A Contract Report for Anya Hindmarch

RCR21-077

ISO 20136 Biodegradability Analysis

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A CONTRACT REPORT

For

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by

pp.  

DIRECTOR and AUTHOR

STRICTLY CONFIDENTIAL

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Executive Summary

Anya Hindmarch approached Eurofins | BLC, with regards to evaluating the degradation of their leather. Anya Hindmarch submitted 2 x leather samples for analysis:

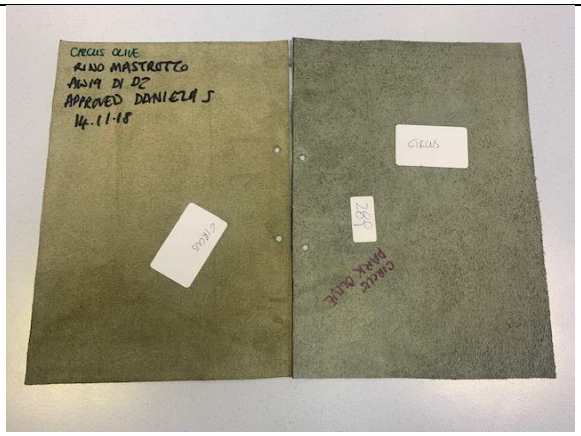
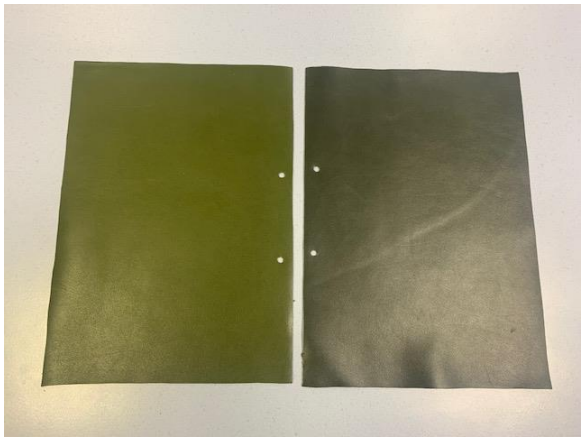
- Sample 1 (TERRA ZEO + WAX)
- Sample 2 (CIRCUS OLIVE)

The samples were analysed in accordance with BS EN ISO 20136:2020 Leather – Determination of the degradability by microorganisms. Ground material was placed into a minimal growth media that contained nutrients, water, and an inoculum from tannery effluent. The incubators were fed a CO_2-free air supply which contained oxygen and normal atmospheric gasses. The material was then biodegraded (for a minimum) of 28 days, to ascertain the relative biodegradability of the material.

Two samples were analysed, and their relative biodegradability compared to a control can be summarised as follows:

Samples	Absolute Biodegradation (%)	Relative Biodegradation (%)
Collagen Control	71.84	100
Sample 1	64.14	89.29
Sample 2	69.95	96.95

Sample Reference

BLC Reference	Customer Reference	Supporting Image	Analysis
Sample 1 (S1)	TERRA ZEO + WAX	 	BS EN ISO 20136
Sample 2 (S2)	CIRCUS OLIVE	 	BS EN ISO 20136

Methodology and Interim Results

BS EN ISO 20136: 2020. Leather - Determination of the degradability by microorganisms

The inoculum used was from the biological tank of the Eurofins BLC tannery in Northampton, UK. The inoculum was obtained on 14/07/2021, stored in a clean plastic bottle and transported/stored at 4°C. The solids were removed using glass wool.

