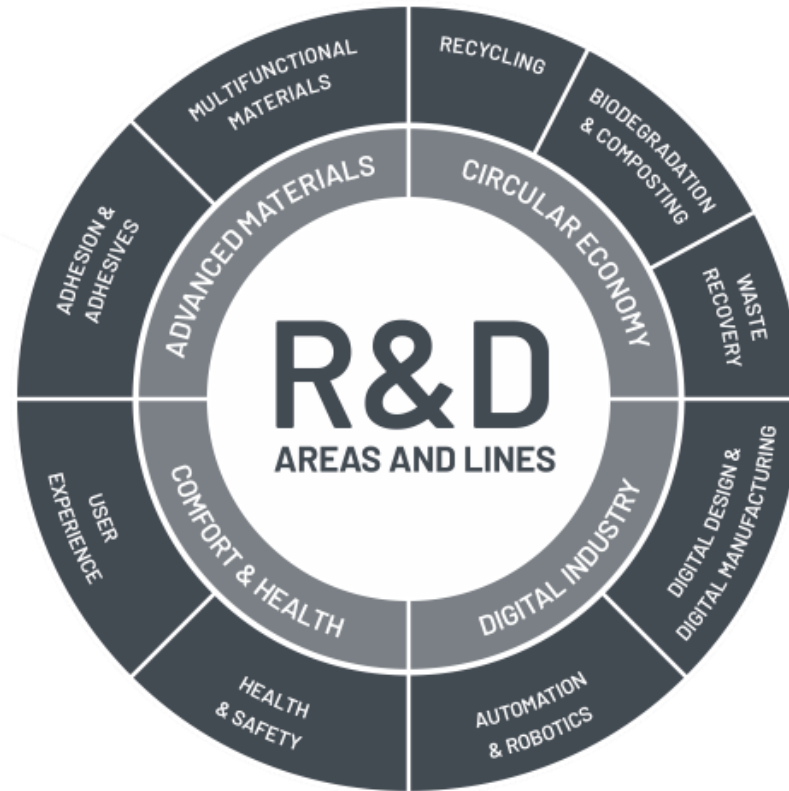


Assessment of the Reproducibility of ISO 20136 for the Biodegradation of Tanned Leather: Longitudinal Analysis and Statistical Validation



PhD. Marcelo Bertazzo
Biotechnology department
mbertazzo@inescop.es

CENTRE FOR
INNOVATION AND
TECHNOLOGY
APPLIED TO
FOOTWEAR



Established in 1971

Leading Innovation & Technology Centre

Specialised in the Footwear Sector and its Entire Value Chain:

- Over 120 highly qualified professionals
- More than 600 associated companies
- Over 3,000 CAD licenses in use across 5 continents
- 35,000 tests and 6,000 technical reports issued annually



