TU Clausthal

# Valorization of degradation products stemming from enzymatic degradation of pre-treated PLA by advanced oxidation processes to bacterial nanocellulose

G. Sourkouni, M. Nenadovic,
V. Jankovic, Ch. Kalogirou, O. Höfft,
D. Rajasekaran, P. Pandis, R. Padamati,
J. Nikodinovic-Runic, Chr. Argirusis

- Materials Research Centre, TU Clausthal, Germany
- Institute of Molecular Genetics and Genetic Engineering, Serbia
- National Technical University of Athens, Greece
- Trinity College, Dublin, Ireland


BioICEP 解塑再用

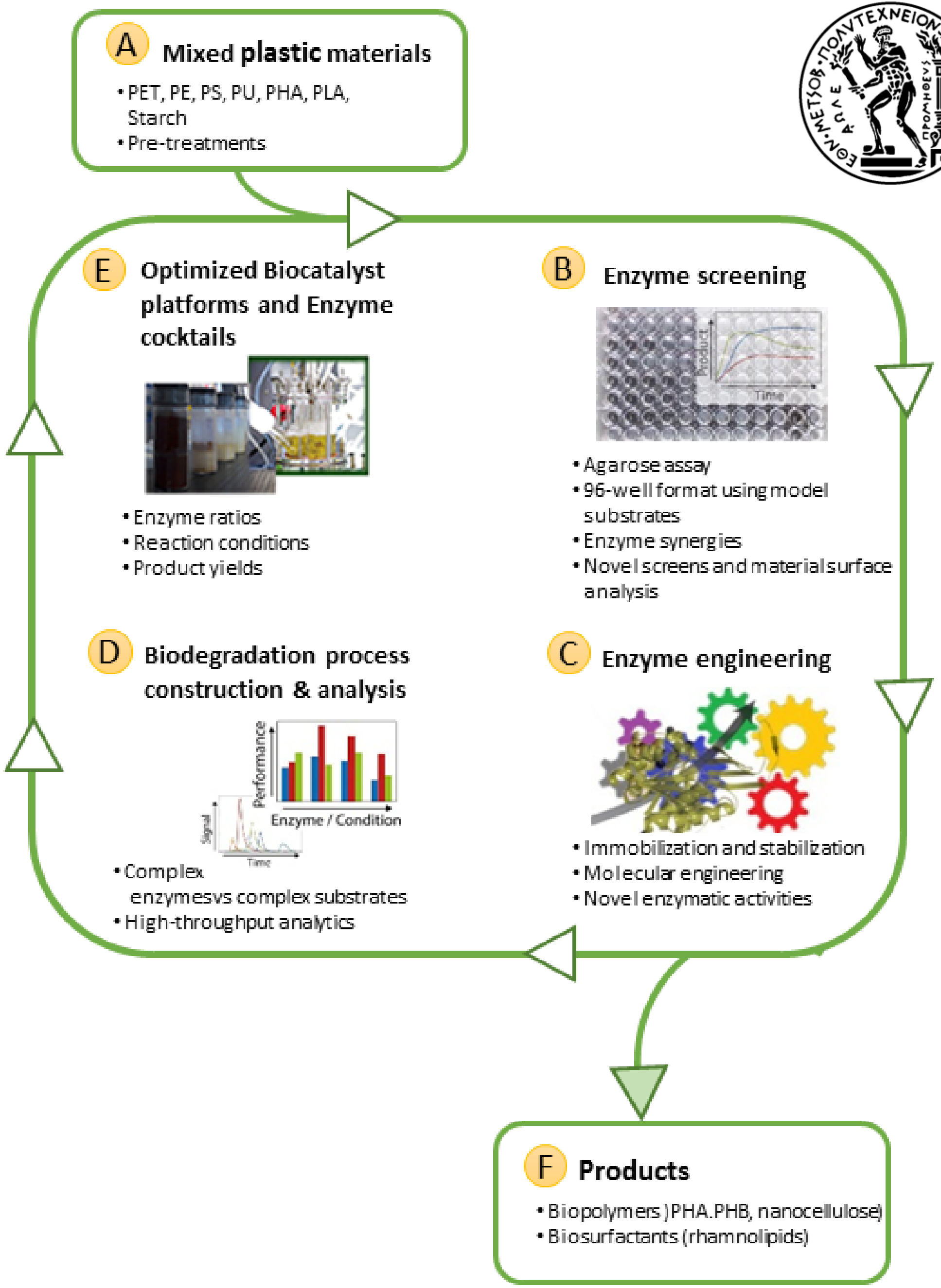

IDEA

# Overall Goals

● Development of pre-treatment processes suitable for surface modification allowing **Cocktails of enzymes** to dock on them and deliver monomers or low molecular weight molecules for valorisation.

● The **development and formation of added value plastic products through enzymatic and biocatalytic treatments.**

● **Biocatalyst improvement** and the generation of all-in-one platforms **with simultaneous high degradation and biopolymer synthetic performance** that will be supplied for **valorisation**.

