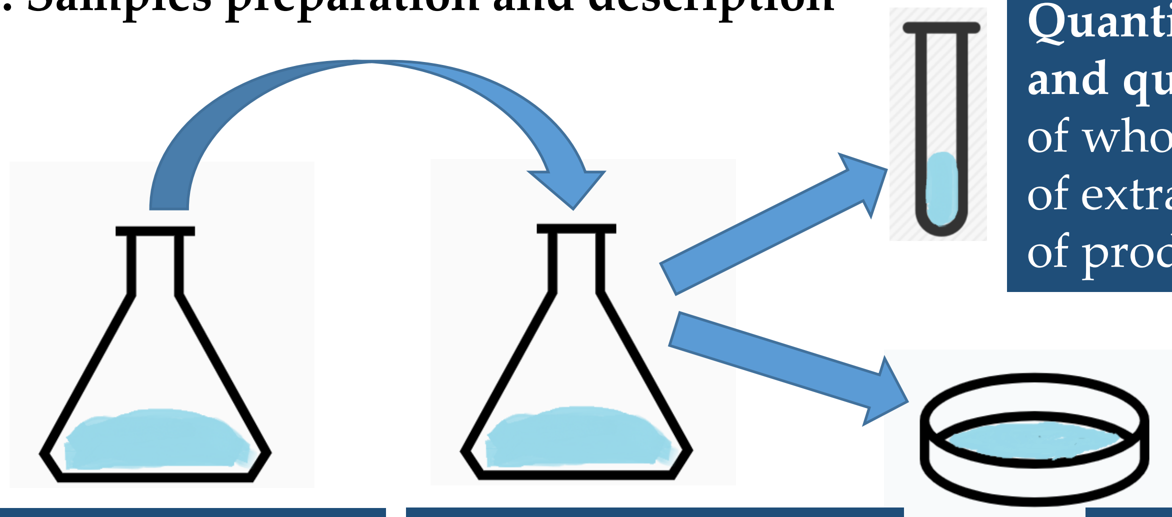
1. Introduction

The scope of this study is orientated to the evaluation of the tribological properties as adhesion, friction and wear behaviors of different polyester films fabricated using the solvent casting method [**1**]. Poly(3-hydroxybutyrate) - PHB is a biodegradable polyester, produced by numerous bacteria. To overcome the negative impact of plastic waste on environment, bio-based and biodegradable substituents are required to replace petroleum-derived plastics [**2**]. Polyhydroxyalkanoates (PHAs) are a class of energy storage compounds produced by prokaryotes, algae, and plants and are considered vital candidates to replace conventional plastics in the packaging field and biomedical sectors [**3**]. These polyesters are fully biodegradable, immunologically inert and have physical properties as conventional plastics [**3**].

The improvement of the PHB material lifetime involves the mechanical and tribological characterization which can be accurately performed by using the atomic force microscopy (AFM) technique. The tribological behavior evaluation of developed PHBh film by AFM is the main research scope of this work. The same method based on the tapping mode of AFM was used to examine the microstructure of polyester films degradation before and after exposure to an alkaline solution [**4**]. The AFM technique was also successfully applied to determine the material behavior of Microelectromechanical (MEMS) components fabricated from soft materials as SU-8 integrated with metallic materials on the same structure [**5**].

2. Samples preparation and description



Quantitative (crotonic acid assay) **and qualitative** (Nile Red staining of whole cells, NMR spectroscopy of extracted powder) **assessment** of produced PHBh

H. elongata starter culture on complete medium (0.1% glucose, 8% NaCl)

H. elongata culture on nitrogen-limited medium (1 % glucose, 8 % NaCl)

PHB extraction by hypochlorite, **purification** and **film preparation (repeated water washing).**

Three polyester films were designed in this study, each containing 1% w/v constituents as follows:

1. **PHBh** (Sample 1) film prepared out of the PHB extracted from the extremely halotolerant bacterium *Halomonas elongata* DSM2581^{T},
2. **PHBc** (Sample 2) film fabricated using a commercially available PHB and
3. **PHBVc** (Sample 3) film generated using the commercial poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV).

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3. Topographical analysis

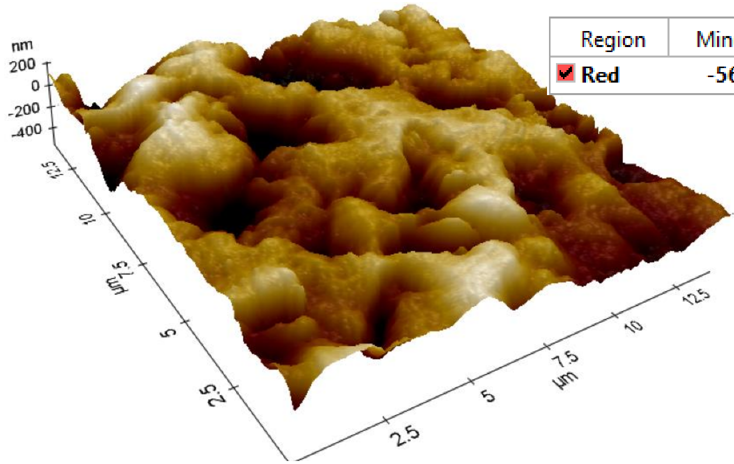
Scope: Analyses of the surface morphology and roughness of investigated samples