INESCOP's BIOTECH.lab

INESCOP's development



• BIO D1



Patented equipment



• BIO D2



• BIO D3



• Fully automatic

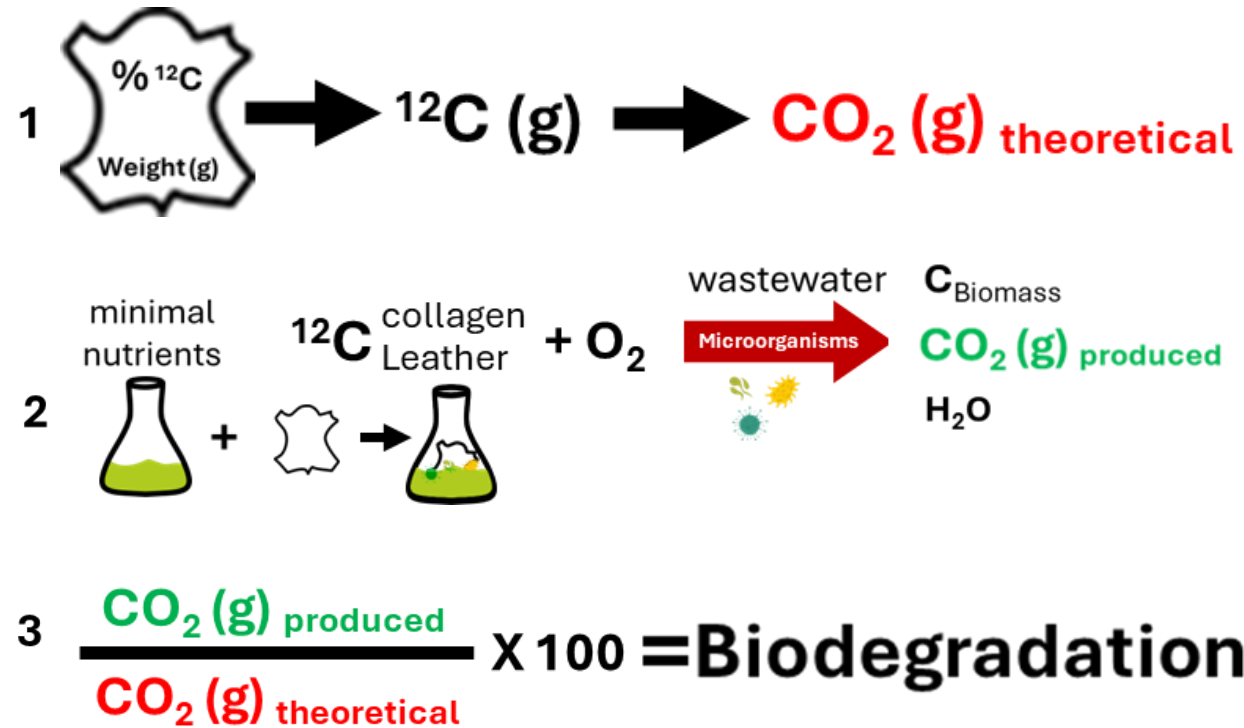
Sensors:

- Infrared (IR) CO₂ detection
- Temperature
- Atmospheric
- Flow rate

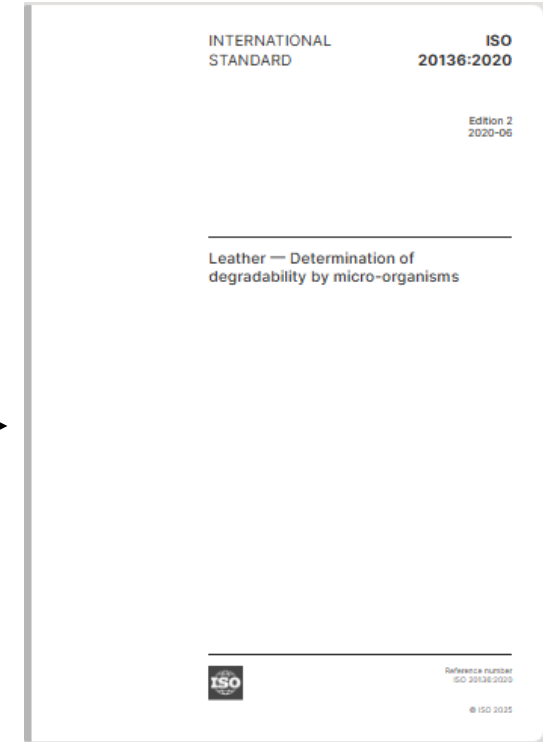
Parameters:

- 30 days trial period
- Duplicate samples
- 70% C+ in 28 days
- Relative % samples

ISO 20136:2020 - Leather — Determination of degradability by micro-organisms



ISO 20136:2017



ISO 20136:2020



2026 ?