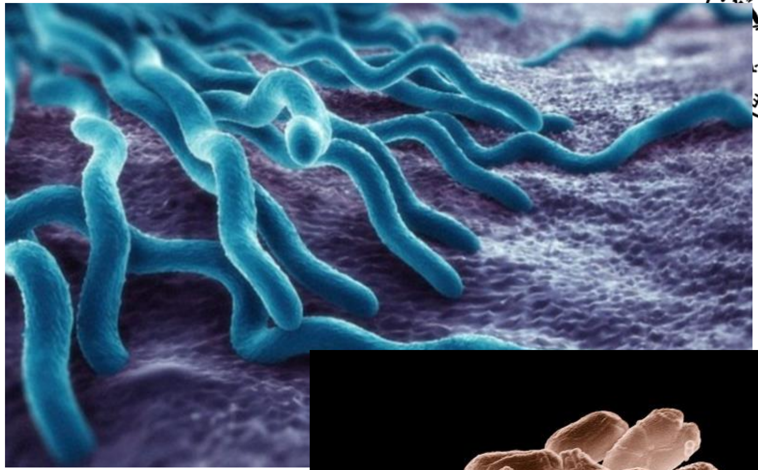


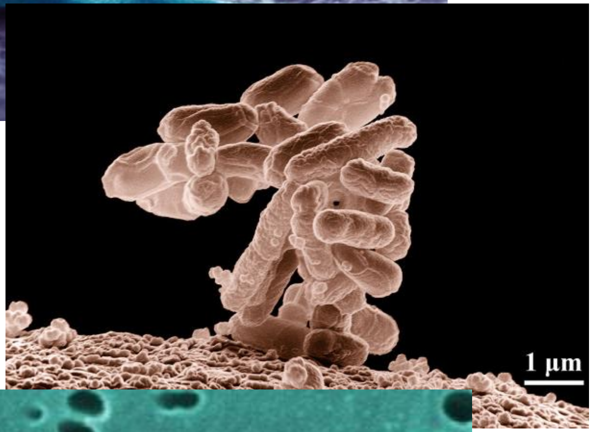
As different enzymes, bacteria, and fungi are about to be tested regarding their ability to degrade plastics, it is of utmost importance to control the surface chemistry of the plastics.

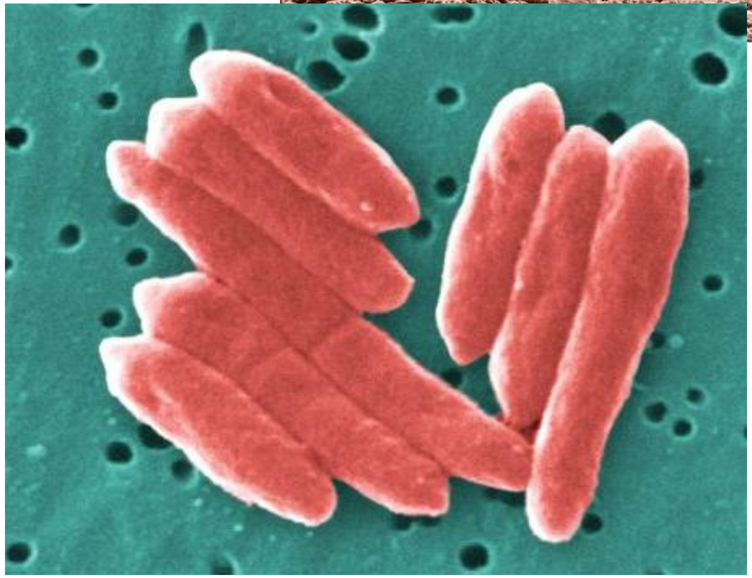
This is important, because each „attacking unit“ must first be adsorbed and dock on the surface of the plastic and then start degradation.

→ Surface chemistry and morphology must match the enzyme/bacteria/fungi needs!

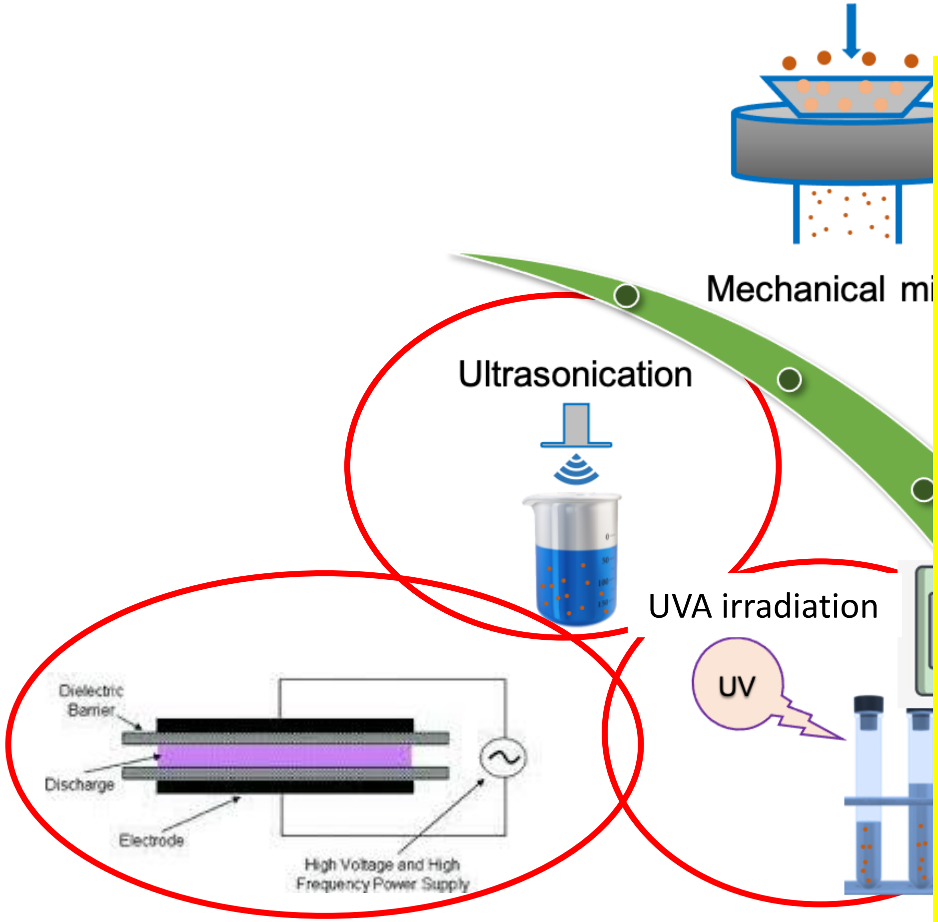
Oxygen content, H/C ratio, hydrophilicity/ hydrophobicity, and roughness are properties of interest, and their control is important.