Using method: The non-contact scanning mode of AFM was applied. The type of AFM probe used in this experiment was PPP-NCHR with a force constant of 42N/m and 330kHz the RF. In this operating tapping mode, the AFM tip is vibrating close to the surface measuring the topography by use the attractive atomic force between the tip and sample surface

Input parameters: Scanning area was of 40μm×40μm

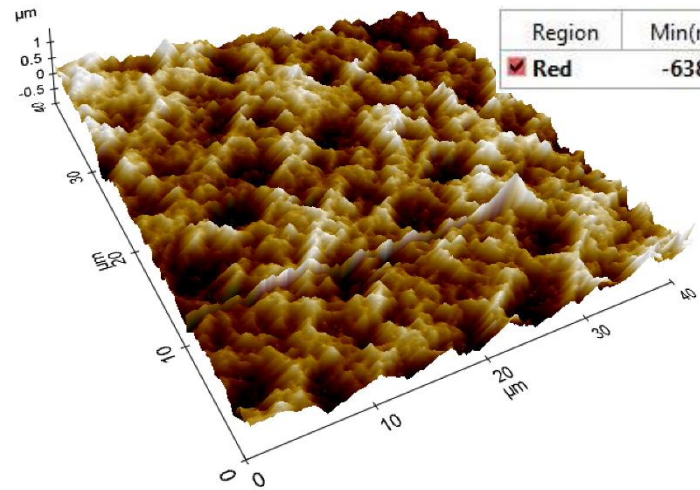
Operating conditions: Controlled humidity of 40%RH and temperature of 20°C; antivibration stage to avoid the external noises

Output results: 3D images, roughness parameters and the grains distribution



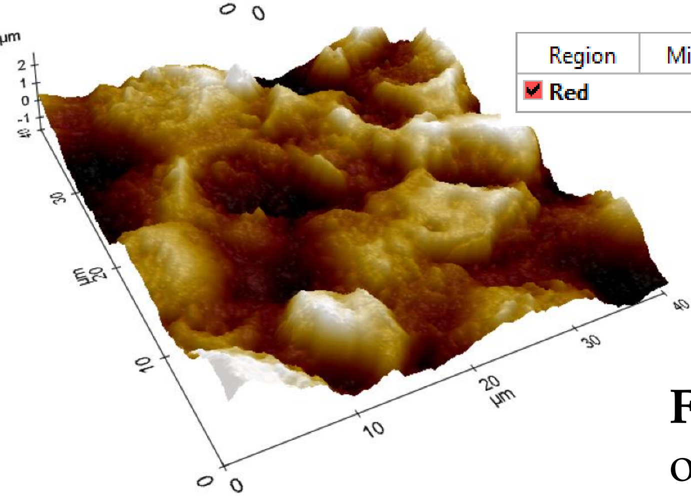
Region	Min(nm)	Max(nm)	Mid(nm)	Mean(nm)	Rpv(nm)	Rq(nm)	Ra(nm)	Rz(nm)	Rsk	Rku
Red	-564.744	212.081	-176.331	0.000	776.824	113.255	88.357	761.983	-1.099	4.380

(**a**) PHBh



Region	Min(nm)	Max(nm)	Mid(nm)	Mean(nm)	Rpv(nm)	Rq(nm)	Ra(nm)	Rz(nm)	Rsk	Rku
Red	-638.039	502.488	-67.775	0.000	1140.526	192.130	154.602	1103.123	-0.281	2.857

(**b**) PHBc



Region	Min(µm)	Max(µm)	Mid(µm)	Mean(µm)	Rpv(µm)	Rq(µm)	Ra(µm)	Rz(µm)	Rsk	Rku
Red	-1.180	1.184	0.002	0.000	2.364	0.420	0.339	2.326	-0.081	2.682

(**c**) PHBVc

Fig. 1 Images (3D) and roughness parameters of samples: (**a**) PHBh; (**b**) PHBc; (**c**) PHBVc

4. Hardness and modulus of elasticity

Scope: To determine the modulus of elasticity and the hardness of PhBh, PHBc and PHBVc investigated biomaterials

