USE OF ACAI SEEDS AS SUBSTRATE IN THE CULTURE OF MICROORGANISMS PRODUCING NEUTRAL LIPIDS

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ABSTRACT

Synthetic plastics are essential due to their widespread availability and low cost. However, the increased usage and improper disposal have led to an accumulation of these wastes in the environment. As an alternative, biodegradable polymers such as polyhydroxyalkanoates (PHAs) have emerged as a promising substitute for fossil-based plastics. PHAs, produced by bacteria, plants, and fungi, exhibit properties similar to synthetic plastics, although the industrial cost-effectiveness remains challenging. The study aimed to devise strategies for producing neutral lipids (NL) through bacterial fermentation using oligosaccharides from açai seeds as the primary carbon source. The residue yielded 32.26 ± 0.49 g/100g of mannose following thermal treatment and acid hydrolysis. Ten bacterial isolates averaged 41 ± 25% NL production. Two isolates and a strain of *C. necator* were selected for production in a 1L bioreactor. NL production for isolates 8C, 2M, and *C. necator* was 16.7%, 15.7%, and 11.7%, respectively. Açai seeds proved effective in NL production; however, further optimization studies are needed to enhance bioprocess productivity.

Keywords: *Euterpe oleracea*, *Bioeconomy*, Bioplastic.

1 INTRODUCTION

The increasing use and disposal of synthetic plastics derived from fossil hydrocarbons have led to a global environmental issue due to their slow decomposition and the formation of microplastics, which affect ecosystems, especially marine ones.[1] In response, the search for sustainable alternatives, such as bioplastics derived from renewable and biodegradable sources, which can be synthesized through biotechnological processes, has intensified. Polyhydroxyalkanoates (PHAs) are a class of biopolymers widely studied due to their properties similar to synthetic plastics and diverse applications; however, the cost of their production remains high.[2] The use of renewable sources, such as agro-industrial residues, for biopolymer synthesis is an alternative to cost reduction.[3] In the Amazon region, the cultivation of açai (*Euterpe oleracea*) produces large quantities of residues, such as seeds (85% to 95% of fruit volume), which contain compounds like mannan, a mannose polymer comprising approximately 53% of dry biomass.[4] This study proposes the use of açai fruit seeds as the main carbon source for the production of neutral lipids (NL) by bacterial isolates from the fruit's endophytic microbiome, aiming at a sustainable alternative for biocompound production.

2 MATERIAL & METHODS

Açaí seeds (10 kg) were acquired from vendors in the city of Belém, PA. The pre-processing of the seeds involved washing with water, drying in a 70°C oven for 12 hours, grinding in a knife mill with size standardization to 1.0 mm (mesh 18), extraction of extractives by solid-liquid extraction with ethanol (50%) at a ratio of 1:9 (w:v), 60°C for 15 min.[5] Subsequently, after vacuum filtration and drying (70°C, 12h), the biomass was subjected to acid hydrolysis. The biomass was subjected to an acid hydrolysis process employing 3% (w/w) diluted sulfuric acid, at a ratio of 1:4 (w:v), and heated to 121°C for one hour.[6] Subsequently, the hydrolysate was cooled in an ice bath, the liquid fraction's pH was neutralized with 20% NaOH (to pH 7.0), filtered again, and stored at -22°C.

The quantification of mannose was analyzed by high-performance liquid chromatography (HPLC) using a Thermo Fisher Scientific UltiMate™ 3000 series instrument. Compound separation was conducted on an HPX 87H 300 x 7.8 mm column (Aminex), operating at 25°C and using H_2SO_4 (5.0 mM) as the mobile phase in isocratic flow (0.6 mL/min) for 20 min. with an injection volume of 10 µL. Quantification of D-(+)-mannose was based on retention time and refractive index data, which were compared with the commercial standard (Sigma-Aldrich®, Brazil) and quantified through a calibration curve.

Ten bacterial isolates belonging to the microorganism bank of CVACBA-UFPA were cultured in 300 mL of medium containing mannose extract (8 g/L), supplemented with 2 g/L of yeast extract at 37°C, 150 rpm, pH 6.5 for 72 hours. The inoculum (10%) was standardized to 10^9 CFU/mL. Two isolates (8C and 2M) were selected for cultivation in a 1L bioreactor, in addition to the standard strain of *C. necator* ATCC 17699 under the same previous culture conditions, but with pH control (6.8) and oxygenation (OD>95%). Quantification of NL was evaluated at the end of the exponential phase for each isolate through gravimetric assays resulting from chloroform extraction.[7] The results of this study are based on single analyses, with no replicates conducted during the methodology employed.