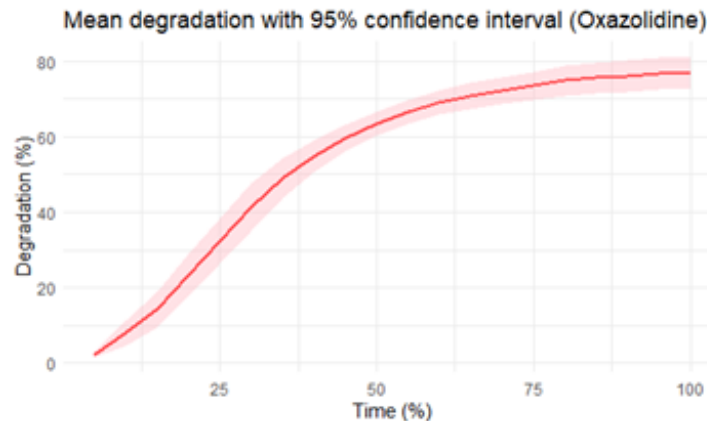
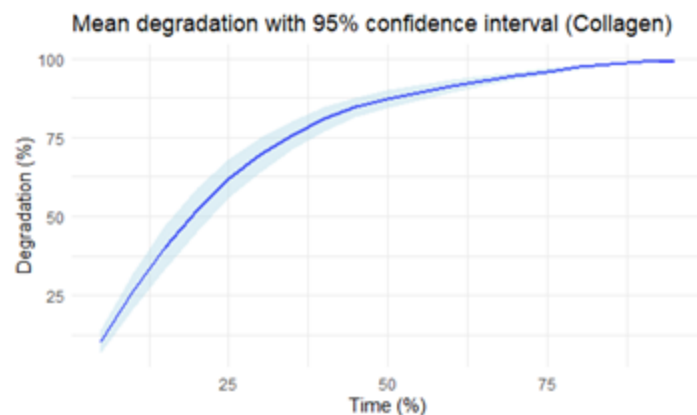
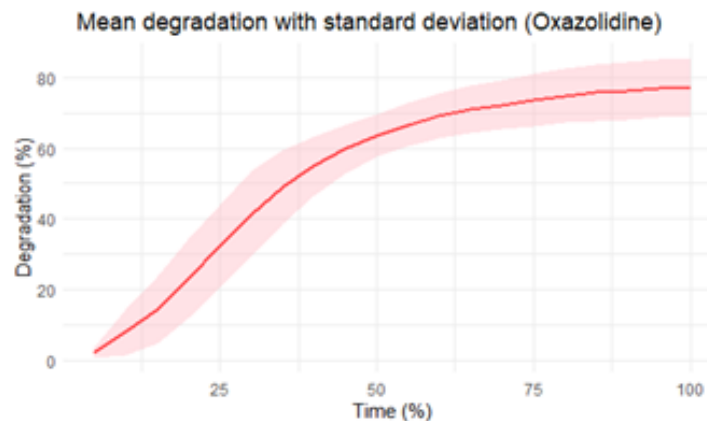
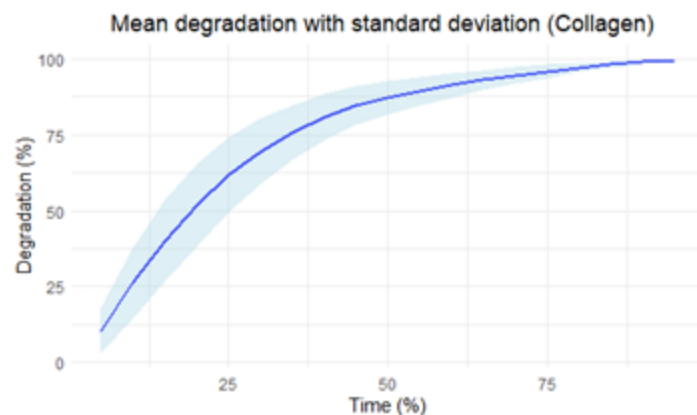
Assessment of the Reproducibility of ISO 20136 for the Biodegradation of Tanned Leather: Longitudinal Analysis and Statistical Validation

| Samples | | Time | | |
|---|--|--|---------------------|--------------------------|
| Oxazolidine  | Collagen (Sigma®)  | Years | Experiments | Tests carried out |
| | | 2019-2025 | 28 – 40 days | 15 |
| Inoculum | | Statistical analysis | | |
| Biological Tannery wastewater | Biological Municipal wastewater | <ul style="list-style-type: none">• Standard Deviation• Coefficients of Variation (CV)• Pearson Correlation Coefficient (r)• Lin’s Concordance Correlation Coefficient (CCC)• Kolmogorov–Smirnov Test• Bland–Altman Plots• Quality Control Charts (QCCs) | | |