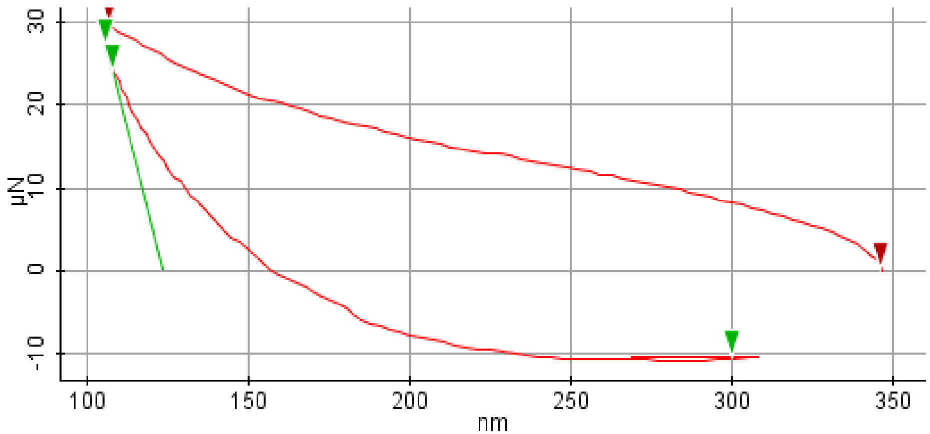
Using method: The nanoindentaion method was applied by using the nanoindentation module of AFM and a Berkovich tip. The results were interpreted based on the Oliver and Pharr Model . The AFM probe used is TD23838 with 272N/m the constant force

Input parameters: The force set-up was selected to 5μm and the indentations were performed in different locations on the material under 30μN the indentation force. The Poisson ration used in the results interpretation was 0.36 [**6**]

Operating conditions: Controlled humidity of 40%RH and temperature of 20°C; antivibration stage to avoid the external noises

Output results: Nanoindentation curves, hardness and modulus of elasticity

Model : Oliver and Pharr Model



Cursors

Cursor	ΔX(nm)	ΔY(µN)	Left X(nm)	Left Y(µN)	Right X(nm)	Right Y(µN)
Force	239.356	-29.443	107.132	29.394	346.488	-0.049
Slope	1.873	-3.130	105.914	26.777	107.788	23.647

Slope Cursor Index 1 : 81
Slope Cursor Index 2 : 84
Contact Depth : 225.4nm
Tip Shape : Berkovich
Poisson's ratio of the tip : 0.07
Poisson's ratio of the sample : 0.36
Hardness : 23.65MPa
Young's modulus : 1.09GPa

Contact Depth : 225.4nm
Tip Shape : Berkovich
Poisson's ratio of the tip : 0.07
Poisson's ratio of the sample : 0.36
Hardness : 23.65MPa
Young's modulus : 1.09GPa

(**a**) PHBh

Contact Depth : 393.46nm
Tip Shape : Berkovich
Poisson's ratio of the tip : 0.07
Poisson's ratio of the sample : 0.36
Hardness : 7.88MPa
Young's modulus : 305.13MPa

(**b**) PHBc

Contact Depth : 323.25nm
Tip Shape : Berkovich
Poisson's ratio of the tip : 0.07
Poisson's ratio of the sample : 0.36
Hardness : 11.29MPa
Young's modulus : 502.54MPa

(**c**) PHBVc

Fig.2 Nanoindentation curves of PHBh sample under a force of 30μN and results of: (**a**) PHBh; (**b**) PHBc; (**c**) PHBVc

5. Adhesion force

Scope: To determine the adhesion force between the AFM tip material and the investigated samples

Using method: The tests are performed using the spectroscopy in point of AFM. The adhesion tests were repeated 4 times and the average values were considered

Input parameters: The normal force was selected at 50μN. The AFM probe was PPP-NCHR type with a constant force of 42N/m and the tip radium of 7 nm

Operating conditions: Controlled humidity of 40%RH and temperature of 20°C; antivibration stage to avoid the external noises

Output results: The adhesion force was measured and interpretated using the XEI software

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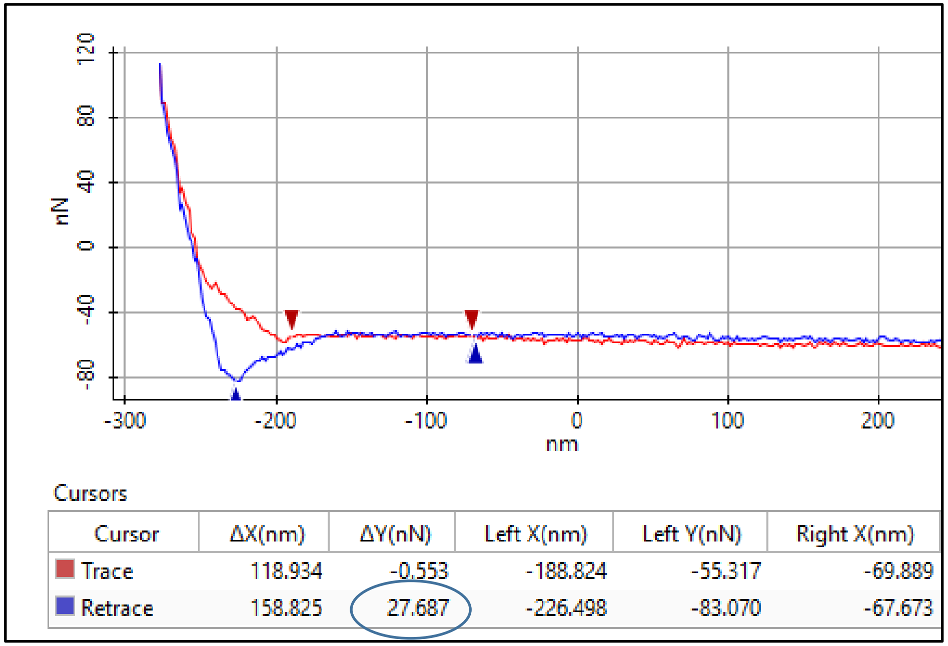

