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Short Communication

Mixed-culture polyhydroxyalkanoate production from olive oil mill pomace

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HIGHLIGHTS

- PHA was produced in non-sterile conditions using olive pomace as the only feedstock.
- Buffer addition to olive pomace increased organic acid production by 75%.
- Highest volumetric productivity was 0.042 g PHA/L-day.
- Highest PHA conversion yield 0.3625 g PHA/L fermentate.
- Maximum %PHA was 39% (on a dry weight cell basis).

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ABSTRACT

Polyhydroxyalkanoate (PHA) was produced in bench-scale sequencing batch reactors (SBRs) fed olive pomace fermentate containing a mixed microbial consortium. Initial anaerobic fermentation in a sequencing batch fermentor (SBF) produced soluble carbon compounds, mainly organic acids. SBF effluent was centrifuged, removing solids, and fed into a SBR where intracellular PHA was produced. Buffer pre-treatment of diluted olive pomace increased organic acid production 75% in SBF fermentate over no pre-treatment. Hydraulic retention time (HRT), solids retention time (SRT), pomace concentration/dilution, and aerobic operation vs. anoxic/oxic cycling were studied to improve PHA formation. Maximum %PHA achieved was 39% (on a dry-weight cell basis), and the highest volumetric productivity was 0.042 g PHA/L-day under fully aerobic conditions. The highest PHA conversion yield was 0.3625 g PHA/L fermentate.

1. Introduction

The goal of this study was to identify operating parameters that maximized conversion of olive pomace, an olive oil production byproduct, to polyhydroxyalkanoates (PHA). Olive pomace currently has limited use and often incurs significant cost associated with disposal. Bacterially produced PHA seems an attractive replacement for petroleum-derived plastic, owing to its biodegradability and ability to be produced from a variety of carbon sources without requiring toxic chemicals.

Current commercial PHA production practices are based on aseptic, single-strain bacterial fermentations that generally rely on refined carbon feedstocks (Khanna, 2004). Investigations of industrial waste products such as whey protein for carbon and nutrient sources have yielded modest cost savings, but considerable energy and cost are expended to maintain sterility (Akaraonye et al., 2010). Mixed bacterial cultures and non-sterile processing methods and feedstocks could allow PHA bio-plastic to compete economically with petroleum-derived plastics.

The proposed process consists of an initial olive pomace fermentation in a sequencing batch fermentor (SBF) to produce soluble organic acids. Centrifuged decant from the SBF, referred to as olive pomace fermentate, is then fed into a sequencing batch reactor (SBR), where organic acids and other compounds are consumed and significant quantities of intracellular PHA are produced. This study's objectives were to (1) evaluate pre-treatment options for increasing organic acid production in SBFs; (2) identify SBF operating parameters that maximize organic acid production; and (3) ascertain SBR operating parameters that maximize PHA production.

2. Methods

2.1. Feedstock materials and microorganisms

2.1.1. Olive pomace

Fresh olive pomace, consisting of olive organic matter and crushed pits, containing 66% moisture content, was stored

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at −20 °C until converted into a prepared feedstock and fed into the SBFs. The frozen olive pomace was thawed and diluted in deionized water at concentrations between 75 and 300 g/L olive pomace. Once thawed, the solution was mixed on a stir-plate for 10 min; then filtered through a 1-mm sieve.

2.1.2. Microorganism source

On SBF and SBR startup days, activated sludge from the University of California, Davis Wastewater Treatment Plant was obtained as an inoculum for the SBFs and SBRs.

2.2. Apparatus setup and operation

2.2.1. 500-mL sequencing batch fermentors

SBFs consisted of 500-mL glass Erlenmeyer flasks placed on stir-plates with a 20-mm stir-bar continuously mixing the contents. Timer-controlled peristaltic pumps drained and fed the SBFs daily. Each SBF had a single decant-and-feed cycle per day with feed volumes determined by the specified HRT. Each SBF startup began with 100 mL activated sludge inoculum and 400 mL olive pomace feedstock. Daily cycling began the following day and SBFs were allowed to stabilize over four HRTs. All SBF experiments were conducted at 20–25 °C.

2.2.2. 12-L sequencing batch fermentor

A 12-L SBF was constructed to produce olive pomace fermentate for SBR experiments. The SBF was operated with a HRT = SRT = 4 days and a 150-g/L pomace concentration with buffer pre-treatment (described below). The 12-L fermentor consisted of a lidded five-gallon high-density polyethylene bucket, fed and drained by two timer-controlled peristaltic pumps. Continuous mixing was provided by two stir plates and two 40-mm stir bars. Daily cycling consisted of a drain cycle that removed 3 L of fermentate and a feed cycle that added 3 L of buffered feedstock. Fermentor decant was centrifuged daily to remove solids and stored at 4 °C until use in downstream SBRs. SBFs were operated at 20–25 °C.