- The media for the test was formulated as follows in Table 1:

Table 1. Formulation as per Standard Method

Materials	Quantity (mL)
Water	788
Inoculum	200
Ferric chloride	2
Magnesium sulfate	2
Calcium chloride	2
Phosphate buffer	4
Ammonium sulfate	2

- The equipment used was an EGA61 respirometer using a multi-channel through-feed infra-red analyser calibrated to 1 ppm resolution. The flow rates were standardised and the time of gas flow through the sensor chamber was optimised.
- A zero CO_2 channel was used to ensure baseline and the positive control was Type 1 collagen supplied by Eurofins BLC. The negative control (Blank) was the above formulation (as detailed in Table 1) without a test material.
- The controls and test samples were run in duplicate.
- The Blank CO_2 respiration is considered the baseline respiration of the growth medium (See Table 1) and this respiration level was removed from the CO_2 data to reveal the CO_2 being released from the material being tested.
- The incubators were shaken on an orbital bed at 150 rotations/minute. The biodegradations were performed without any light.
- The carbon content of the materials was analysed (see Table 2):

Table 2. The carbon content as per analysis.

Materials	Carbon (%)
Collagen	50.9
Sample 1	53.6
Sample 2	47.9

- The maximum theoretical carbon dioxide ($ThCO_2$) was calculated for controls and the materials to ascertain $ThCO_2$, allowing the viability of experiment using the collagen control reaching 70% as the minimum (See Table 3).

Table 3. Calculation of the theoretical CO_2 that could be evolved.

Sample	Carbon in the sample (%)	Dry Weight (%)	Weight of the sample (g)	Theoretical maximum of C in the sample (g)	Theoretical maximum of CO_2 – $ThCO_2$ (g)
Collagen	50.9	85.1	0.1574	0.0801	0.2938
Sample 1	53.6	88.2	0.1645	0.0882	0.3233
Sample 2	47.9	89.8	0.1666	0.0798	0.2926

- The CO_2 monitored was plotted over time and after 28-days (see Figures 4-6), if the control had biodegraded more than 70%, then the test was terminated for the controls and the experiments. The positive control for this test proceeded as normal and the test was terminated after 28 days.
- The absolute biodegradation (%), i.e., the amount of cumulative CO_2 released over the 28 days, was taken from the end point of the test.
- The relative biodegradability of the test sample compared to the positive control (Type 1 collagen). The relative biodegradability indicates whether the sample is easily biodegradable or resistant to biodegradability.

Table 4. Biodegradation results of the test samples as compared to the collagen control.

Samples	Absolute biodegradation (%)*	Relative biodegradation (%)**
Collagen control	71.84	100
Sample 1	64.14	89.29
Sample 2	69.95	96.95

*The absolute biodegradation is the amount of CO_2 evolved, recorded at the end of the test.

**The relative biodegradation is the % biodegradation if the control was normalised to 100%, i.e., how much biodegradation would take place when the control is at 100%.

- The samples tested biodegraded at a rate lower (in absolute terms) than the positive control, but only marginally. Untanned collagen can be largely degraded by micro-organisms very easily because the inoculum bacteria are collagenolytic species and thus a leather degrading at the same rate as a collagen control is notable.
- When the biodegradability is increased relative to a positive control biodegradation of 100%, the biodegradability rates indicate to what extent the test samples would be at the same timestamp. The data indicates that Sample 1 would be 89.29% degraded and Sample 2 would be 96.95 degraded.
- Figure 4 shows that the rate of breakdown had not reached a plateau by the 28th day. In biodegradability of chemicals, the plateau of the cumulative relative biodegradability curve should occur on or after the 90% has been reached - to help determine a biodegradable chemistry or not. The rate of increase (and extrapolation of the natural logarithmic curve) in Sample 1 suggest that it will exceed the 90% threshold before plateauing.
- Figure 4 shows that Sample 2 biodegrades easily and shows no plateauing of the cumulative relative biodegradability curve. The relative biodegradability calculation suggests that Sample 2 will reach 96.95% at the same point that the collagen control does – this indicates that Sample 2 is slightly lower, but comparable, to the collagen control in terms of biodegradability.