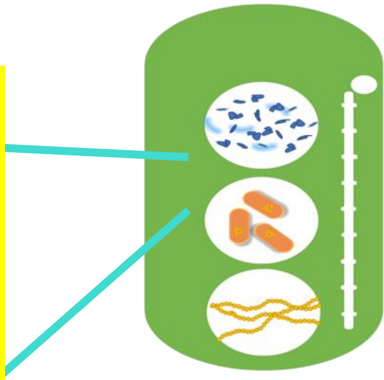
# Mechanical, green chemical and photo irradiation pre-treatments

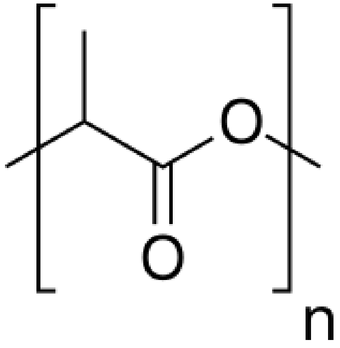



Dielectric Barrier Discharge Plasma
(DBD (atmospheric) Plasma)

**The pre-treatment should not decompose the plastic.**

**It should only optimize the surface and accelerate the docking reaction of the enzyme on the plastic surface.**






## Example → Poly-Lactic Acid (PLA)

- Which methods can affect the surface chemistry
- Which parameters are important
- Characterisation of the surfaces in order to categorize them and use the appropriate enzymes, bacteria or fungi (or mixtures of them) in order to have the best degradation results

## Reminder

Goal is *not the total mineralization* of the plastics but their *conversion to molecules* with lower molecular weight, meaning to obtain „fresh“ raw materials and recycle the plastics in a green way.

De-la-Pinta, I., Cobos, M., Ibarretxe, J. *et al.* Effect of biomaterials hydrophobicity and roughness on biofilm development. *J Mater Sci: Mater Med* **30**, 77 (2019). https://doi.org/10.1007/s10856-019-6281-3

# Experimental procedure

treatment →

High power
Ultrasounds (US)
at 20 and 860 kHz

or/and

Ultraviolet
(UVA) irradiation

or

DBD Plasma

characterization →

XPS (surface layer)

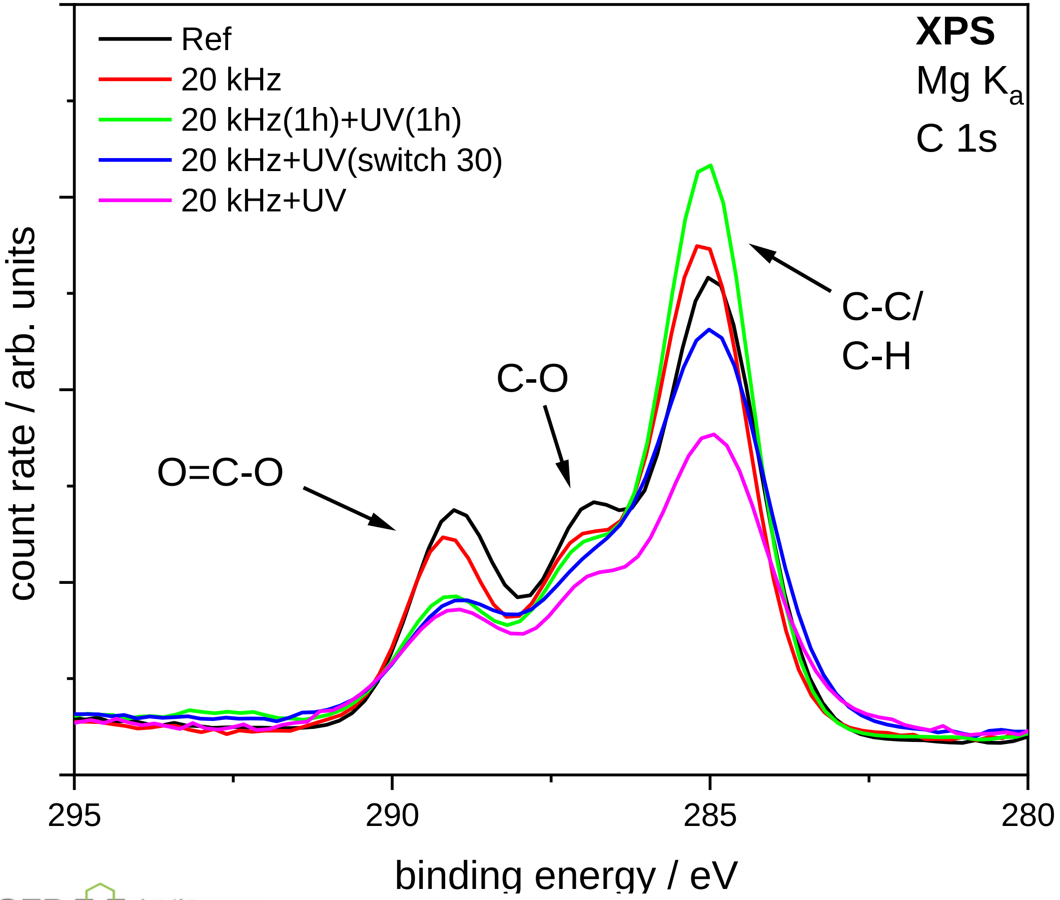
FTIR (bulk )

