

The power of synergistic interactions between organisms for plastic recycling and degradation

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Horizon 2020
European Union Funding
for Research & Innovation



OUTLINE

- Background
- Objectives
- Microbial consortia for multiplastic degradation
- Combination *Galleria mellonella* with microorganisms
- Combination *Eisenia fetida* with microorganisms
- Conclusions

Agri-food Waste Plastics

Food packaging and Agriculture
consume ~44% of worldwide
production of plastics



Polyethylenes (PE), Polystyrene (PS), Polyethylenthereptalate (PET)

End of life



Sorting

Management
Systems

Waste



Plastics



Organic fraction +plastics

Released to the
environment



31 % recycled



Non-recyclable:
Mixed or
multilaminated

Incinerated
Landfiled

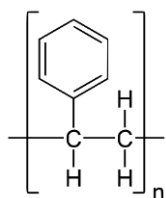
New  **biorecycling
routes**

Compost
contamination

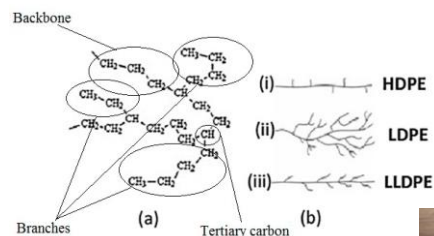
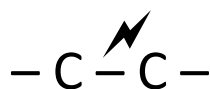


Soil contamination





Polystyrene

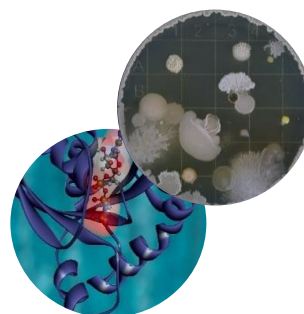


Polyethylene

Sen & Raut (2015) J. Environ.
Chem. Eng., 3, 462–473



Yang et al. (2015).
Environ Sci Technol,
49, 12080



Earthworms & Insects
(+microbiome)

Casts/faeces (microplastics)

Microorganisms
(Lignin)

Enzymes
(esterases, lipases,
hydrolases)