Fig. 3 Adhesion force between AFM tip (Si) and the PHBh material

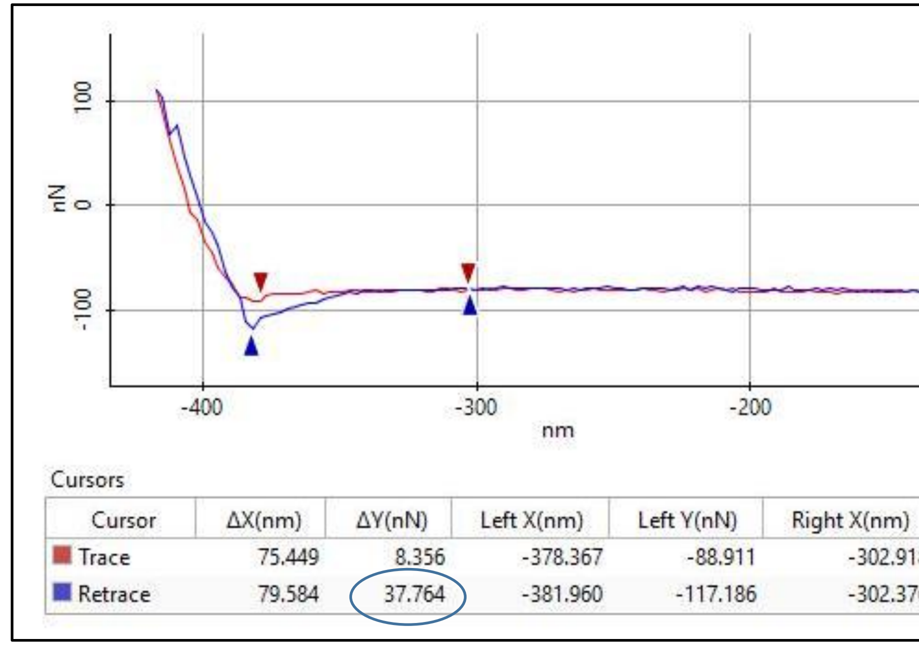

Fig. 4 Adhesion force between AFM tip (Si) and the PHBc material

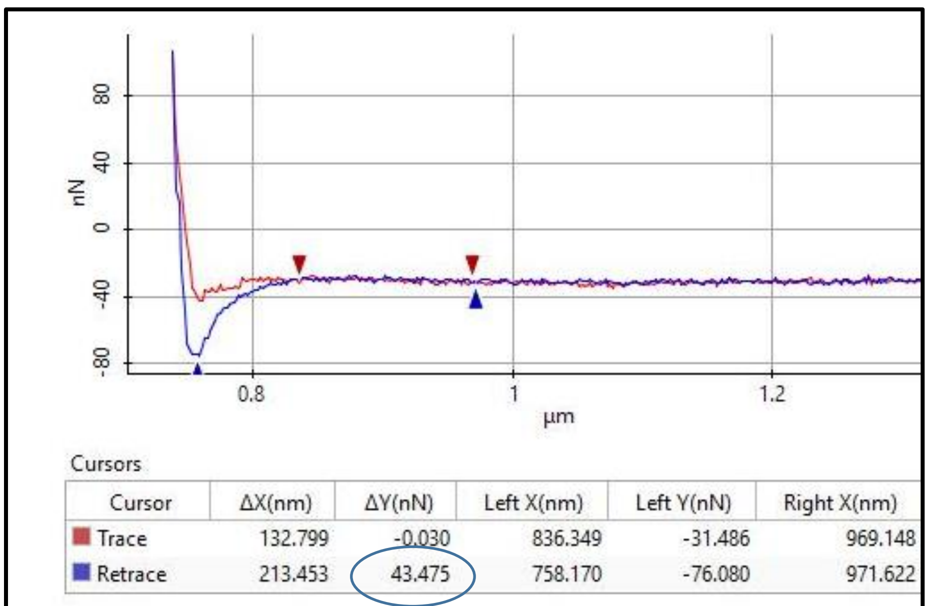

Fig. 5 Adhesion force between AFM tip (Si) and the PHBVc material

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6. Friction analysis

Scope: To measure the friction force between investigated materials and AFM tip (Si)

Using method: The AFM lateral mode is used for this measurements. The friction map gives information about the torsional deflection of the AFM probe during lateral movement on the direct contact with samples surface.