Assessment of the Reproducibility of ISO 20136 for the Biodegradation of Tanned Leather: Longitudinal Analysis and Statistical Validation

- Standard Deviation

- Coefficient of Variation (CV)

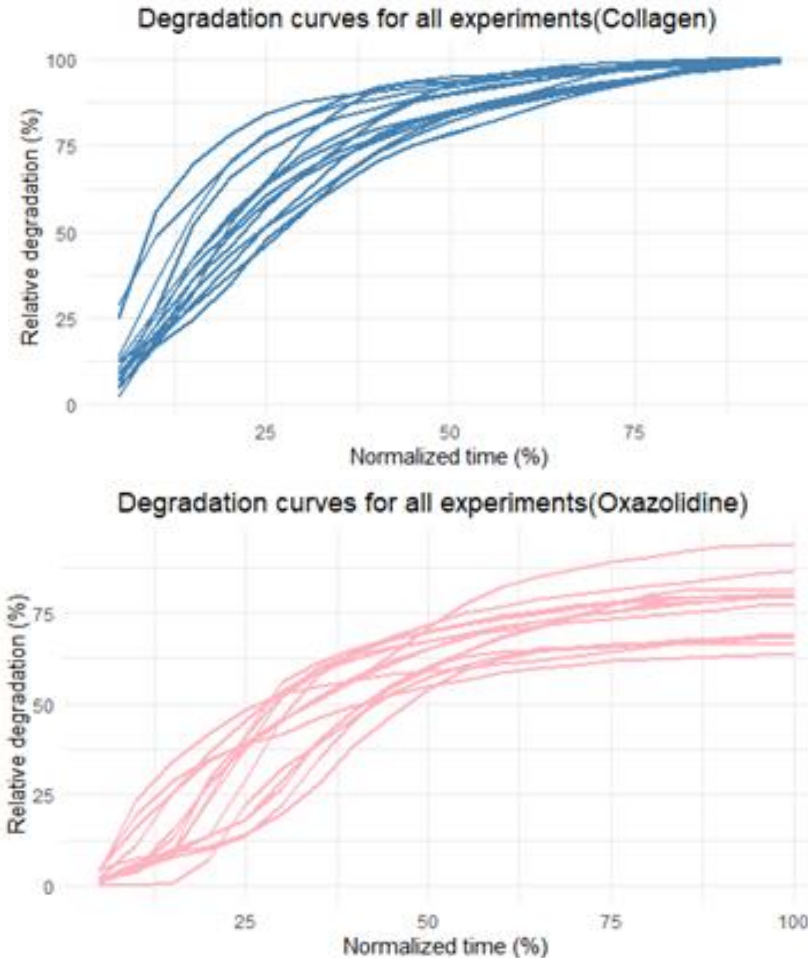


| Collagen | |
|----------|--------|
| Time (%) | CV (%) |
| 5 | 70.7 |
| 10 | 44.3 |
| 15 | 33.2 |
| 20 | 26.2 |
| 25 | 19.8 |
| 30 | 15.5 |
| 35 | 12.0 |
| 40 | 9.29 |
| 45 | 7.59 |
| 50 | 6.34 |
| 55 | 5.33 |
| 60 | 4.37 |
| 65 | 3.42 |
| 70 | 2.87 |
| 75 | 2.32 |
| 80 | 1.59 |
| 85 | 1.08 |
| 90 | 0.71 |
| 95 | 0.31 |