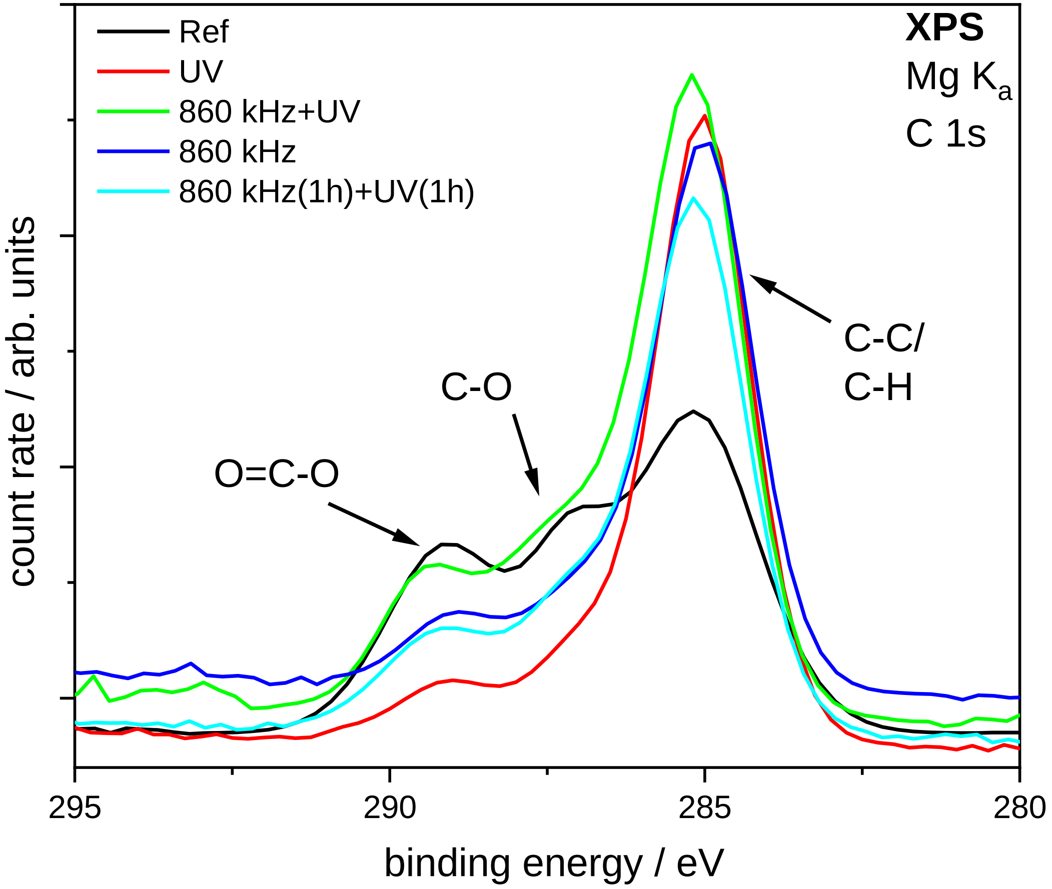
DCA (wettability)

CLSM/AFM (roughness)

# XPS Results

As a result of the treatment procedures a change of the surface elemental composition is expected, and in the particular case of plastic materials a change in the C/H and C/O ratio as compared to the untreated surface.

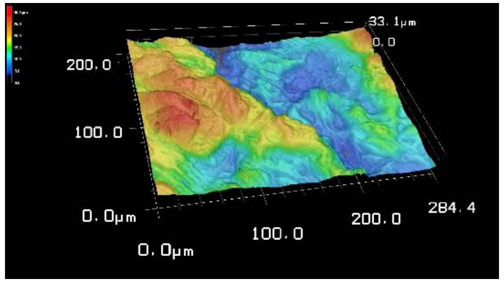


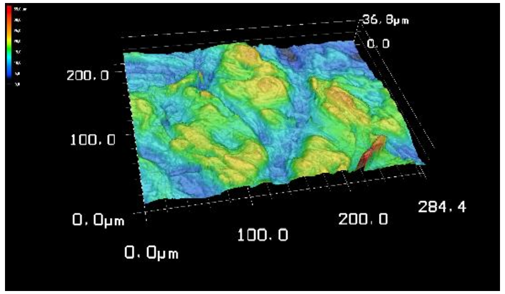
# Confocal Laser Scanning Microscopy (CLSM)