Input parameters: The AFM probe used in friction characterization is PPP-NCHR with the following parameters: force constant 42N/m, length 125μm, width 30μm, thickness 4μm, the tip height 15μm. The normal applied force (set-point) was 200nN

Operating conditions: Controlled humidity of 40%RH and temperature of 20°C; antivibration stage to avoid the external noises

Output results: The friction maps and the friction coefficients

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Based on torsion beam theory, the friction force between AFM probe and investigated polymers can be computed as:

$$F_f = \frac{dz \cdot r \cdot G \cdot h^3 \cdot b}{l^2 \cdot s}$$

where dz[μm] is the calibrated deflection of AFM probe determined based on the difference between profiles toward and backward Y/2 (**Fig.6)** in volts [V] and divided to the sensitive factor 98.97 V/μm (provided by manufacturer), r = 0.33, G – shear modulus of the AFM cantilever material, l – cantilever length, h – cantilever thickness, b – cantilever width, s – tip height of the AFM probe.

Then, the friction coefficient is determined as the friction force divided by the sum between the normal applied force and the adhesion force presented in Figs. 3 - 5.

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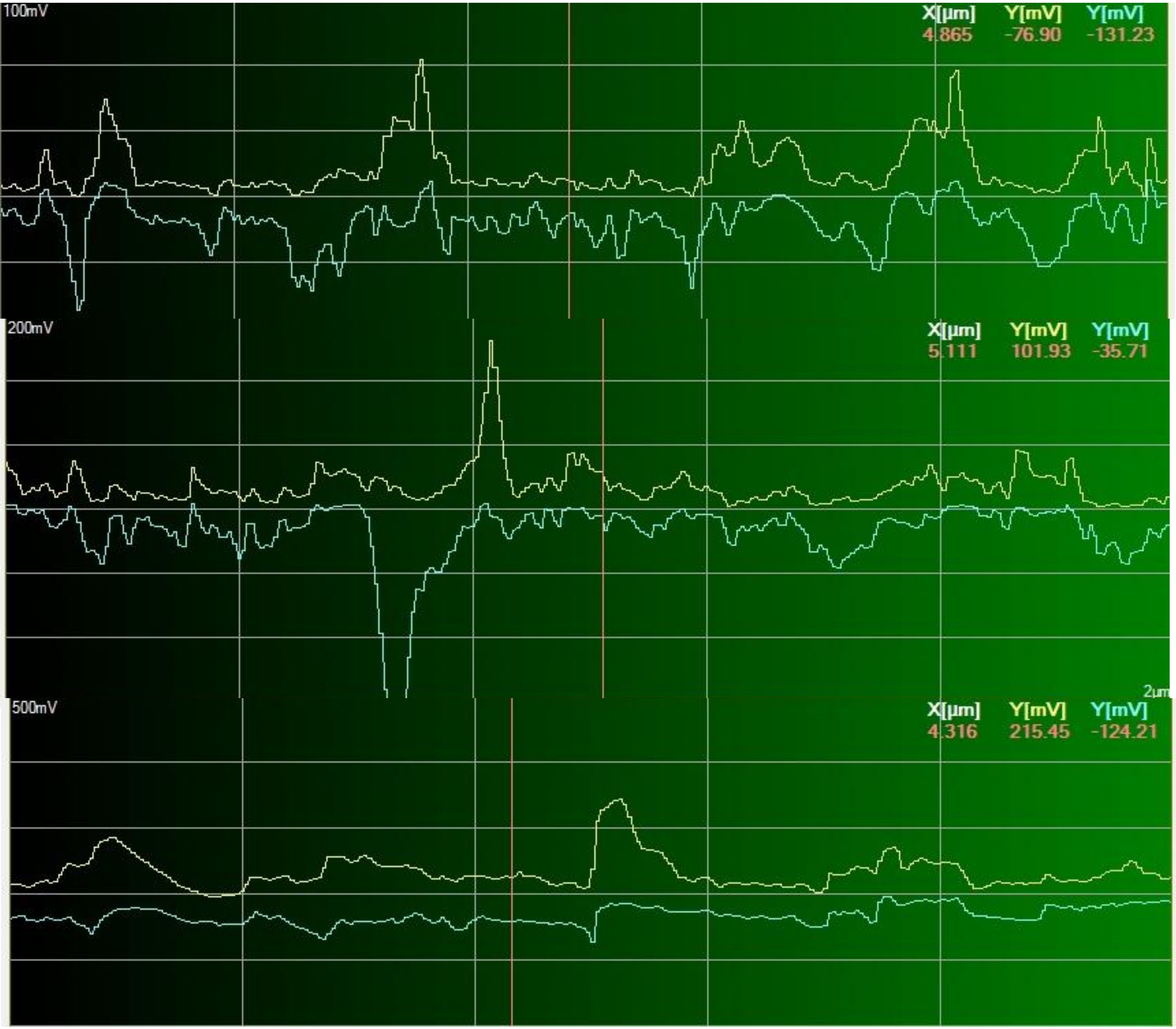

