Bioresource Technology 166 (2014) 194–200

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Production of poly(hydroxybutyrate–hydroxyvalerate) from waste organics by the two-stage process: Focus on the intermediate volatile fatty acids

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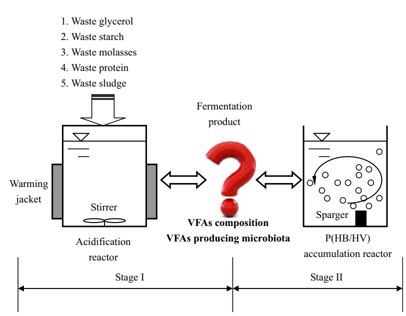
HIGHLIGHTS

- Five waste organics were studied to produce P(HB/HV) in one two-stage process.
- Changing materials brought more effects on VFAs composition other than the yield.
- VFA composition and the P(HB/HV) content had a good, positive linear correlation.
- Special bacteria were required to produce VFAs from different wastes in this process.
- Potential competition among acidogenic bacteria producing different VFA was proposed.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 15 April 2014
Received in revised form 12 May 2014
Accepted 13 May 2014
Available online 20 May 2014

Keywords:
P(HB/HV)
VFAs
Waste organics
Pyrosequencing

ABSTRACT

The two-stage process, coupling volatile fatty acids (VFAs) fermentation and poly(hydroxybutyrate–hydroxyvalerate) (P(HB/HV)) biosynthesis, was investigated for five waste organic materials. The overall conversion efficiencies were glycerol > starch > molasses > waste sludge > protein, meanwhile the maximum P(HB/HV) (1.674 g/L) was obtained from waste starch. Altering the waste type brought more effects on VFAs composition other than the yield in the first stage, which in turn greatly changed the yield in the second stage. Further study showed that even-number carbon VFAs (or odd-number ones) had a good positive linear relationship with P(HB/HV) content of HB (or HV). Additionally, VFA producing microbiota was analyzed by pyrosequencing methods for five wastes, which indicated that specific species (e.g., *Lactobacillus* for protein; *Ethanoligenens* for starch; *Ruminococcus* and *Limnobacter* for glycerol) were dominant in the community for VFAs production. Potential competition among acidogenic bacteria specially involved to produce some VFA was proposed as well.

Abbreviations: PHAs, polyhydroxyalkanoates; PHB, poly-β-hydroxybutyrate; P(HB/HV), poly(β-hydroxybutyrate-co-β-hydroxyvalerate); PHV, poly-β-hydroxyvalerate; HB, β-hydroxybutyrate; HV, β-hydroxyvalerate; VFAs, volatile fatty acids; HAc, acetic acid; HBu, n-butyric acid; HPr, propionic acid; HVa, n-valeric acid; i-HBu, isobutyric acid; i-HVa, isovaleric acid; P_{VFAs}, VFAs production amount; R_{HAc}, HAc percentage; R_{HPr}, HPr percentage; R_{HBu}, HBu percentage; V_{VFAs}, VFAs production rates; $Y_{P(HB/HV)}$, the yield of P(HB/HV) in the second stage; Y_{total}, the yield of P(HB/HV) in the whole process; Y_{VFAs}, the yield of VFAs in the first stage.

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http://dx.doi.org/10.1016/j.biortech.2014.05.038
0960-8524/

1. Introduction

Polyhydroxyalkanoates (PHAs) are a group of polyesters that are promising alternatives to conventional plastics due to their biodegradability and ability to be produced from renewable resources. Two common PHA monomers are hydroxybutyrate (HB) and hydroxyvalerate (HV). Pure PHB is stiff and brittle, whereas with the introduction of HV, the elasticity and flexibility increased, providing the polymer with properties similar to polypropylene (Albuquerque et al., 2011; Arcos-Hernández et al., 2013). Thus, the production of the HB and HV copolymer, namely P(HB/HV), is currently attracting much interest from researchers.

In the literature, pure culture microorganisms are well-known to accumulate P(HB/HV) in their wild form or by genetically recombinant strains (Lee, 1996; Pan et al., 2012). Meanwhile, because the mixed culture process is easier to manipulate and does not require sterile conditions, using mixed culture, particularly activated sludge, has turned to be a trend to produce P(HB/HV) in industrial scale (Salehizadeh and Van Loosdrecht, 2004; Chang et al., 2012; Wang et al., 2013). Apart from microorganisms, the main obstacle hindering the mass production of P(HB/HV) is the cost of the carbon substrate, which accounts for 28–50% of the total production cost during microbial fermentation (Choi and Lee, 1999). Hence, it is necessary and important to develop alternative low-cost carbon sources such as agricultural and industrial waste organic residues. However, unlike pure cultures, waste organics are not directly stored as PHA by mixed cultures but preferentially accumulated as glycogen (Dircks et al., 2001). PHA production from carbon-enriched raw materials requires a previous anaerobic fermentation step for their transformation into short-chain volatile fatty acids (VFAs). For this reason, the majority of the studies related to PHA production by mixed cultures are based on the use of VFAs (Dias et al., 2006).