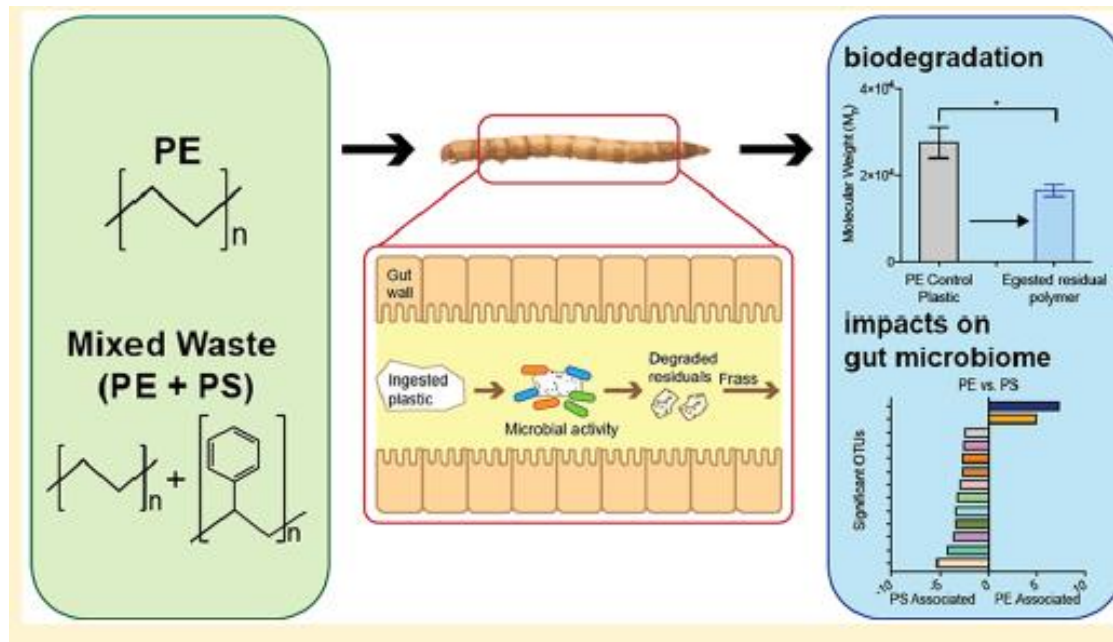
$\text{CO}_2 + \text{H}_2\text{O}$

CO_2

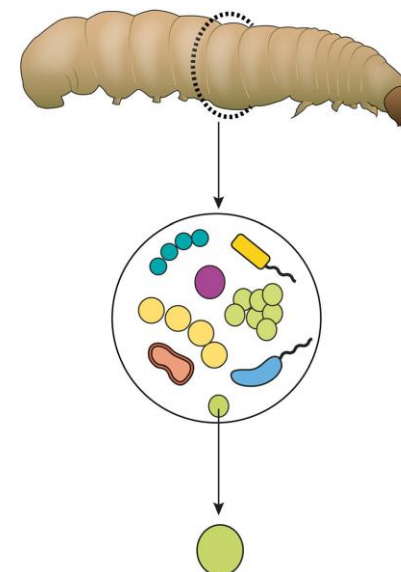
Partial biodegradation

AWP-adapted

Consortia
“ad hoc”



Cassone et al. (2020). Proc. Royal Society B, 287.
Brandon et al (2018). Environ. Sci Technol. 52, 6526



Evidence for role in plastic degradation

By higher organisms (e.g. insect larvae)

- survival of germ-free insect larvae on plastic as the sole food source

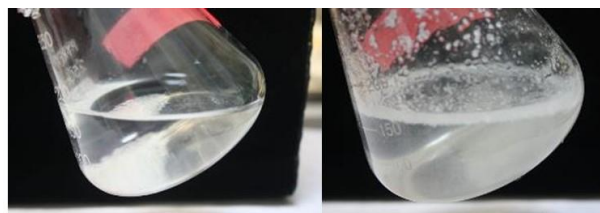
By gut microbiota

- increased microbial abundance in the gut
- disruption of microbiome (e.g., with antibiotics) reduces plastic losses
- identification of putative plastic degradation products in the gut/faeces
- isolation of putative plastic-degrading microbes from gut and frass

By gut-independent microbes

- growth of isolated colonies on plastic as a sole carbon source

Lear et al. (2021). Environmental Microbiome, 16(1), 1-19.



Microbial
Consortia



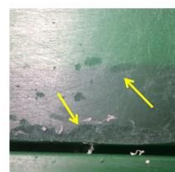
Tenebrio monitor "Gusano de la harina"
(Fuente: UAL)



Galleria mellonella "Gusano de la cera"



Insects



Earthworms



Combination of biocatalytic systems (hydrolytic enzymes, microbial, insects, and earthworms)

- maximize the transformation yields
- allowing the treatment of mixed plastic waste streams
- to convert fossil fuel plastic into biodegradable counterparts in a single step.



- Develop microbial consortia for multiplastic degradation
- Increase the plastic conversion efficiency of *Galleria mellonella* through the incorporation of microorganisms
- Increase the plastic conversion efficiency of *Eisenia fetida* through the incorporation of microorganisms