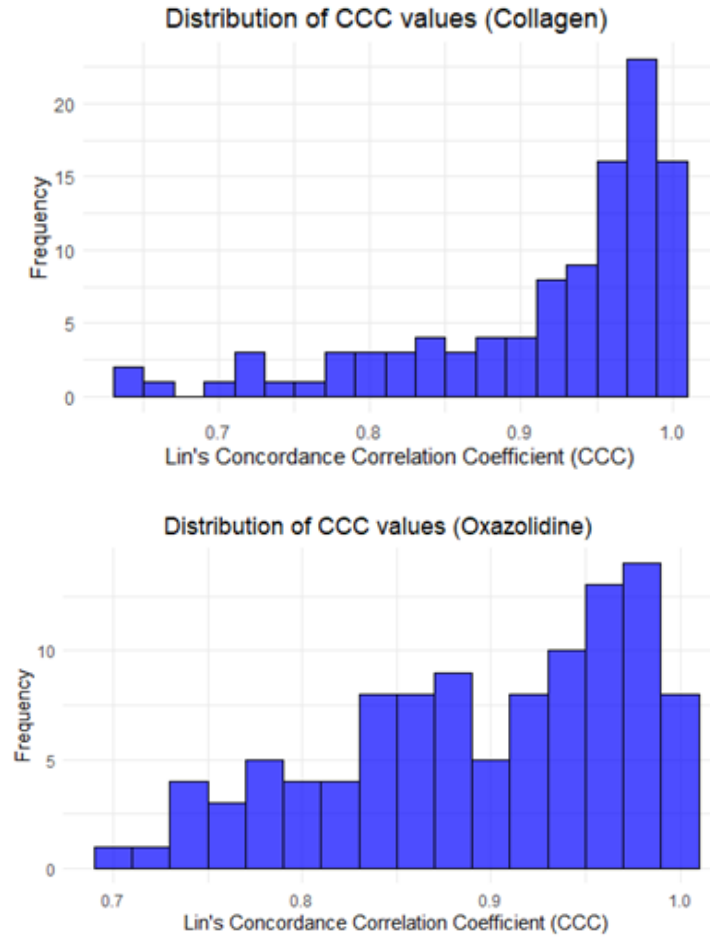
| Oxazolidine | |
|-------------|--------|
| Time (%) | CV (%) |
| 5 | 62.7 |
| 10 | 80.1 |
| 15 | 65.7 |
| 20 | 47.6 |
| 25 | 36.7 |
| 30 | 29.5 |
| 35 | 21.2 |
| 40 | 15.0 |
| 45 | 11.3 |
| 50 | 9.38 |
| 55 | 9.17 |
| 60 | 9.25 |
| 65 | 9.44 |
| 70 | 9.73 |
| 75 | 10.0 |
| 80 | 10.4 |
| 85 | 10.5 |
| 90 | 10.6 |
| 95 | 10.7 |
| 100 | 10.8 |

Assessment of the Reproducibility of ISO 20136 for the Biodegradation of Tanned Leather: Longitudinal Analysis and Statistical Validation

- Pearson Correlation Coefficient (r)

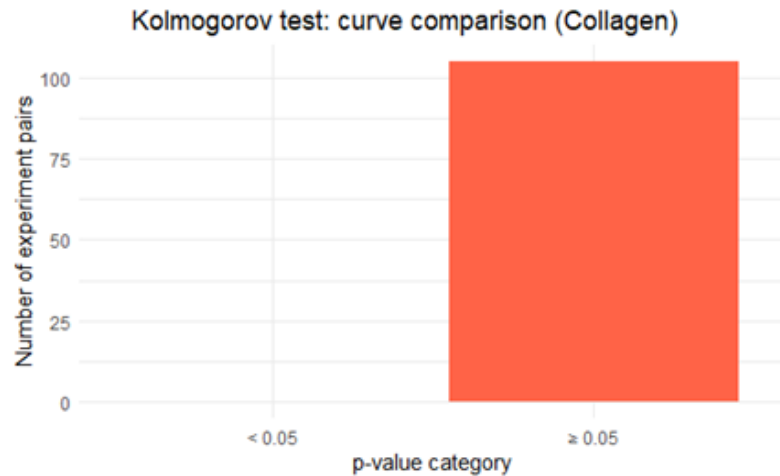


- Lin's Concordance Correlation Coefficient (CCC)