Figure 1: Sample 1 ground for ISO 20136 testing

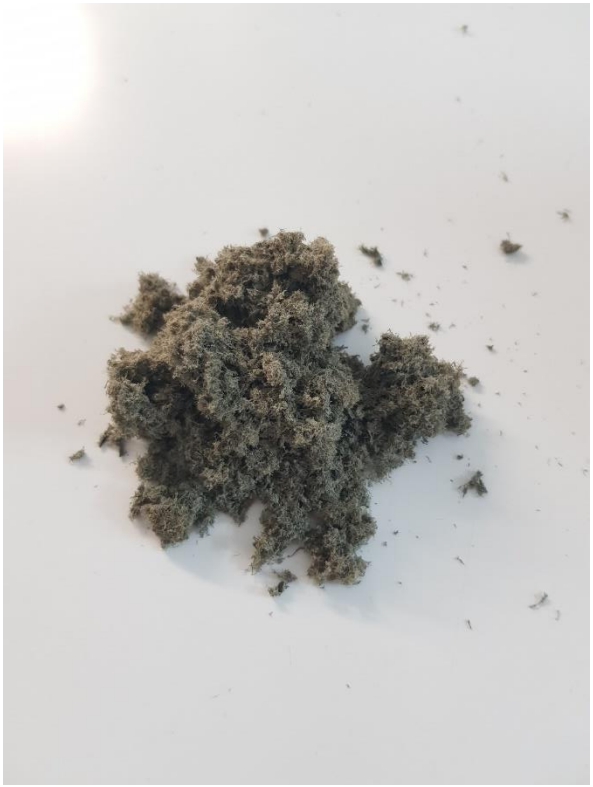


Figure 2: Sample 2 ground for ISO 20136 testing

Figure 3: Reactors in shaker with CO_2 free air supply.

Figure 4: CO_2 monitored was plotted over time and after 28-days – positive control (grey), sample 1 (orange) and sample 2 (blue).