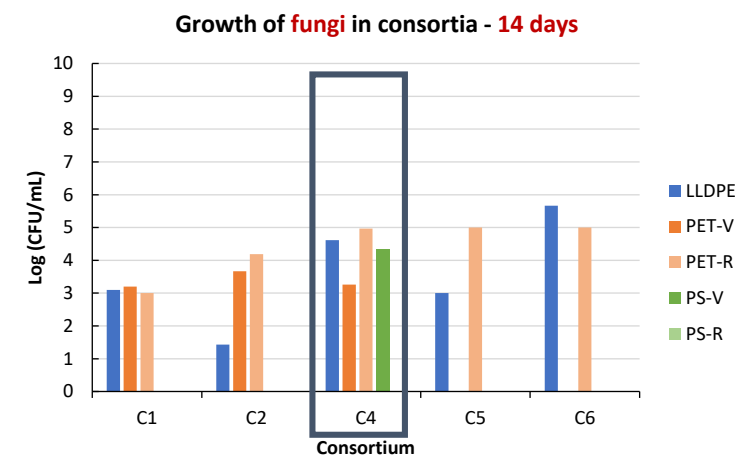
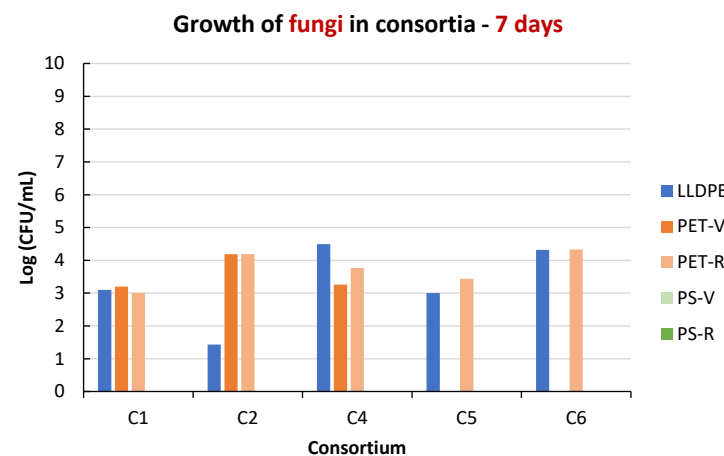
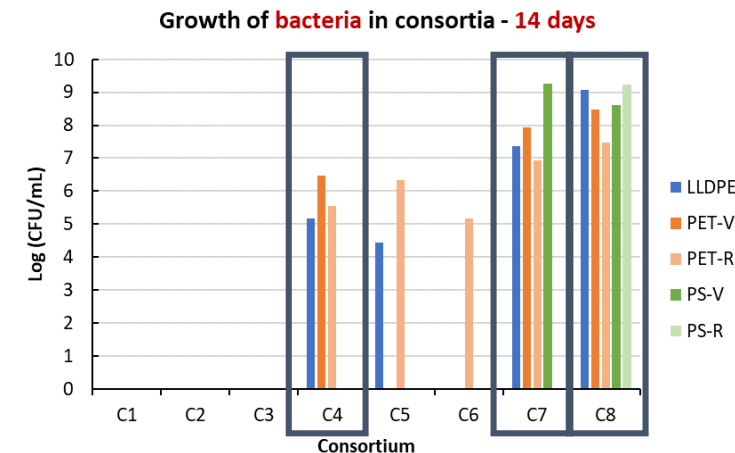
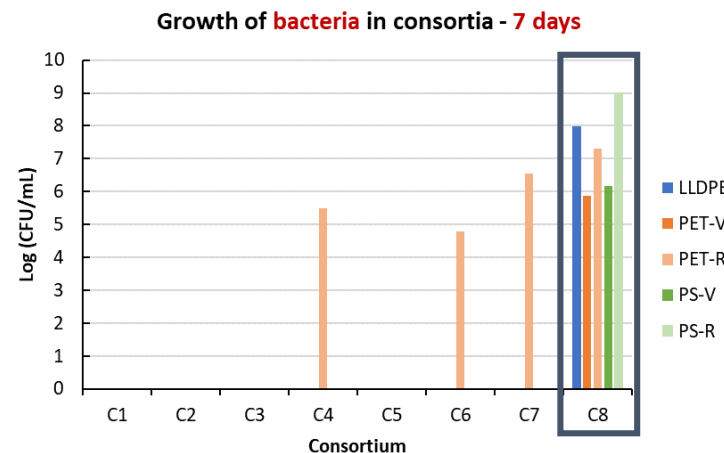
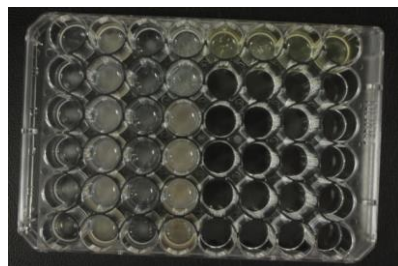
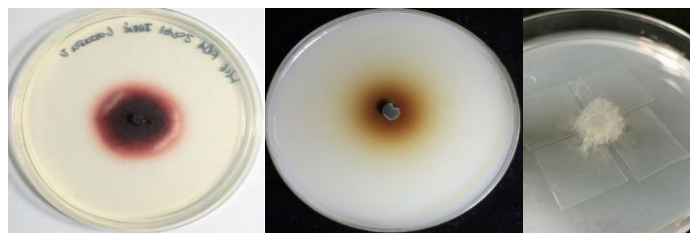
Galleria mellonella "Greater wax moth"