2.2.3. 300-mL sequencing batch reactors

300-mL working volume SBRs were constructed with 2.5″ clear acrylic pipes connected to 2.5″ Schedule 80 PVC unions. 2.5″-diameter rubber membranes were cut from 7″ Sanitaire fine-bubble disc diffusers and mounted between the two union halves in each SBR for a watertight seal. 2.5″ Schedule 80 PVC caps were used as caps atop each SBR and as bottoms below each union. A 1/4″ brass barb fitting was threaded into each bottom cap and connected to the gas inlet. Air solenoid valves controlled the type and timing of gas (atmospheric air or nitrogen) entering each SBR. Air flow rates were maintained by Cole-Palmer PMR4 flow regulators. Peristaltic pumps drained and fed each SBR based on their designated HRT and SRT durations. SRT durations were maintained with 15-min settlement periods at the end of each feed cycle to retain the correct biomass percentage. A digital timer controlled each solenoid valve and peristaltic pump. The SBRs were operated at 20–25 °C.

2.3. Analytical methods

This experiment required measurement of pH, inorganic nitrogen (ammonia, nitrate), mixed-liquor suspended solids (MLSS) and mixed-liquor volatile suspended solids (MLVSS), organic acids, and the polyhydroxyalkanoates polyhydroxybutyrate (PHB) and polyhydroxyvalerate (PHV). All were measured according to standard and accepted scientific practice. Interested parties may contact us directly to review supplier lists and analytical techniques.

2.4. Experimental

2.4.1. SBF feedstock pre-treatments

Pre-treating the olive pomace feedstock was investigated to identify treatments that increased organic acid concentrations in the SBF fermentate. Buffer, centrifugal, and combined buffer/centrifugation pre-treatments were examined. Centrifugation of mixed olive pomace and olive mill effluent was previously shown to increase organic acid production in batch fermentations (Dionisi et al., 2005). Centrifuged feedstock was produced by centrifuging standard feedstock at 3750 rpm for 10 min to remove suspended solids before placing the centrifugate into feed containers. pH adjustment was also examined because low fermentor pH levels (down to 4.1 pH) were observed in initial SBF experiments; low pH could possibly inhibit organic acid production (Zeng et al., 2006). Potassium carbonate was added to standard feedstock at 6.9 g/L and mixed on a stir plate for 10 min before placement into feed containers to raise the pH and buffer the media. A pH of 10.0–10.5 was achieved in the SBF feedstock, depending on pomace concentration. SBFs fed buffered pre-treated feedstock had a fermentate pH of around 6.5–7.5, compared to 4.0–4.5 pH in SBFs fed standard feedstock. Combined pre-treatment by centrifugation and buffer addition was performed by adding 6.9 g/L potassium carbonate to centrifuged feedstock and mixing on a stir plate for 10 min prior to placement into feed containers. Four separate 500-mL working volume SBFs with different feedstock pre-treatments were operated with one fermentation cycle per day, HRT = SRT = 4 days, and a pomace concentration of 150 g/L. These operating parameters were based on results from initial SBF experiments that displayed good organic acid production (data not shown). SBFs were sampled after a four-HRT stabilization period by drawing samples at the end of a 24-h fermentation cycle to measure fermentate pH, chemical oxygen demand (COD), and organic acid concentrations.

2.5. SBF operating parameter optimization

The effect of pomace concentration and HRT on organic acid concentrations and pomace conversion yields (g organic acid/g olive pomace) was investigated to determine SBF operating parameters that maximized organic acid production. 2-, 4-, and 8-day HRT values and 75-, 150-, and 300-g/L pomace concentrations were examined. HRT values above 8 days have been shown to increase methanogensis in other waste streams (Bengtsson et al., 2008), thus reducing organic acid concentrations in anaerobic fermentors. Nine separate 500-mL fermentors were operated and sampled after a four-HRT stabilization period. SBF fermentate samples were drawn at the end of the 24-h fermentation cycle for pH, COD, organic acid, and inorganic nitrogen analyses.

2.6. SBR anoxic/oxic cycling

We investigated the necessity of anoxic/oxic cycling for PHA production in SBRs across a range of operating parameters, with and without anoxic/oxic cycling, as such cycling may only be beneficial under certain reactor operating parameters. HRT, SRT, and pomace concentration were the operating parameters examined. A low HRT value of 0.5 days was chosen to prevent any feast-famine response during the feed cycle and a high value of three days was chosen to promote the feast-famine response. 0.5-, 1-, and 3-day HRTs had feed-cycle durations of 6, 12, and 24 h. The low SRT value was set to 3 days and the high value to 12 days, parameters typical of wastewater operations and known to elicit PHA production with high-COD feedstocks. Feed concentrations were expressed as the concentration of pomace fermentate; levels below 100% were diluted with deionized water. Full strength (100%) had a