# Atomic Force Microscopy (AFM)

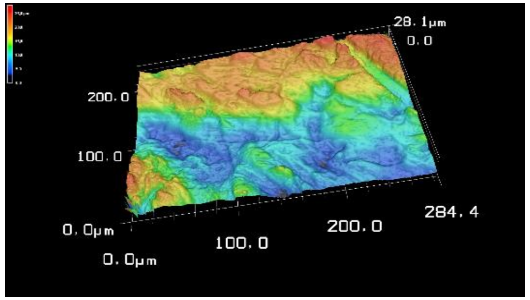
## Reference



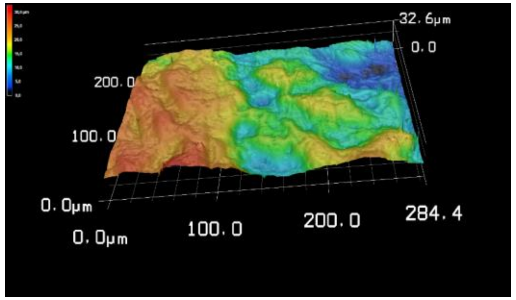
## US 860 kHz



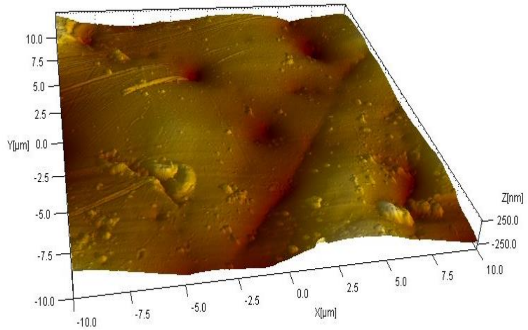
## PLA UV



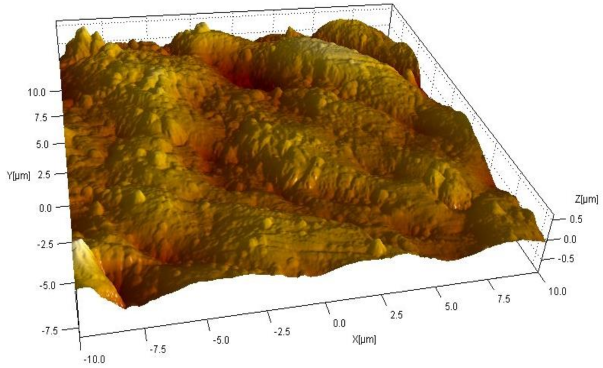
## US 20 kHz



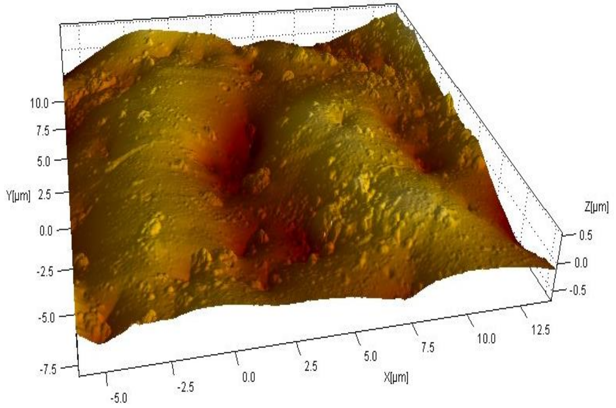
## PLA Reference



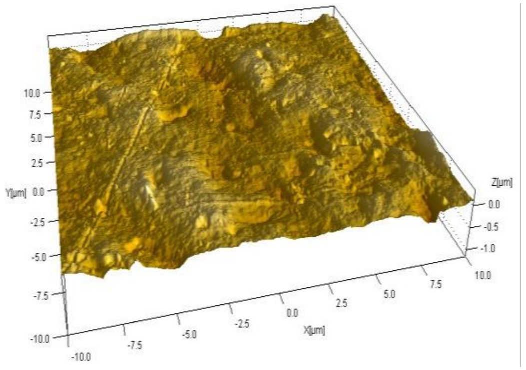
## US 860 kHz



## PLA UV



## US 20 kHz

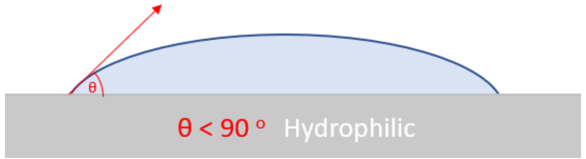


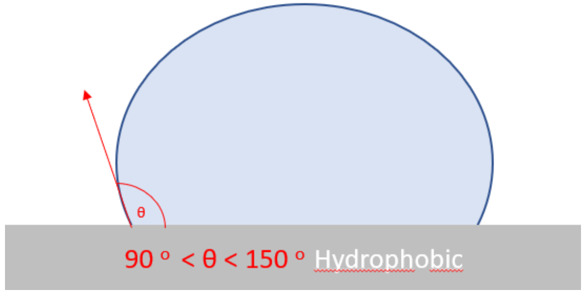
De-la-Pinta, I., Cobos, M., Ibarretxe, J. *et al.* Effect of biomaterials hydrophobicity and roughness on biofilm development. *J Mater Sci: Mater Med* **30**, 77 (2019). https://doi.org/10.1007/s10856-019-6281-3

# Drop Contour Analysis Results

## Plasma treated samples

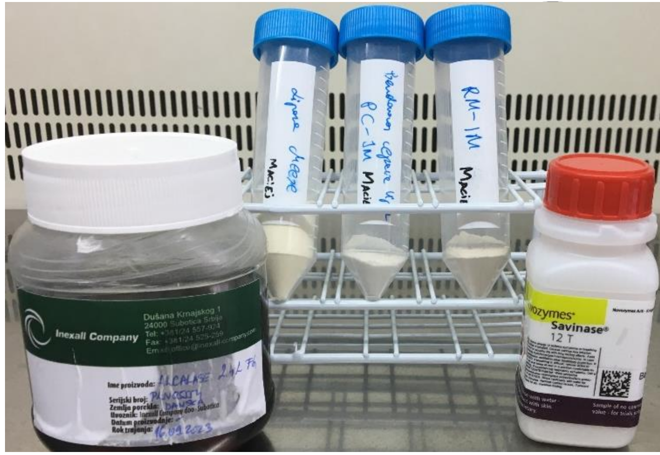
DCA shows that the **hydrophilicity is increased**




| DCA overview of all plasma treatment methods | | |
|---|---|---|
| Samples name | overview | |
| | Angle 1 [°] | Angle 2 [°] |
| As received | 69,2 | 70,2 |
| 5 s | 53,4 | 52,9 |
| 10 s | 50,8 | 50,1 |
| 20 s | 49,7 | 53,8 |
| 60 s | 53,6 | 53,7 |

XPS results confirm the increase in oxygen content in plasma treated PLA samples.



# Enzyme mix of

- Alcalase 2.4 L FG (Novozymes, batch PLN05554),
- Savinase (Novozymes, GHSFS-1-02-1) and
- 3 lipases (Serowar PL; Sigma, cat.no. 54327 and Sigma, cat. no. 52001).

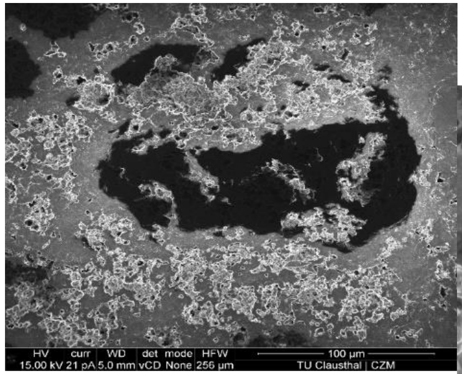
Enzyme mix contained 5 mg/mL of each alcalase and savinase, and 1.5 mg/mL of each of lipases in a buffer (pH 8.5)

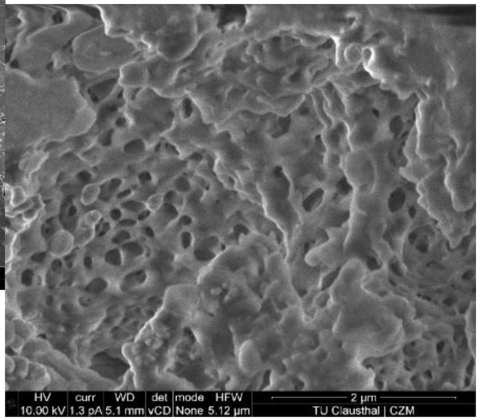
- Control reactions contained no enzyme mix.
- Samples were incubated at 42 °C at 150 rpm for 16 weeks.
- Weekly, aliquots (1 mL) were taken and stored at -20 °C for further analyses