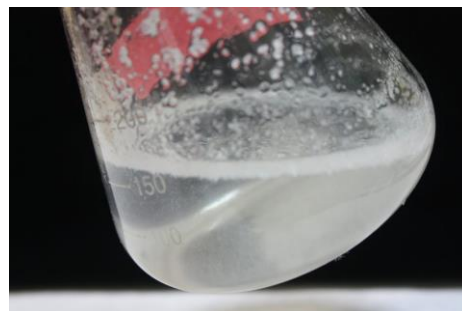
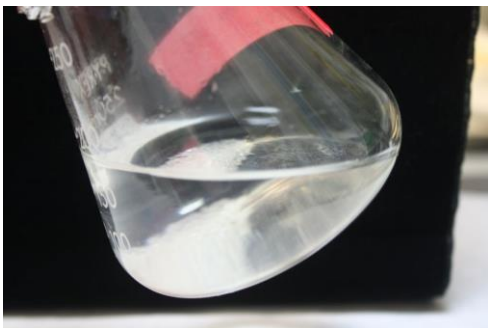
E. andrei/foetida





- Bacteria and fungi **screened for plastic-degrading related enzymes**: lipases, cutinases and ligninases.
- Six strains: **combined in pairs or trios to build up eight consortia**
- Plastic biodegradation: **Capability to grow in virgin or recycled LDPE, LLDPE, PET or PS as the sole carbon source.**

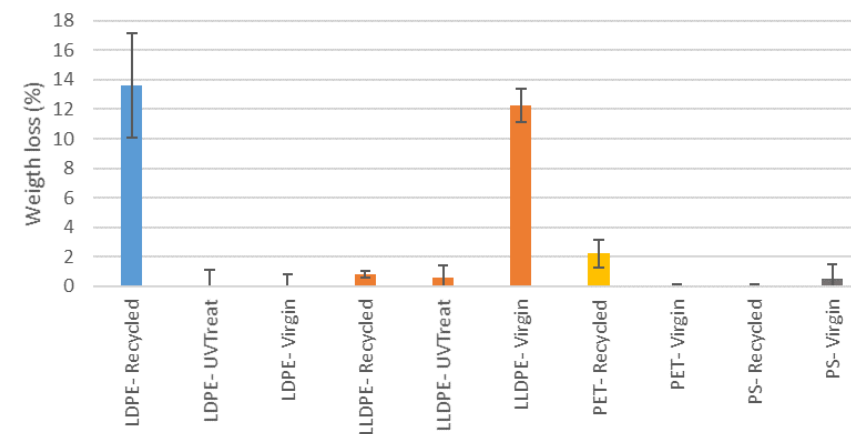




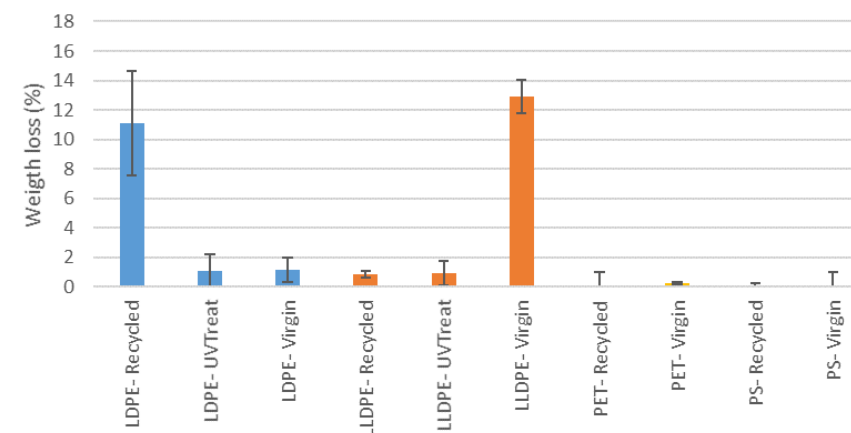
Plastic weight loss (%)

- Most - Non significant or <2%
- Consortia C2:
 - LDPE - recycled 14%
 - LLDPE - virgin 12%
 - PET-recycled 2%
- Consortium C4:
 - LDPE - recycled 11%
 - LLDPE – virgin 13%