3 RESULTS & DISCUSSION

The extraction of mannose from açai seeds by acid hydrolysis yielded a 32.2 ± 0.49% yield with 8.12 ± 0.1 g/L of mannose. In other studies, the substitution of sulfuric acid with maleic or oxalic acid resulted in a hydrolysate with concentrations of 6.4 g/L and 24.4 g/L, respectively. Furthermore, the use of oxalic acid under more severe conditions (150°C for 40 min.) yielded a mannose concentration of approximately 49 g/L, which is higher than those obtained with sulfuric acid.[9] Employing a sequential acid and enzymatic extraction, a mannose yield of 95% was obtained.[4]

The production of NL by ten bacterial isolates is presented in Table 1. Isolates 8C, 2M, and 11M exhibited an NL yield above 50% relative to cell biomass, with concentrations of 506 mg/L, 286 mg/L, and 116 mg/L of NL, respectively. In contrast, isolates 4M, 6M, and 13C showed no detectable accumulation of NL. A study using brewery wastewater containing maltose (30 g/L) achieved, after 90 hours of fermentation, a biomass concentration of 1.5 g/L with 450 mg/L of NL. [8]

Table 1. Production of neutral lipids by bacterial isolates using mannose from açai fruit seeds as the main carbon source.

Bacterial isolate	Time (h)*	Cellular biomass (g)	Neutral lipids (%)
8C	17	0.205	74.15
2M	17	0.117	73.50
11M	17	0.067	52.24
14M	17	0.086	25.58
9M	10	0.092	23.91
7M	10	0.092	20.65
2C	12	0.112	16.96
4M	17	0.015	nd
6M	17	0.039	nd
13C	17	0.029	nd

*: Neutral lipid was evaluated at the end of the exponential phase; nd: Undetected

After ranking, bacterial isolates 2M and 8C, along with the commercial strain *C. necator* (ATCC 17699), were selected for batch fermentation in a 1L bioreactor. Figure 1 shows the cellular growth of the microorganisms after 8 hours. The cellular growth rate (μ) was 2.1 h^{-1} for 2M and *C. necator*, and 1.7 h^{-1} for 8C. The results demonstrated that the microbial isolates grew in the medium derived from açai seeds as well as the standard strain, indicating the carbon source (8 g/L mannose) was effective.

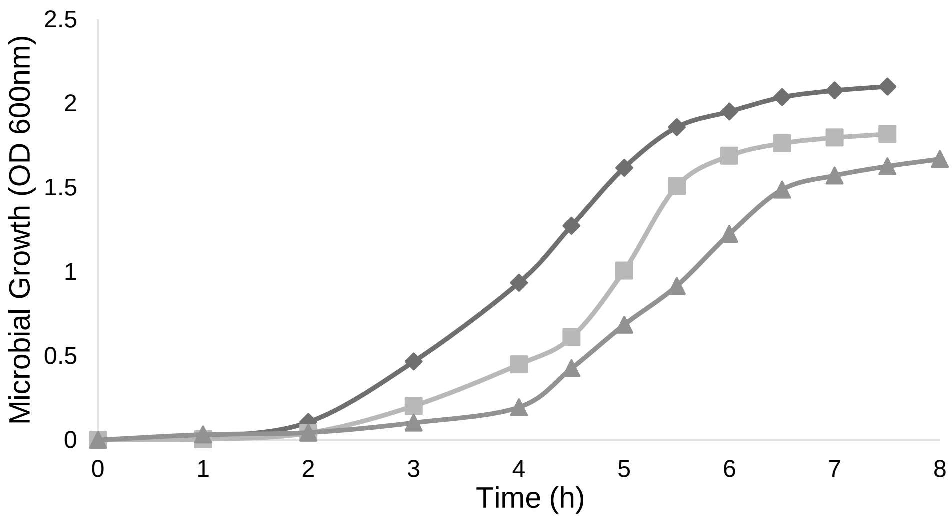

Figure 1. Microbial growth curve in a 1L bioreactor medium with the main carbon source derived from açai seeds. (♦) isolate 2M (■) isolate 8C e (▲) *C. necator* strain.

Table 2 presents the production of NL by the two microbial isolates and the standard *C. necator* strain using mannose from açai seeds as the main carbon source. Isolate 8C stood out among the others by exhibiting superior NL production (230 mg/L), representing a yield of 16.72%. In contrast, the commercial strain, which is the benchmark in NL production, registered only 160 mg/L (11.72% yield). A study conducted cultivation of *C. necator* in shaken flasks under different nutritional conditions and obtained 350 ± 0.01 mg/L of PHAs after 24 hours of fermentation.[9]

Table 2. Production of neutral lipids by microorganisms in a 1L bioreactor using mannose from açai fruit seeds as the main carbon source

Microorganism	Biomass (g)	Neutral lipids (mg/L)	Neutral lipids (%)
8C	0.957	230	16.72
2M	0.896	200	15.74
C. necator	0.964	160	11.72

4 CONCLUSION

In conclusion, the valorization of açai seeds as a source of fermentable sugars for the microbial fermentation production of bioproducts such as bioplastics was presented. The results demonstrated that acid hydrolysis was effective in releasing mannose, enabling its utilization by the studied microorganisms. The selected bacterial isolates showed the ability to produce NL from açai seed mannose, with isolate 8C standing out by exhibiting higher production compared to the standard strain *C. necator*. However, despite promising results, further studies are required to optimize the NL production process and explore the potential of polyhydroxyalkanoates, using açai residues as a carbon source.

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