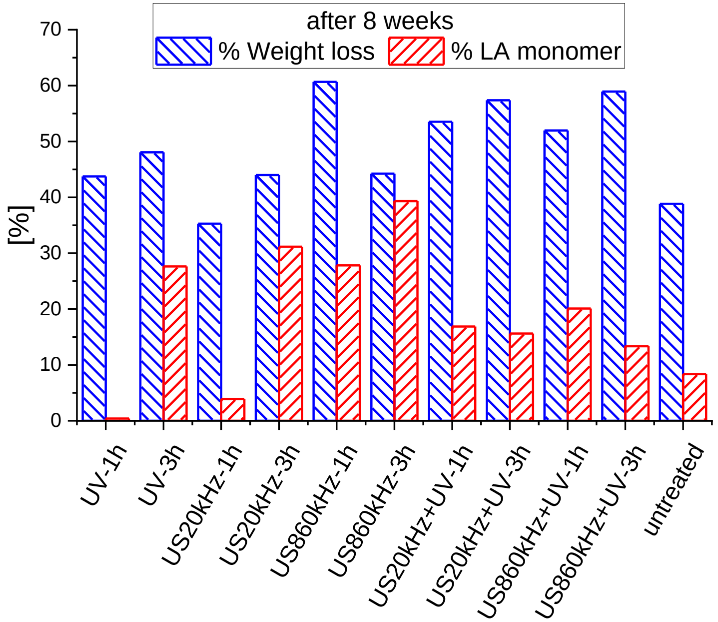
## PLA – films in

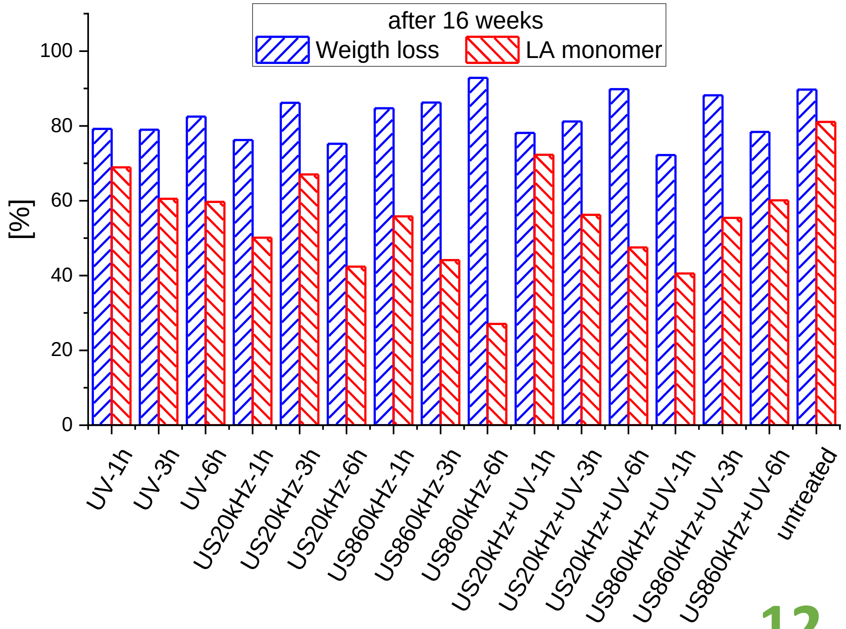
- **Enzymatic cocktail (Alcalase, Savinase, Lipozyme RM IM, Novozyme 435)**

  - Pretreatment: ultrasounds, ultraviolet irradiation, and DBD plasma
  - Model compost @37C
  - Enzymatic hydrolysis @42C
  - Weight loss of 90% achieved with commercial enzyme cocktail containing alkaline proteases and lipases!
  - Hydrolysis product supported growth and bacterial nanocell accumulation