C2: RHM4+RHM1+RBM1



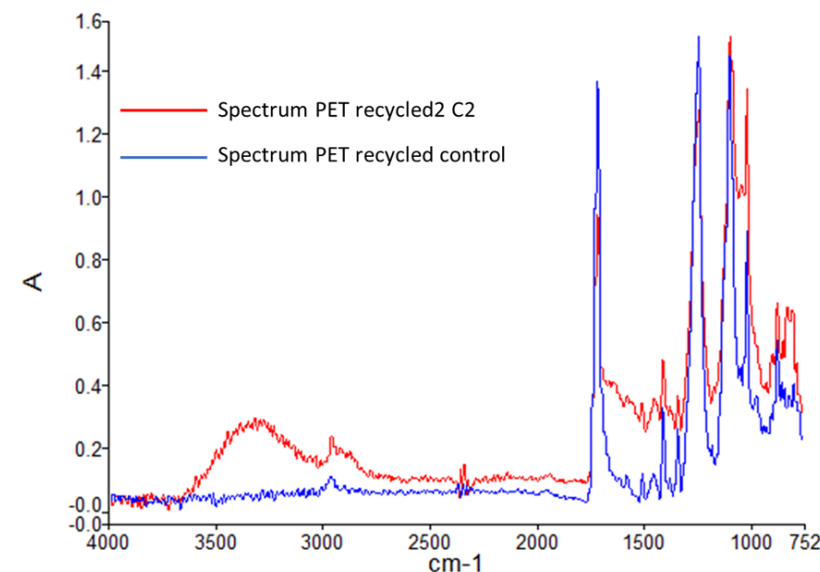
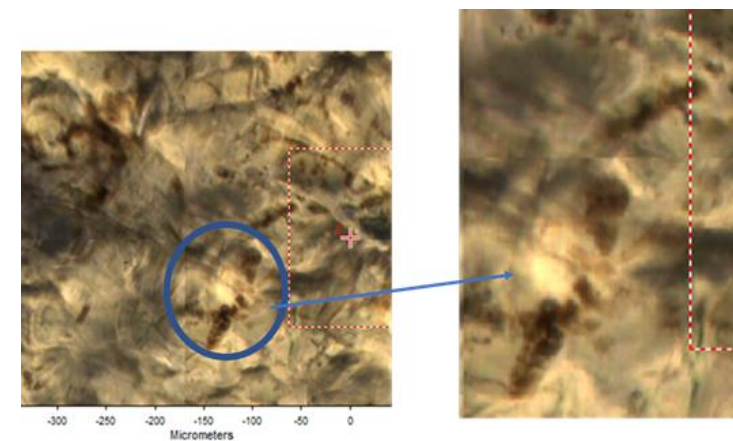
C4: RBM2+E7





PET recycled treated with Consortium C2: Chain scission through the hydrolysis of the ester bond.

- Changes in chemical composition in the surface area
- A band at $3500\text{--}3100\text{ cm}^{-1}$ (-OH)
- Decrease of bands in the range where the ester bond of PET resonates: 1712 cm^{-1} (carbonyl stretching) and 1240 cm^{-1}
- Spectrum modification $1700\text{--}1500\text{ cm}^{-1}$ (carboxyl or carboxylate groups) spectral range, likely related to microbial degradation.



