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Kinetic and Morphological Analysis of *C. necator* Cells During the Synthesis and Degradation of Intracellular Polyhydroxyalkanoates (PHAs).

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The increasing commercialization of PHAs is due to the excellent combination of their properties: thermoplasticity, biodegradability and biocompatibility, which allow the design of products for diverse applications [1], [2]. PHAs are accumulated by microorganisms in the form of intracellular granules under conditions of nutritional stress [3]. The aim of this work is the study by Transmission Electron Microscopy (TEM) the formation, growth and degradation of the PHAs granules during bacteria fermentation.

The study was carried out in three stages. In stage 0, the inoculum of *C. necator* in TYF medium (Tryptone, Yeast, Fructose) was cultivated during 24 h. In stage 1, washed cells were incubated during 72 h in a medium statistically optimized for the PHB production [4]. Finally, in stage 2, cells were incubated for 98 h in a carbon-free medium such that *C. necator* degrades PHB. Thus, the whole incubation of stages 1 and 2 lasted 170 h (Fig. 1).

In stage 0, *C. necator* synthesized from 1 to 6 spherical PHA granules/cell with a maximum area of 0.18 μm^2. After 24 h of stage 1, some cells grew up to 3-times their length and 5-times their area, with a variable granule content up to 17. Furthermore, TEM images favored the "budding model" [5], in which nascent granules are located adjacent to or even bound to the inner side of the cytoplasmic membrane (Fig. 2). Then, a granule coalescence was observed, leading even to a single granule of 0.43 μm^2. Finally, the TEM micrographs clearly showed the granules degradation and the cell size decrease along Stage 2.

In conclusion, TEM allowed studying the kinetics and morphology of the different stages of accumulation and degradation of the PHAs granules, essential issues for scaling up the production of these biodegradable polyesters.

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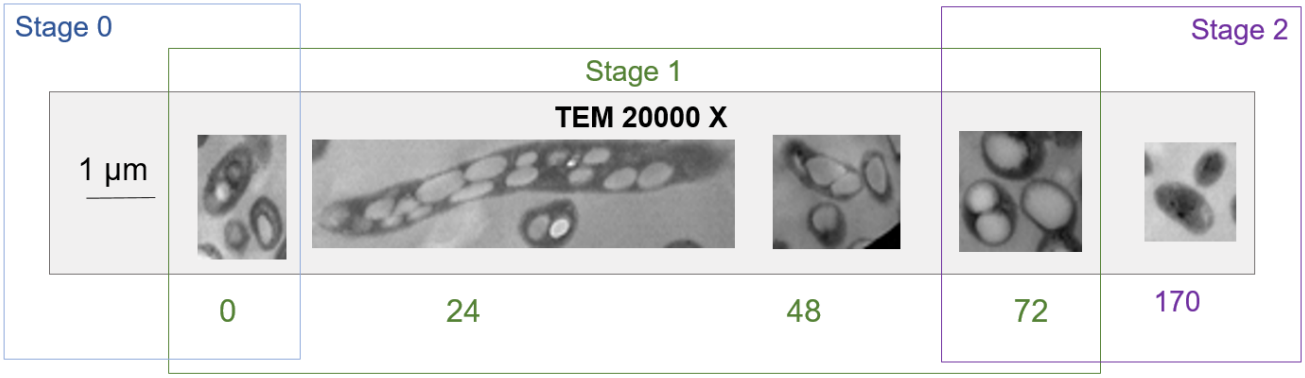

Time (h)	0	24	48	72	170
Maximum cell area (µm^2)	0.51	2.54	0.68	0.75	0.37
Maximum granule area (µm^2)	0.18	0.18	0.13	0.43	0,17
Maximum number of granules per cell	6	17	5	3	2

Figure 1: TEM micrographs of cells obtained in different fermentation stages with theirs corresponding areas (20 000 X);

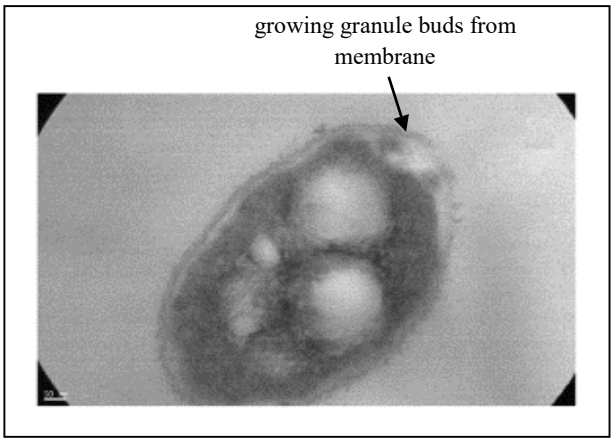

Figure 2: TEM image of a cell favored the “budding model” (140 000 X).

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