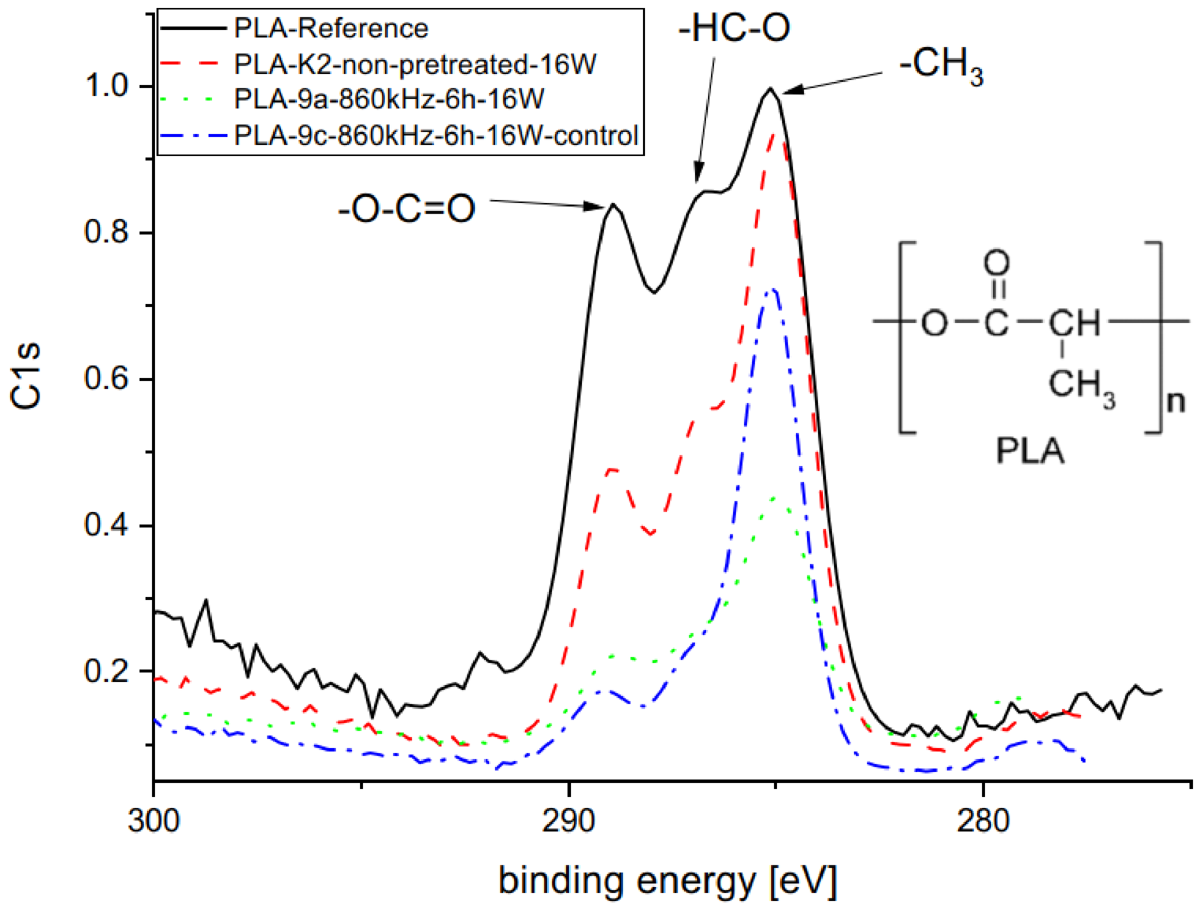


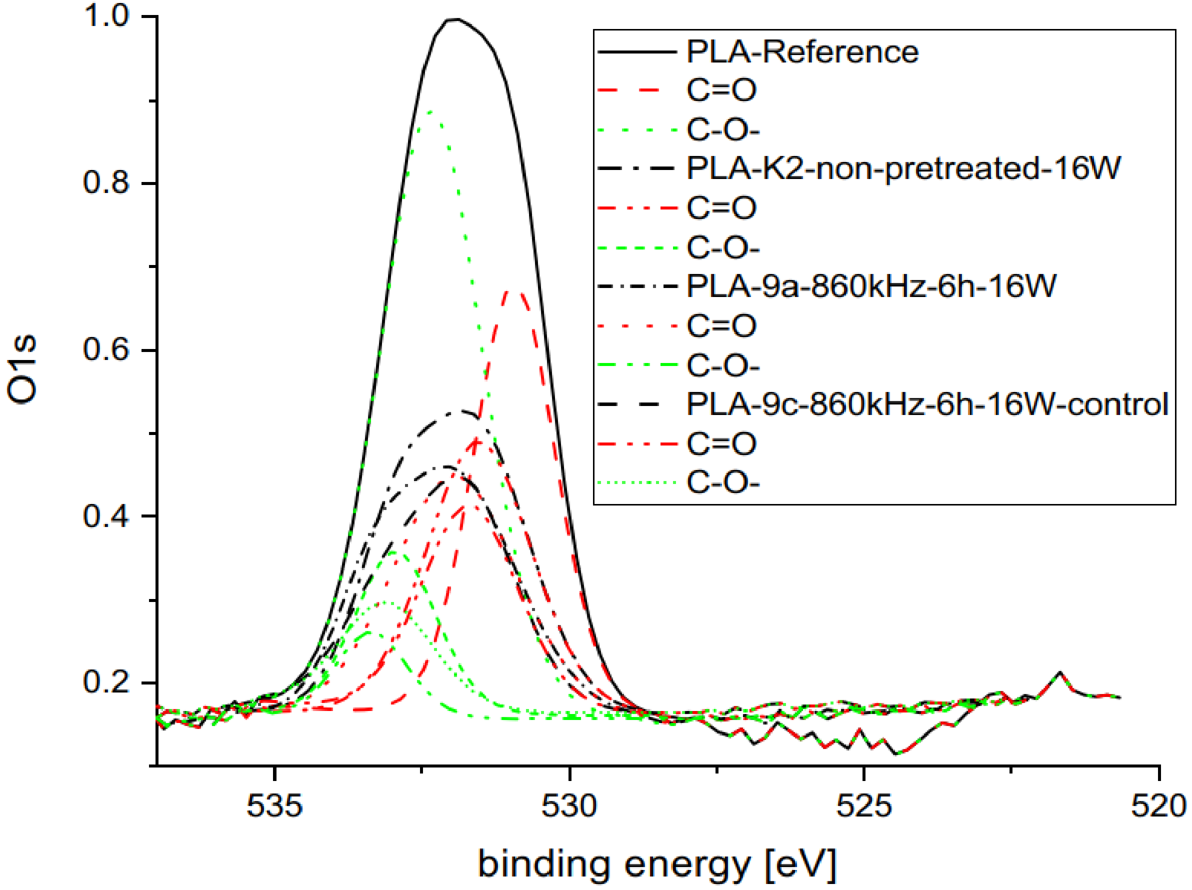



# Surface chemistry changes due to enzymatic degradation of pre-treated PLA samples




XPS signals of carbon 1s and oxygen 1s orbitals

# Bacterial nanocellulose production

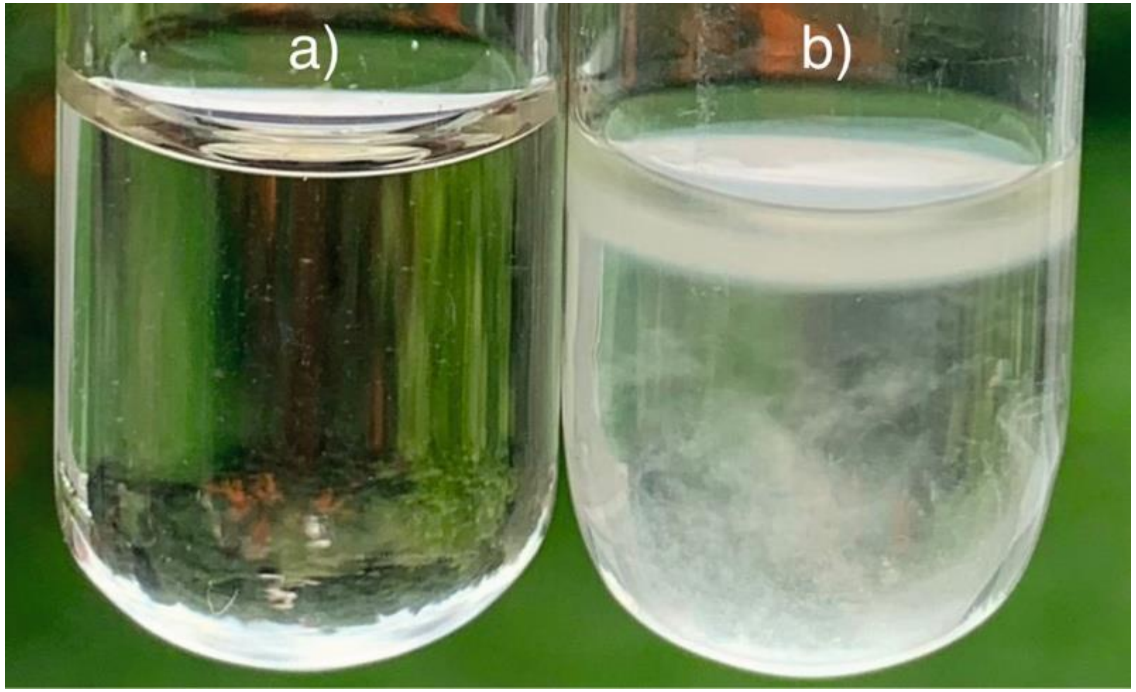
PLA hydrolysates from enzymatic biodegradation were used as substrate for nanocellulose production using Komagataeibacter medellinensis* (ID13488).