(a) Si/PHBh
F_f = 47 nN
μ = 0.15

(b) Si/PHBc
F_f = 119.14 nN
μ = 0.35

(c) Si/PHBVc
F_f = 295.15 nN
μ = 0.85

Fig.6 Friction maps, friction force F_f and friction coefficient μ between AFM tip (Si) and investigated materials

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7. Wear tests

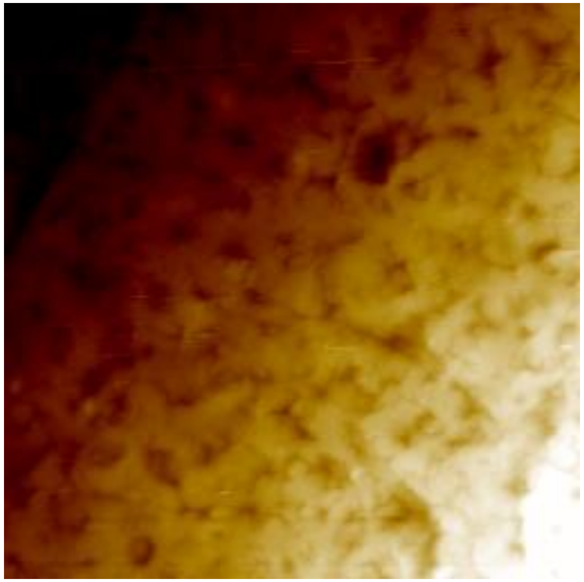
Scope: To determine the difference between the wear resistance of investigated bio-materials PHBh and PHBc with higher and smaller hardness (Fig.2)

Using method: The scratching of material by using the contact mode of AFM and a diamond Berkovich tip. After, the scanning of the scratched area by AFM was done for the interpretation of the removed material volume. XEI software used to measure the dimensions of the triangular section of the removed area. After by considering the length of scratch, the volume of the removed material was estimated

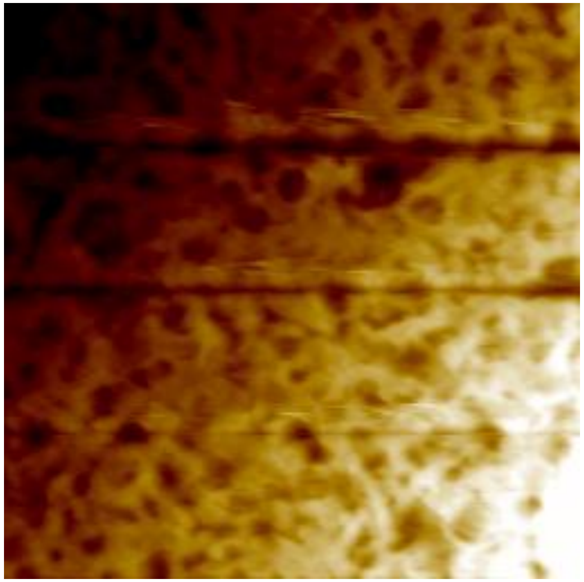
Input parameters: Normal load = 10μN, 20μN, 30μN; Scanning rate 1Hz, Scratching time = 5 minutes; Length of scratching 40μm

Operating conditions: Humidity of 40%RH; temperature of 20°C

Output results: Variation of the materials wear as a function of applied loads

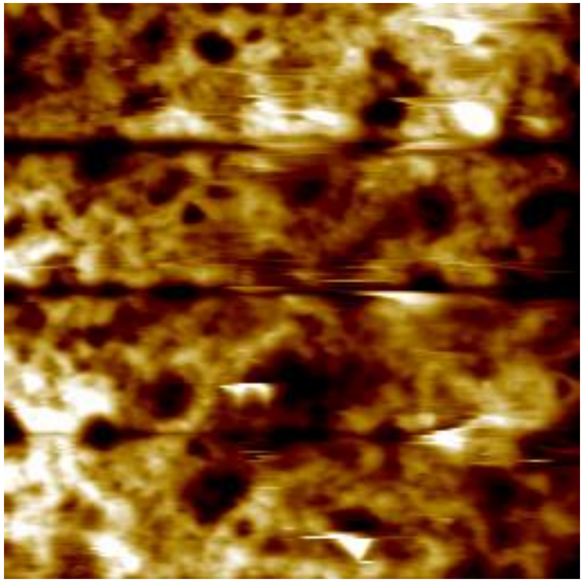


(a) PHBh



(b) PHBh

Fig.7 The wear test under different scratching forces: (**a**) is the initial surface of PHBh, after scratching the same probe is shown in (**b**), and the PHBc material is represented in (**c**)