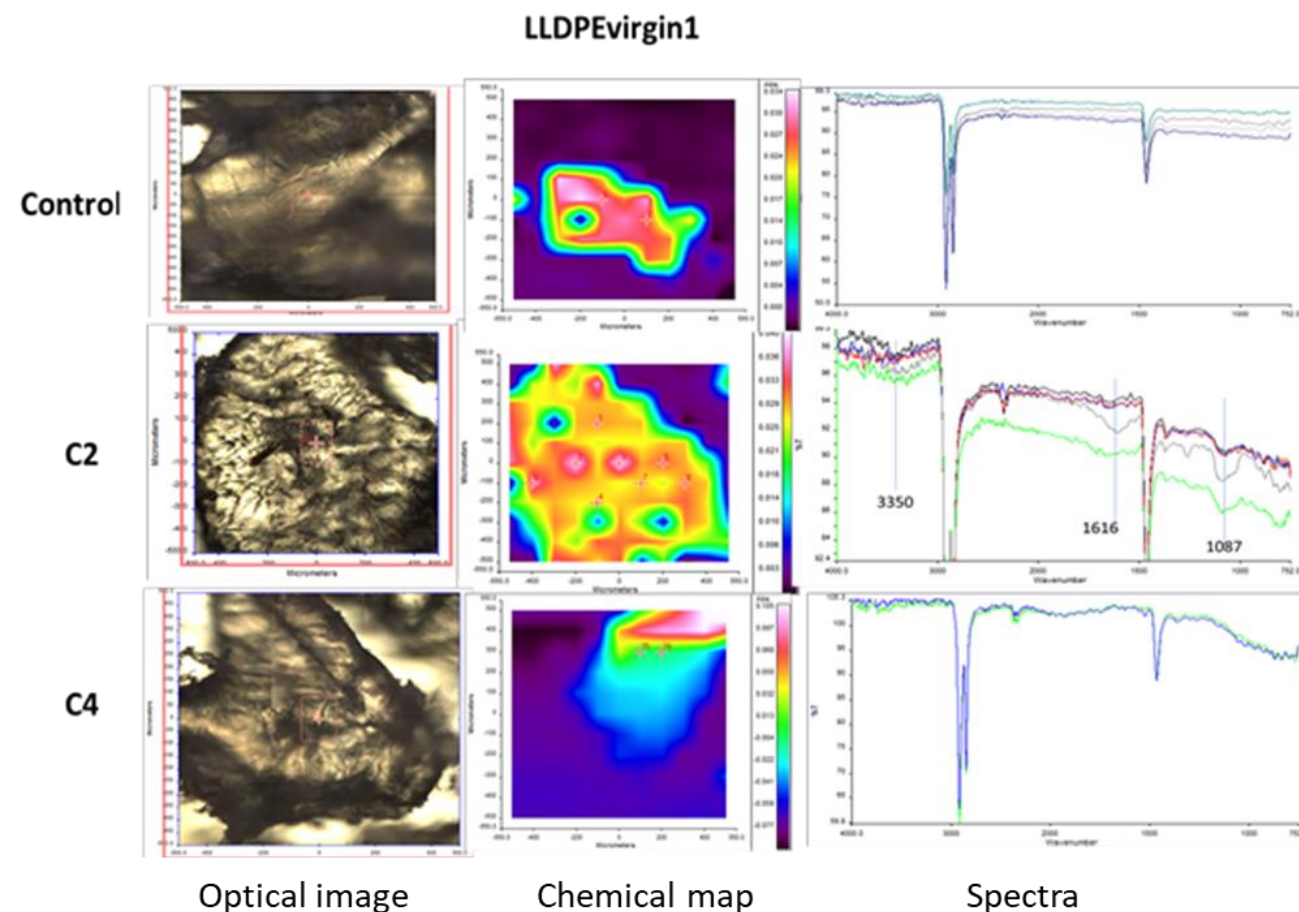
FTIR Chemical Imaging analysis



Small changes in chemical composition in the surface area of LLDPE virgin after C2 treatment were observed

Incorporated oxygen functional groups was observed at $3500\text{--}3100\text{ cm}^{-1}$ (-OH) and $1800\text{--}1500\text{ cm}^{-1}$ (C=O).

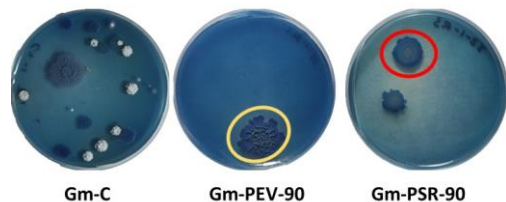
A broad adsorption band centered at 1087 cm^{-1} related to C-O was evident.