Komagataeibacter medellinensis strain transforms glucose to bacterial nanocellulose, in the presence of glucokinase, phosphoglucomutase and uridine triphosphate (UTP)-glucose-1-phosphate uridylyltransferase, with the aid of cellulose synthases.

It is expected that during PLA degradation lactic acid is transformed to fructose through pyruvaldehyde and glyceraldehyde intermediates, and then isomerized to glucose, which is used by bacteria for nanocellulose production.

* Molina-Ramírez, C., Castro, C., Zuluaga, R. *et al.* Physical Characterization of Bacterial Cellulose Produced by *Komagataeibacter medellinensis* Using Food Supply Chain Waste and Agricultural By-Products as Alternative Low-Cost Feedstocks. *J Polym Environ* **26**, 830–837 (2018). https://doi.org/10.1007/s10924-017-0993-6

# Nanocellulose production from enzymatic PLA hydrolysate by Komagataeibacter medellinensis




Bacterial nanocellulose production from PLA hydrolysates after 3 days of incubation:
a) control and b) hydrolysate obtained after enzymatic degradation.

Bacterial nanocellulose production from PLA hydrolysates after 7 days of incubation

G. Sourkouni, S. Jeremić, Ch. Kalogirou, O. Höfft, M. Nenadovic, V. Jankovic, D. Rajasekaran, P. Pandis, R. Padamati, J. Nikodinovic-Runic, Chr. Argirusis, "Study of PLA pre-treatment, enzymatic and model-compost degradation, and valorization of degradation products to bacterial nanocellulose",
World Journal of Microbiology and Biotechnology, (2023) 39:161, https://doi.org/10.1007/s11274-023-03605-4

# CAN ADVANCED OXIDATION PROCESSES ENABLE BIODEGRADATION OF PLASTICS BY SURFACE ACTIVATION?

Chr. Argirusis[1,2] , G. Sourkouni[2] ,
Ch. Kalogirou[1,2] , A. Gödde[2] , P.K. Pandis[1]

[1]National Technical University of Athens, Greece
[2]Clausthal University of Technology, Germany

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# Summary

- Advanced oxidation processes are useful as pre-treatment step in the biotechnological degradation and valorization of plastics.

- They can activate the plastics´ surface either chemically or morphologically depending on the method.

- Dielectric Barrier Discharge (DBD) Plasma is oxidizing the surface (when oxygen plasma is used). Roughness increased after 60 s of treatment. 10-20 s sufficient for surface oxidation/activation.

- Photocatalysis is a powerful tool in the degradation of plastic surfaces.
  It increases the roughness, but it reduces the oxygen content of the upper surface and its wettability.

- High power ultrasonication acts mostly through mechanical impact of cavitation collapse on the surface, which then induces chemical reactions.
  US increase the roughness, but they also reduce the oxygen content of the upper surface and its wettability.


# *Thank you!*

## A SUSTAINABLE WAY TO A BETTER WORLD

BioICEP 解塑再用
Bio Innovation of a Circular Economy for Plastics

European Commission

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## Recent related publications


1. G. Sourkouni, S. Jeremić, Ch. Kalogirou, O. Höfft, M. Nenadovic, V. Jankovic, D. Rajasekaran, P. Pandis, R. Padamati, J. Nikodinovic-Runic, Chr. Argirusis, “Study of PLA pre-treatment, enzymatic and model-compost degradation, and valorization of degradation products to bacterial nanocellulose”, World Journal of Microbiology and Biotechnology, (2023) 39:161, https://doi.org/10.1007/s11274-023-03605-4
2. Ch. Kalogirou, G. Sourkouni, O. Höfft, A. Gödde, N. Papadimitriou, P. Pandis, Chr. Argirusis, “Assessing the time dependence of AOPs on the surface properties of Polylactic Acid”, Journal of Polymers and the Environment, published online 29.10.2022, https://doi.org/10.1007/s10924-022-02608-w
3. M.G. Savvidou, D. Mamma, P.K. Pandis, G. Sourkouni, Chr. Argirusis, *“Organic wastes for bioenergy production via microbial fuel cells: A review”,* Energies 2022, 15, 5616. https://doi.org/10.3390/en15155616
4. G. Chatziparaskeva, I. Papamichael, I. Voukkali, P. Loizia, G. Sourkouni, Chr. Argirusis, and A.A. Zorpas *“End-of-Life of Composite Materials in the Framework of the Circular Economy”,* Microplastics 2022, 1, 377–392
5. Th. Kamperidis, P.K. Pandis, Chr. Argirusis, V. N. Stathopoulos, G. Lyberatos and A. Tremouli, *“Comparative Study of Different Production Methods of Activated Carbon Cathodic Electrodes in Single Chamber MFC Treating Municipal Landfill Leachate”,* Appl. Sci. 2022, 12, 2991. https://doi.org/10.3390/app12062991
6. Th. Kamperidis, P.K. Pandis, Chr. Argirusis, G. Lyberatos, and A. Tremouli, *“Effect of Food Waste Condensate Concentration on the Performance of Microbial Fuel Cells, with Different Cathode Assemblies”,* Sustainability 2022, 14, 2625. https://doi.org/10.3390/su14052625