(c) PHBc

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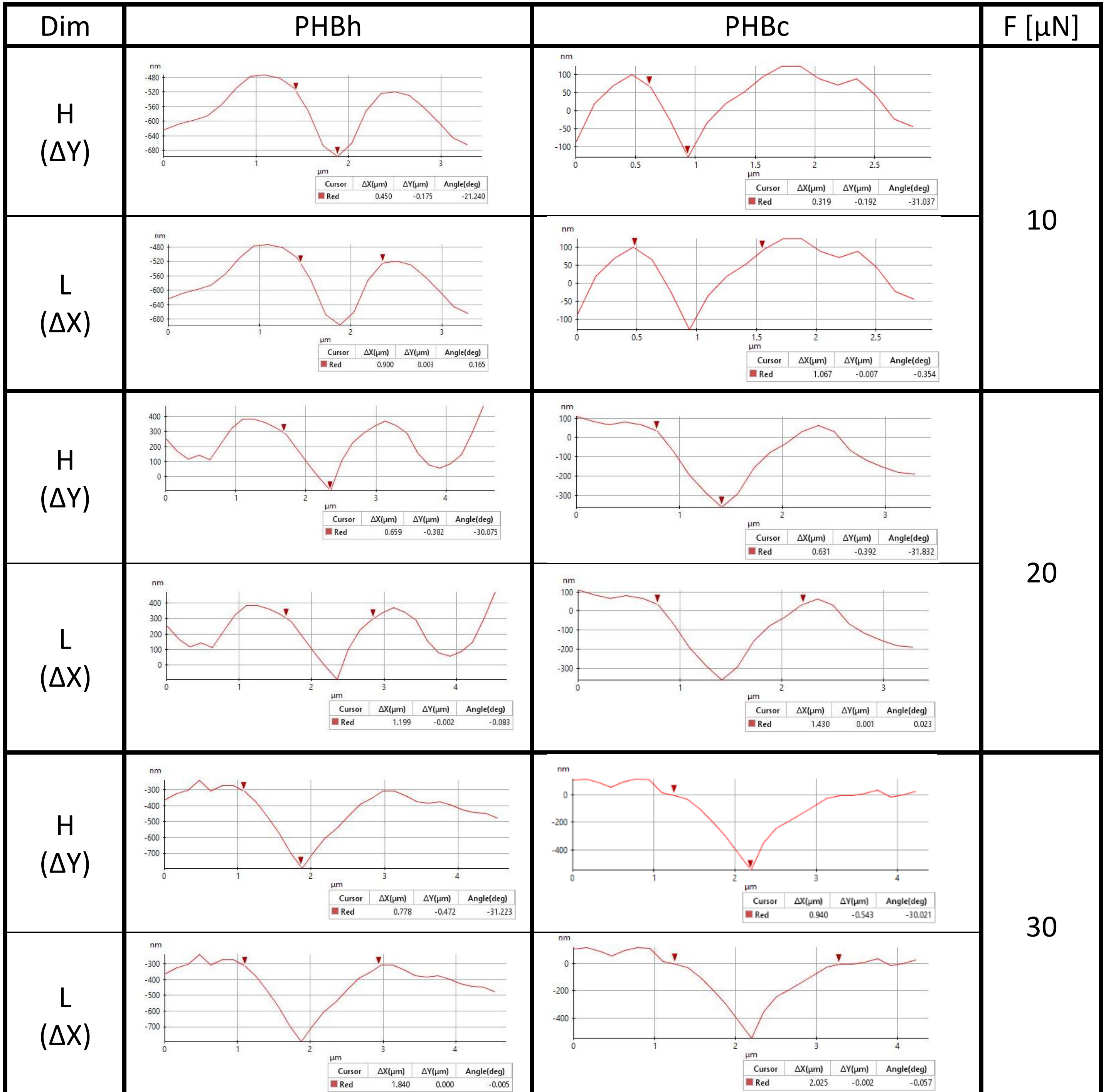
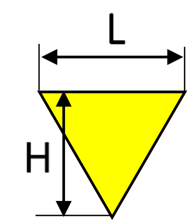


Fig.8 Wear area dimensions (high and length) of samples for different scratching forces F