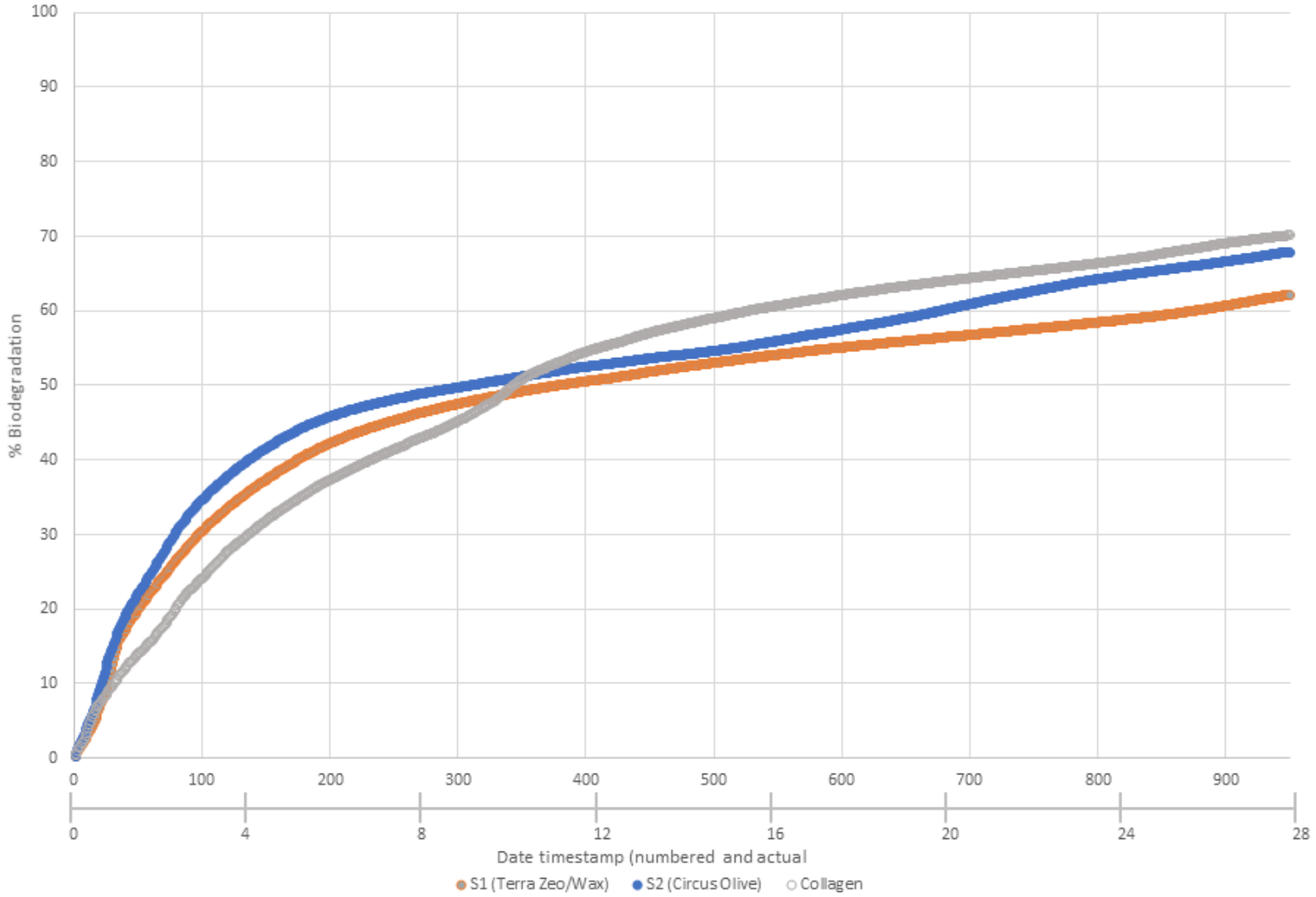


Figure 5: The biodegradability profiles (mean) of the positive control (grey) compared to Sample 1 (blue).

