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In vivo rumen microbial degradation of polyhydroxyalkanoate biopolymers

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Polyhydroxyalkanoates (PHAs) are biopolyesters which are synthesised and stored in the cell cytoplasm as water-insoluble inclusions by various microorganisms (Anderson *et al.* 1990). PHA biopolymers, are water insoluble, stable at a variety of pH and have been shown to be degraded and used as nutrients by soil bacteria and fungi (Megaert *et al.* 1993), making them ideal candidates as novel delivery systems to provide controlled slow release of included compounds into the rumen of cattle. To understand the biodegradation characteristics of PHA within the rumen environment, a 122-day animal trial was undertaken in a repeated-measures, randomised-block design with three fistulated animals used to determine between animal variations. Two replicate sets of PHA biopolymer samples were placed in the rumen of each animal allowing measurement of within animal variation (Animal Ethics Committee approval SA 2020/03/737).

Extruded 'solid' cylinders or 3D printed 'porous' cylinders were prepared from PHA biopolymer using a two-step process; dry mixing followed by melt compounding and extrusion using a co-rotating twin screw extruder with a diameter of 16 mm for the solid cylinder. To enable 3D printing, the PHA, it was first extruded as a 1.6 mm filament which was used in a 3D printer. The biopolymer pieces were dried, weighed and placed into numbered nylon bags secured with nylon fishing line. These bags, containing the allocated sets of biopolymer pieces, were placed into two nylon mesh bags per animal, inserted into the rumen of each animal via the rumen cannula and secured with a nylon rope. The nylon mesh bags were withdrawn via the cannula on days 30, 62, 92 and 122, and the specific numbered bags of biopolymer pieces assigned for removal at each timepoint removed. The biopolymer pieces were recovered, rinsed with reverse osmosis water and dried. Percentage weight loss, and analysis by scanning electron microscope (SEM), differential scanning calorimetry (DSC) and gel permeation chromatography (GPC) was undertaken for each biopolymer piece.

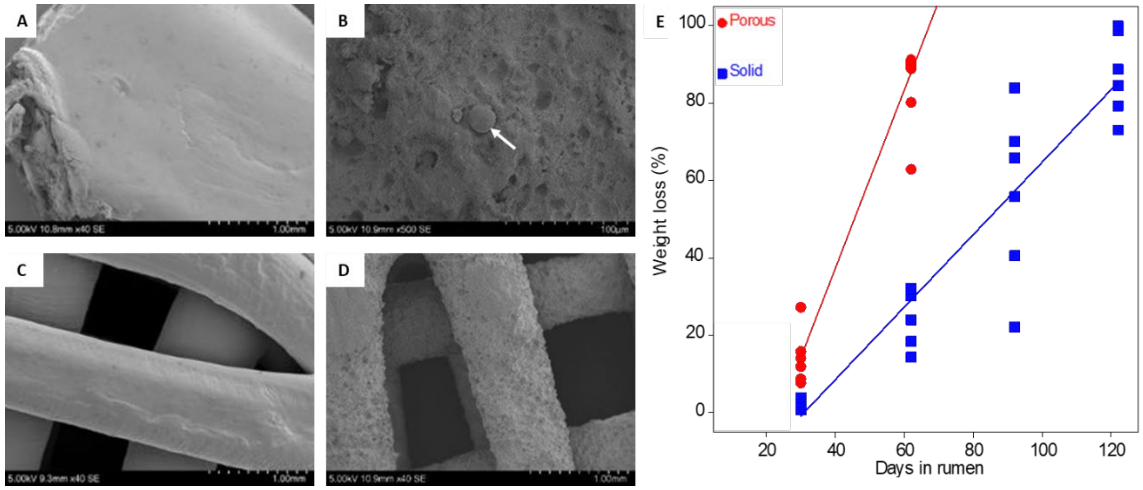

Fig. 1. SEM images of pieces of (A) solid PHA prior; (B) solid PHA after 30 days in the rumen (with a bacterial cell indicated with an arrow); (C) porous PHA prior; (D) porous PHA after 30 days in the rumen; and (E) linear regression analysis of degradation rate of solid (blue square) and porous (red circle) biopolymer pieces in the rumen over time.

The surfaces of representative solid and porous samples were analysed using SEM which showed that surface erosion was the dominant pattern of biodegradation. Prior to placement in the rumen, all the samples presented a smooth surface (Fig. 1A, C) whereas after 30 days in the rumen, both the solid and porous PHA samples had a large number of holes and hemispherical divots on the surface, indicative of bacterial enzymatic degradation (Fig. 1B, D). Linear regression analysis showed that the solid and porous PHA biopolymers degraded at significantly different rates ($P < 0.001$) with the porous samples degrading 2.46 times more rapidly than the solid biopolymer (Fig. 1E). DSC analysis revealed consistency in the melting behaviour of PHA and the GPC showed molecular weight consistency in the highly degraded samples, both confirming that in the rumen environment surface erosion is the dominant degradation pattern. The surface erosion biodegradation of the PHA biopolymer pieces *in vivo* in the rumen over the 122 days is a very promising first step for the use of biopolymers as novel delivery platforms for the controlled slow release of compounds into the rumen of cattle.

References

Anderson *et al.* (1990) *Microbiological Reviews* **54**(4), 450–472
Megaert *et al.* (1993) *Applied and Environmental Microbiology* **59**, 3233–3238.

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