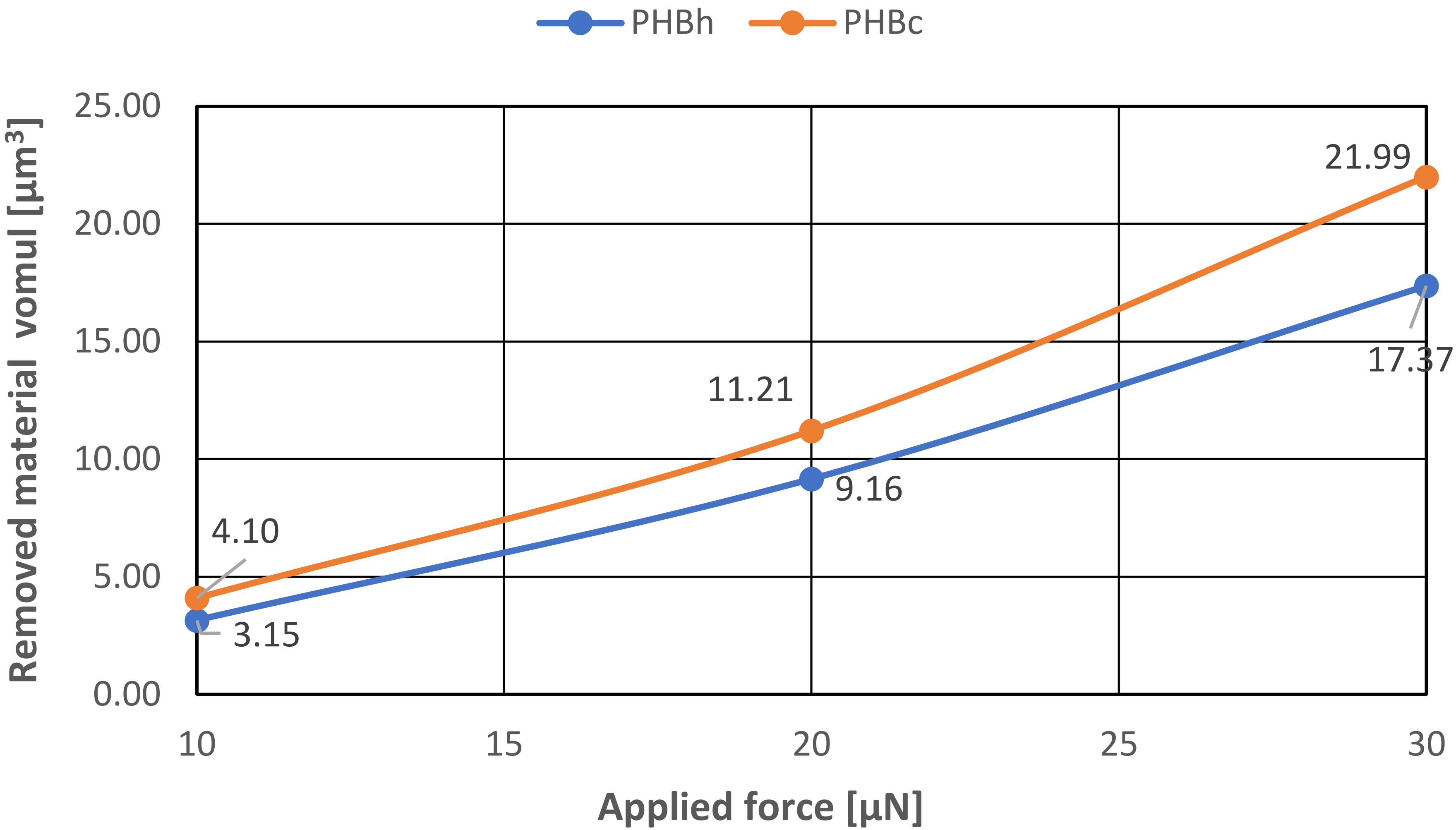


Fig.9 Variation of the volume of the removed material based on wear under different loads and for a controlled sliding time (5 minutes) for PHBh and PHBc materials

8. Results and Discussion

PHBh film prepared out of the PHB extracted from the extremely halotolerant bacteria Halomonas elongata DSM2581T has superior tribological and mechanical properties compared with a PHBc film fabricated using a commercially available PHB and a PHBVc film generated using the commercial poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV). The roughness of PHBh is smaller than the roughness paramenter of the PHBc and PHBVc sample and its modulus of elasticy and hardness are higher. In the same way, the adhesion and the friction forces is decreasing as well as the wear of the material removed by scratching. The AFM technique is an adequate testing technology to predict the wear behavior and the lifetime of biodegradable materials starting from nanoscale analysis.

9. Conclusions

Halomonas elongata DSM 2581^T used to produce PHB undergoing single nutrient limitation in nonsterile culture medium with high salinity (8% w/v NaCl) is an adequate material to obtain biodegradable samples with proper tribological and mechanical properties. The extensive experimental tests performed in this study demonstrated an improved tribo-mechanical properties of bacterial PHBh material compared to the films made of commercial PHB and poly(3-hydroxybutyrate-co-3-hydroxyvalerate (PHBV).

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Acknowledgments

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