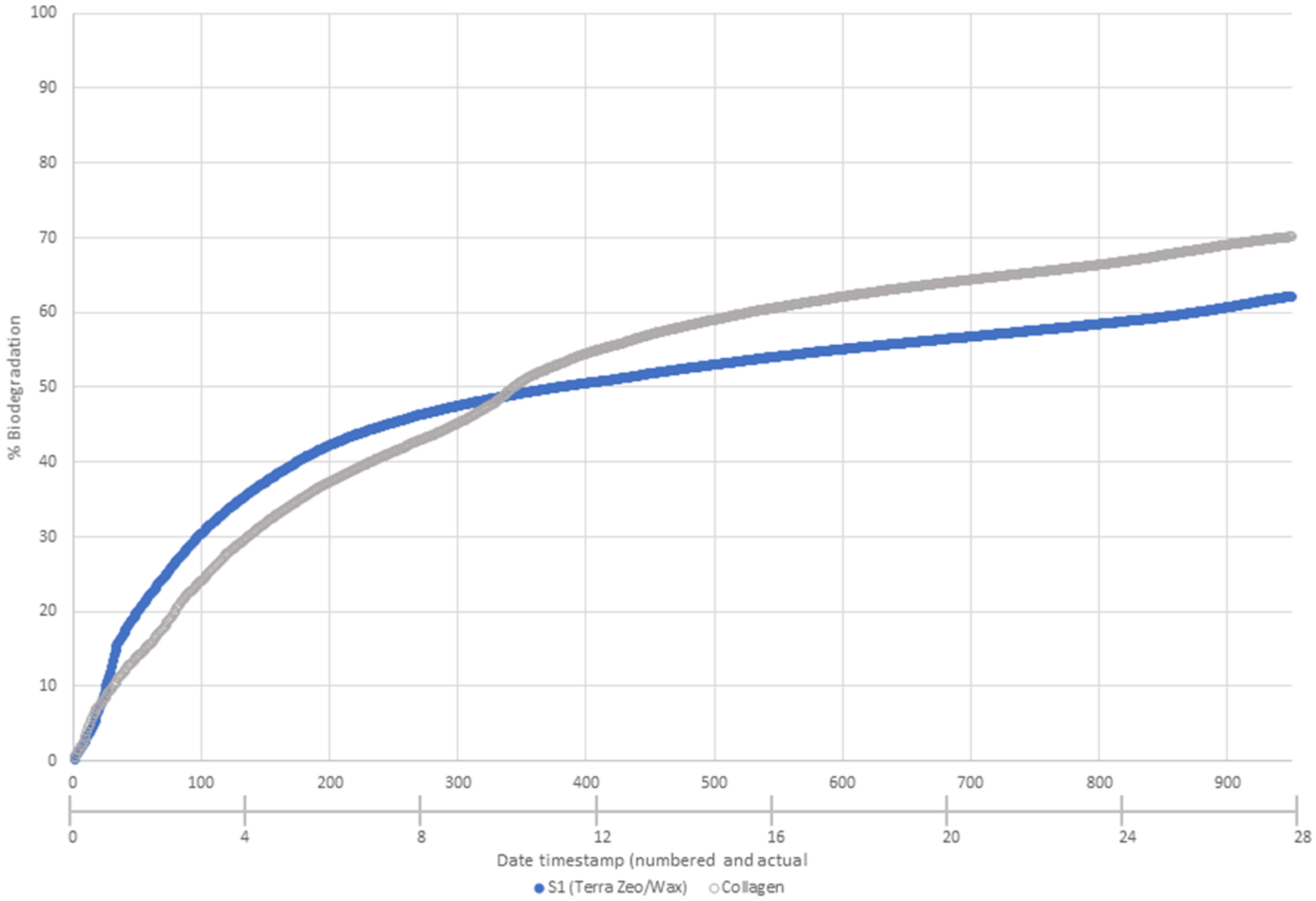
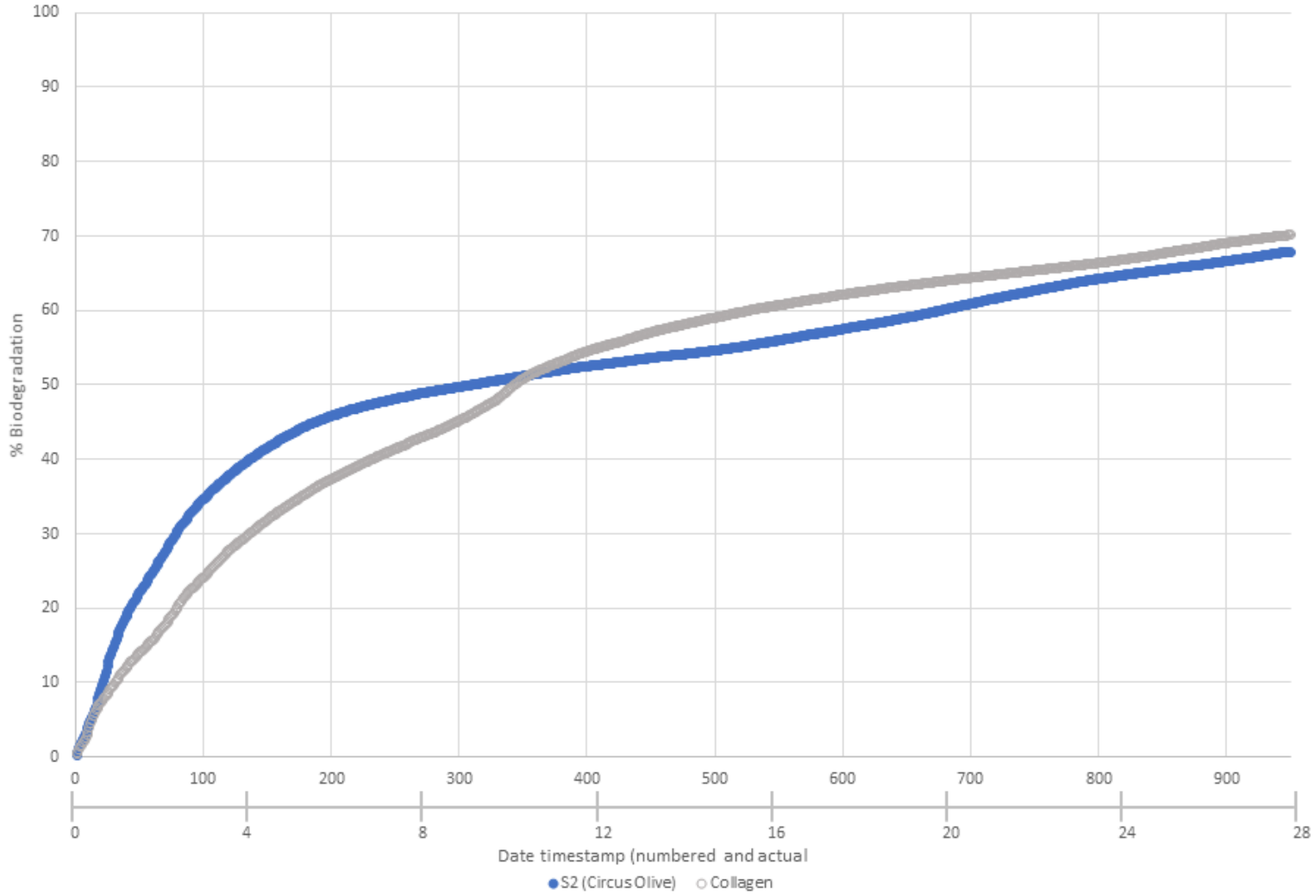