Consequently, a two-stage process has been developed to produce PHA using mixed cultures from waste organics in recent decades. In detail, the waste organic is fermented under acidogenic conditions to obtain VFAs in the first anaerobic stage, which provide suitable substrates for the PHA biological synthesis that occurs in the following second aerobic stage. This sequential conversion of “organics–VFAs–PHA” has been fulfilled for various raw materials, such as biodegradable wastes (Majone et al., 1999), olive oil mill effluents (Dionisi et al., 2005), sugar molasses (Albuquerque et al., 2010) and olive oil mill effluents (Md Din et al., 2012), indicating that this two-stage process is a feasible industrial technique to produce PHA from waste organics. Meanwhile, the performance in the aforementioned cases varied in a wide range due to the inconsistent operation conditions, which makes it difficult to consider that how diverse types of waste organics affect the PHA production for an established two-stage process. Currently, there is little information of the correlation between the fermentation material and P(HB/HV) production ability in the two-stage process. The intermediate VFAs appear to be the key to understanding this problem, as the content of HB and HV is largely determined by the availability and composition of VFAs (Albuquerque et al., 2011). In addition, although VFAs are commonly recognized as the acidification products of the anaerobic digestion which results in biogas production, the microbial characterization of the acidogenic microbes and their mutual relationship regarding different fermentation wastes are seldom reported. These microbes may be crucial for VFAs production and composition.

Therefore, the aim of this paper was to examine the production of P(HB/HV) using activated sludge from five representative organic wastes through this two-stage process, focusing on the role of VFAs in this conversion. The effect of the waste type on the performance of each stage will be evaluated based on peer comparison. Additionally, microbiota of the VFAs production stage will be analyzed using the pyrosequencing technique to look into the correlation between acidogenic microbes and VFAs output. Finally, the relationship among VFAs composition, VFA producing microbes and P(HB/HV) productivity in one two-stage process will be revealed.

2. Methods

2.1. Experimental setup and operation

A two-stage reactor system was setup to produce P(HB/HV) via VFAs, as shown in Fig. 1. In the first stage, the VFA production experiments were conducted under anaerobic conditions, each with 1.2 L prepared solution and 20 g seed sludge, in which the COD concentration of each substrate was 10,000 mg/L. The initial pH was adjusted to 10.0, and the temperature was maintained at 35 °C. Oxygen in these flasks was removed from the headspace by nitrogen gas sparging for 3 min. The flasks were sealed with rubber stoppers. All flasks were stirred at a speed of 120 rpm during the entire test period.

In the second stage (Fig. 1), aerobic batch tests were performed to investigate the effects of substrate composition on P(HB/HV) synthesis. The 1 L supernatant containing VFAs was transferred into a glass reactor in each batch test, along with a defined volume of the acclimated aerobic sludge to keep the content of the MLSS at approximately 2 g/L. The tests were operated under continuous aerobic conditions with an aeration rate of 2 L/min at room temperature. DO content of approximately 80% in the mixed liquid was maintained by aeration through a ceramic membrane disperser with an air compressor. The stirring rate was maintained at 400 ± 5 rpm using a mechanical agitator. The initial pH value of the mixed liquid was adjusted by adding diluted sulfuric acid or diluted sodium hydroxide.

2.2. Sludge and waste organics

Anaerobic sewage sludge collected from a local wastewater treatment plant was used as seed inoculums for VFAs production from different substrates in the first stage of the process, as shown in Fig. 1. The five typical waste organics used for fermentation were the following: cane molasses generated from a sugar-manufacturing process was used as a nutrient source (73.2% of the total organic

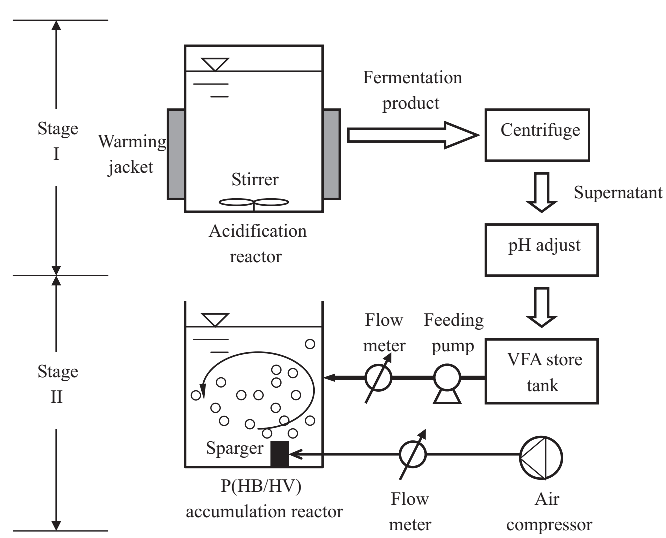

Fig. 1. Schematic of the two-stage process for P(HB/HV) production via VFAs.