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Pilot-Scale Fermentation of Cacao Liquid Residues for Sustainable PHA Biopolymer Production

ABSTRACT

Theobroma cacao L. is a tropical crop of major global importance. While increased cacao production offers substantial economic advantages for producing areas, it also generates significant waste and environmental drawbacks, since only about 10% of the cacao fruit is used and the rest is discarded (Battegazzore, Bocchini, Alongi, & Franche, 2014). These residues include cacao mucilage, a liquid by-product rich in fermentable sugars and organic acids. This characteristic makes it a valuable carbon source for biotechnological uses, such as producing polyhydroxyalkanoates (PHAs).

PHAs are intracellular biopolyesters produced by microorganisms under conditions of excess carbon and limited nutrients. They offer a sustainable alternative to traditional petrochemical plastics because of their biodegradability and biocompatibility. However, one of the main obstacles to their industrial use is their low productivity at scale. Therefore, scaling up PHA production processes through intermediate pilot-stage phases is highly valuable. Successful scale-up depends on selecting appropriate criteria that ensure high productivity at larger volumes without compromising product quality. Over 90 bacterial genera have been identified as PHA producers. *Priestia megaterium* was chosen for this study because of its rapid growth, metabolic versatility, adaptability to various ecosystems, and high genetic and physiological stability. This aerobic, spore-forming bacterium can produce PHAs under nutrient-limited conditions, making it a promising candidate for industrial uses.

This study examines the biotechnological production of PHAs using liquid residues from cacao bean processing as a carbon source and *Priestia megaterium* as the production strain. Valorization of these liquid effluents through microbial fermentation helps reduce waste and enhances resource efficiency. The process's performance was assessed based on substrate consumption, PHA accumulation, and volumetric productivity.

Initially, a nutritional and operational study was performed at a 4 L scale. The microorganism was first adapted to the cacao-derived carbon source. Then, a culture medium was developed through an experimental design where the carbon-to-nitrogen (C/N) ratio was varied to improve PHA production. Results from the small-scale study showed that an optimal C/N ratio of 10.61 produced a volumetric productivity of 0.139 $g \cdot L^{-1} \cdot h^{-1}$. Once optimal conditions were established on a small scale, the process was scaled up to 50 L. The oxygen transfer coefficient (kLa) served as the scale-up criterion to determine the aeration and agitation conditions. The theoretical kLa value was estimated using the Van´t Riet (1979) model, yielding 34.97 h^{-1}. Meanwhile, the experimental kLa, determined via the dynamic method, was 26.20 h^{-1}. An airflow rate of 14 $L \cdot min^{-1}$ was applied to the 50 L bioreactor, and agitation was varied throughout the process between 202 and 630 rpm.

Finally, a validation run was performed under the scaled-up conditions. The results showed a volumetric productivity of 0.179 $g \cdot L^{-1} \cdot h^{-1}$, representing a 22% increase on the productivity obtained at the 4 L scale. The integration of cacao processing liquid residues into PHA production shows the potential of agro-industrial waste streams to be converted into high-value biopolymers. This method supports circular bioeconomy strategies and encourages the development of sustainable practices materials.

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