Assessment of the Reproducibility of ISO 20136 for the Biodegradation of Tanned Leather: Longitudinal Analysis and Statistical Validation

- Kolmogorov–Smirnov Test

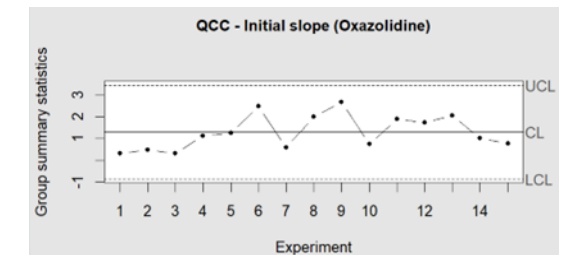
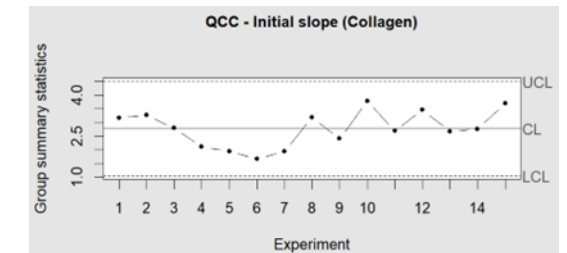


- Bland–Altman Plots

| Collagen | | | | | | |
|--------------------|---------|--------------------------|--------|-------|--------------------------|---------|
| Statistic | Minimum | 1 st Quartile | Median | Mean | 3 rd Quartile | Maximum |
| Points outside (%) | 0.000 | 0.000 | 0.000 | 2.807 | 5.263 | 10.526 |

| Oxazolidine | | | | | | |
|--------------------|---------|--------------------------|--------|-------|--------------------------|---------|
| Statistic | Minimum | 1 st Quartile | Median | Mean | 3 rd Quartile | Maximum |
| Points outside (%) | 0.000 | 5.000 | 5.000 | 5.238 | 10.000 | 15.000 |

- Quality Control Charts (QCCs)



Assessment of the Reproducibility of ISO 20136 for the Biodegradation of Tanned Leather: Longitudinal Analysis and Statistical Validation

Main Conclusions:

- Over 5 years and 15 trials, **stable and repeatable degradation patterns** were observed.
- Collagen, used as the reference material, showed **high reproducibility** across all trials, supported by multiple statistical tests.
- Oxazolidine-tanned leather showed **expected biological variability**, but with similar curve shapes and dispersions.
- Differences between trials **remained within acceptable statistical limits**, confirming data consistency.
- The repetition of degradation patterns across different trials confirms system stability and rules out random effects.
- In summary, the biodegradation tests following ISO 20136 are **reproducible and reliable**.

CERTIFICADO



Inescop, Centro Tecnológico del Calzado, certifica que la empresa:

COMPANY, S.L.
 Street Name, Number, Neighborhood
 CEP - CITY, COUNTRY

is authorised to use the "BiOdegradable" label on the products mentioned below for the same period as the validity period of this certificate.

ARTICLE
 "SAMPLE 1"

The degradation of the above-mentioned material has been assessed according to the ISO standard – DETERMINATION OF DEGRADABILITY BY MICRO-ORGANISMS (ISO 20136:2020) at INESCOP's laboratories. The degradation percentage obtained is as follows:
SAMPLE 1: 76.7%.



Biodegradable

76.7 % ISO / 20136



inescop.es

Certificate No.: CT02 XXX 202X This certificate shall remain valid until DD/MM/YYYY



Firmado digitalmente
 por BERTAZZO PERES
 MARCELO - 35708490R
 Fecha: 2025.07.22
 12:16:41 +02'00'

SHAPING
 THE FUTURE
 OF FOOTWEAR
 inescop.es

This certificate is complementary with the report: C-220245XX
 INESCOP takes responsibility only for the article or sample analysed

Thank you!!



mbertazzo@inescop.es