Figure 6: The biodegradability profiles (mean) of the positive control (grey) compared to Sample 2 (blue).



Discussion

- Sample 1 and Sample 2 had marginally less biodegradability compared to a collagen control.
- Colouration of the samples seen in Figure 10 and 11 compared to Figure 8 and 9, suggest that the colourants (which would appear as Total Carbon in both) were released by the material breakdown. Biodegradability of the dyestuffs in the leather may yield a better result if that carbon could be released to the bacteria.
- Sample 1 had a lower assimilation rate than Sample 2, but calculation of the relative biodegradability show that it is exceedingly close to the 90% threshold where the consideration of any plateau effects is considered.
- Figures 10 and 11 show the differences between bacterial biomasses between the two samples. Figure 10 shows greater bacterial mass (particularly the left replicate). The bacterial inoculum has responded well to the overall leather chemistry seen in Sample 2 – the tannage type, dye chemistry, retanning type, fatliquor choice, auxiliaries, and other leather treatments all must be biodegradable if they are to be assimilated by the bacteria.
- If the chemistry is difficult for the bacteria to breakdown, then the bacteria will not assimilate the carbon and will not be able to respire the carbon out as carbon dioxide (which is detected by the test).
- Another major consideration for this test is the presence of any anti-microbial chemicals, which can be certain natural or synthetic ingredients. Many natural chemicals that are included in leather have anti-microbial properties that may need to be screened for anti-microbial activity. Synthetic anti-microbials such as bactericides (often added in pigments, protein fillers, fatliquors, or certain retanning chemistries will negatively affect the biodegradability tests.
- Fungicides added to prevent mould growth in the leather are known to have anti-bacterial properties in addition to their anti-fungal attributes. To ensure that a full biodegradability is seen in the leather the anti-microbial action of the chemistry should be fully considered.
- The biodegradability levels seen in Table 4 and Figures 4 to 6 indicate that Samples 1 and 2 are ultimately biodegradable through comparisons to a collagen control.

Conclusion & Recommendations

The samples tested biodegraded at a rate lower (in absolute terms) than the collagen positive control; **64.14%** (Sample 1) and **69.95%** (sample 2) compared to 71.84% (positive control). When the biodegradability is increased relative to a positive control biodegradation of 100%, the biodegradability rates indicate to what extent the test samples would be at the same timestamp. The data indicates that Sample 1 would be **89.29%** degraded and Sample 2 would be **96.95%** degraded.

