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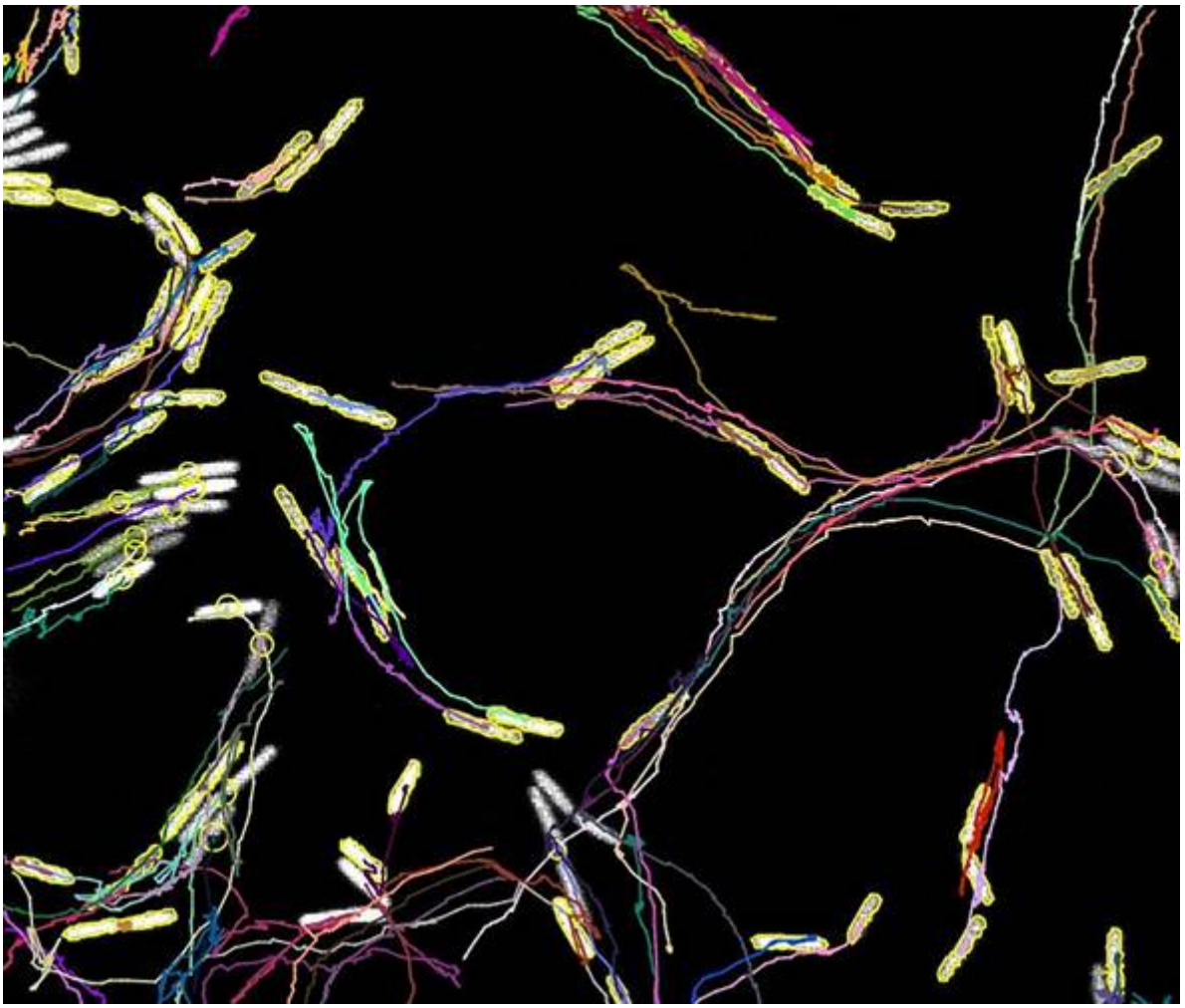

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

BIOFILM GROWTH KINETICS OF *Pseudomonas monteilii* ON A POLYCARBONATE SURFACE IN A CDC REACTOR

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Microbial biofilms are composed of a highly organized and structured community of microorganisms encased within a protective matrix of exopolymeric substances (EPS). This matrix forms the scaffold for the three-dimensional architecture and is responsible for adhesion to surfaces and cohesion within the structure. The biofilm formation process is multistage and influenced by various factors, including hydrodynamic conditions, environmental conditions, microbial interactions, and the availability of nutrients. The objective of this study was to evaluate the biofilm growth kinetics of *P. monteilii* on polycarbonate coupons in a CDC Biofilm Reactor (CBR) (BioSurface Technologies, USA) under dynamic conditions with controlled temperature and flow rate. For this purpose, *P. monteilii* was pre-cultured in JJP broth (24 h, 30°C, 180 rpm) and inoculated in a CDC biofilm bioreactor. After a 24-hour batch condition (180 rpm, 35°C), continuous conditions were maintained for 72 hours (150 rpm, 35°C). At different times during the continuous conditions (0, 8, 24, 32, 48, and 72 hours), coupons were extracted, and each coupon was washed with 0.9% PS and analyzed. Microbial viability was determined by the drop plate method on LB agar plates (24 h, 32°C). For the intended purpose, the biofilm attached to the coupons was resuspended in 2 mL of 0.9% phosphate-buffered saline (PBS). Subsequently, serial dilutions were prepared, and the resulting dilutions were expelled onto a 10 µL drop on an agar plate. To determine the biofilm composition, the coupons were analyzed using confocal laser fluorescence microscopy (CLFM) with three specific stains: Syto 9, Propidium Iodide (IP), and Calcofluor White (CFW). The resulting images were analyzed using Fiji software through an *ad hoc* workflow designed to extract information related to the biofilm depth and volume, as well as to analyze the spatial distribution of living cells, dead cells, and EPS. The system exhibited considerable biofilm growth up to 32 hours, indicating the major biovolume occupied by the EPS and the viable cell, which reached up to 94% of the volume measured in µm3. After this period, a noticeable decline of biofilm was observed in the subsequent hours. Consistent with this trend, the CLFM results indicated that at 32 hours, an increase in the IP and CFW signals suggested the formation of a mature biofilm. This study provides evidence that the optimal time for the growth of *P. monteilii* biofilm on a CDC reactor is 32 hours under our incubation conditions (30°C, 180 rpm).

Palabras clave: Biofilm - CDC biofilm reactor- Pseudomonas monteilii