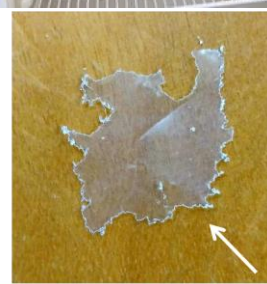
FTIR Chemical Imaging analysis



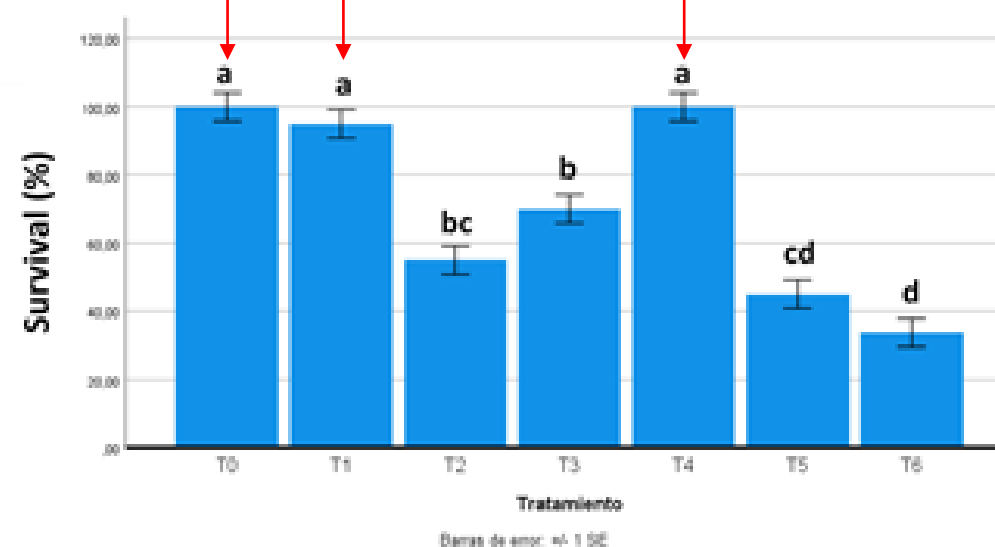
Galleria mellonella



Galleria mellonella "Greater wax moth"



Galleria mellonella – GLM (43,638; 6; $P < 0,01$)



T0: Standard diet (no plastic)

T1: Bacteria 1 – LLDPE 100%

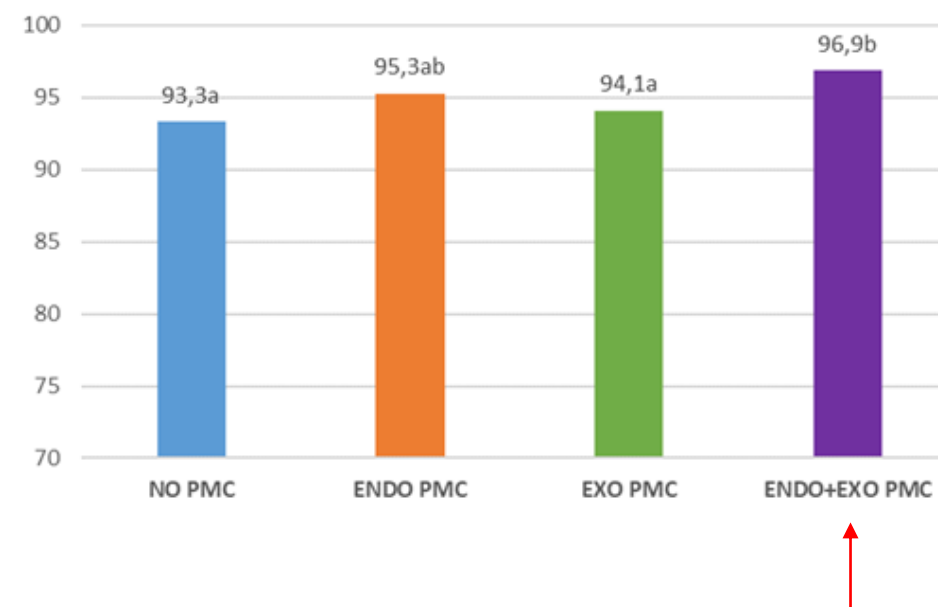
T4: Bacteria 2 – LLDPE 100%



E. andrei/foetida



EF Survival % (GLM-RM, F-anova 6.1**)



- The microbial consortia selected in this work are very promising tools for the treatment of heterogeneous mixtures of plastic wastes.
- The combination of microorganisms and *G. mellonella* or *E. fetida* allows higher survival when feeding on plastic, which can be used for plastic biological recycling.
- Further analyses are required to determine the fate of plastic bioconversion by the combinations insect or earthworm with microorganisms

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Thanks for your attention!

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