Appendix 1 – Samples at the Start of the Test

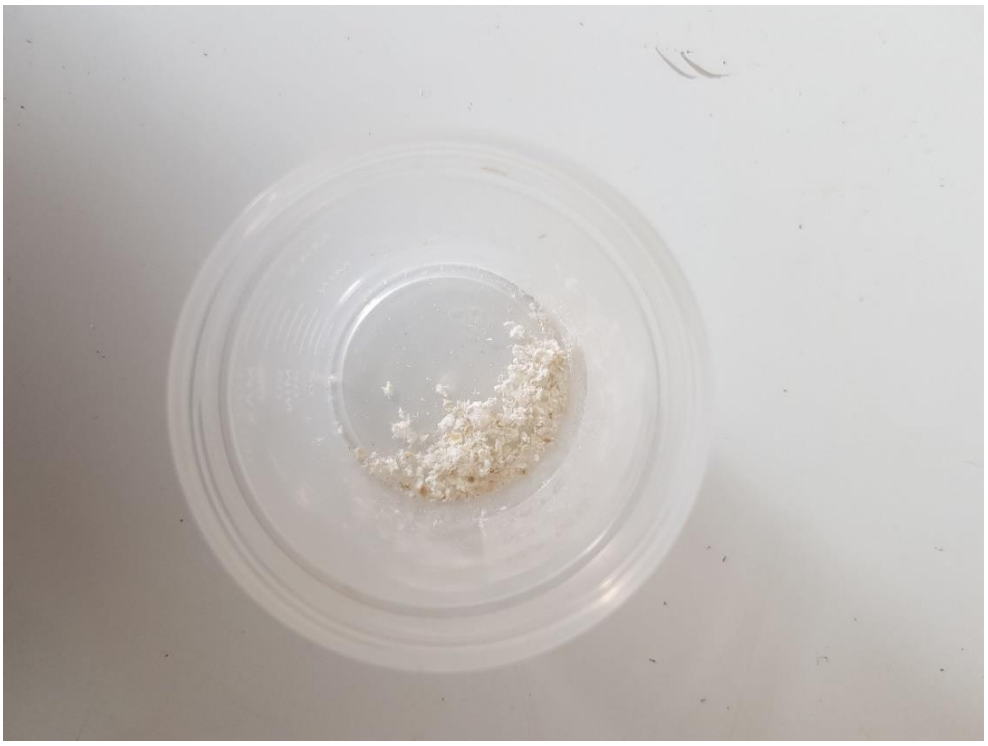


Figure 7. Positive control, collagen sample.

Figure 8. Sample 1 replicates at the start of the test.

Figure 9. Sample 2 replicates at the start of the test.

Appendix 2 – Samples at the End of the Test

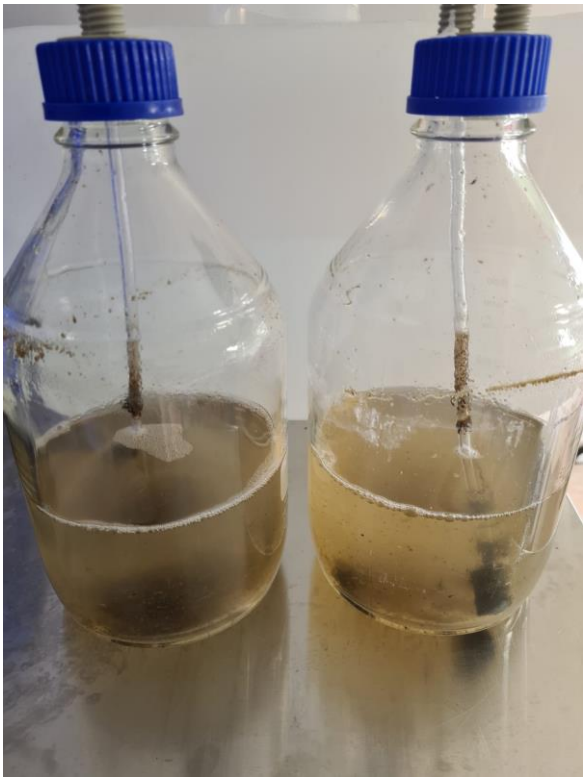


Figure 10. Sample 1 at the end of the 28 days degradation.

Figure 11. Sample 2 at the end of the 28 days degradation.