Bioresource Technology 175 (2015) 23–33

Contents lists available at ScienceDirect

Bioresource Technology

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

Influence of organic loading rate and solid retention time on polyhydroxybutyrate production from hybrid poplar hydrolysates using mixed microbial cultures

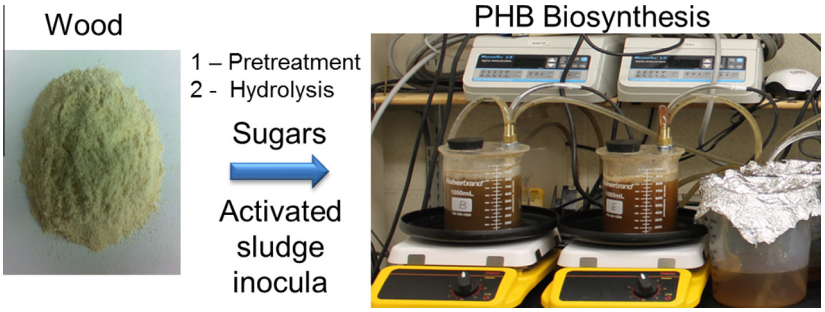
Jing Dai [a], Karol Gliniewicz [b], Matthew L. Settles [b], Erik R. Coats [c], Armando G. McDonald [a,*]

[a] Department of Forest, Rangeland and Fire Sciences, University of Idaho, Moscow, ID 83844-1132, United States
[b] Department of Biological Sciences and the Institute for Bioinformatics and Evolutionary Studies, University of Idaho, Moscow, ID 83844, United States
[c] Department of Civil Engineering, University of Idaho, Moscow, ID 83844, United States

HIGHLIGHTS

- Polyhydroxybutyrate was biosynthesized from poplar wood hydrolysates.
- Fed-batch bioreactors were seeded using mixed microbial cultures.
- Microbial community evolution was monitored using 16S rRNA Illumina sequencing.
- *Actinobacteria*, *Alpha-* and *Beta-proteobacteria* were dominant groups in bioreactors.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 5 September 2014
Received in revised form 8 October 2014
Accepted 9 October 2014
Available online 17 October 2014

Keywords:
Wood hydrolysates
Mixed microbial cultures
Polyhydroxybutyrate
16S rRNA sequencing

ABSTRACT

The aim of this study was to investigate the potential of using wood hydrolysates (enzymatically hydrolyzed from hybrid poplar) as substrate to produce polyhydroxybutyrate (PHB) using mixed microbial cultures. The optimal operational conditions for fed-batch bioreactors were 4 d solid retention time with an organic loading rate of 2.5 g/Ld. The maximum PHB accumulated was 27% of cell dry weight with a yield of 0.32 g/g (g PHB produced per g sugars consumed). Microbial community analysis was done at the genus level by 16S rRNA sequencing on an Illumina system and community evolution was observed among different samples and initial seed. *Actinobacteria*, *Alpha-* and *Beta-proteobacteria* were found to be the dominant groups in all the bioreactors. Several PHB-storing microorganisms were characterized belonging to *Alpha-* and *Beta-proteobacteria*.

1. Introduction

Petroleum-based plastics are widely used in a variety of applications, including food packaging, communication, transportation, clothes, shelter and health care (Chen, 2010). Due to their large amount of consumption, petro-plastics present a serious disposal problem in the landfills causing by their very slow rate of degradation. Therefore, bio-based and biodegradable plastics are gaining a lot of interest and being developed as suitable alternatives for petroleum-based plastics. Polyhydroxyalkanoates (PHAs) are a class of biodegradable and biocompatible polyester thermoplastics that can be synthesized by various microbial strains under unbalanced growth conditions, such as the presence of excess carbon source and limitation of at least one essential nutrient (e.g. phosphorous, nitrogen or oxygen) (Anderson and Dawes, 1990; Chen, 2010). These polymeric chains are stored in the cytoplasm as granules and function as bacterial carbon and energy storage

* Corresponding author.
E-mail address: armandm@uidaho.edu (A.G. McDonald).

http://dx.doi.org/10.1016/j.biortech.2014.10.049
0960-8524/

materials (Anderson and Dawes, 1990). The most prevalent PHA is poly-3-hydroxybutyrate (PHB) and can be produced by many bacterial species.

Most of carbon sources used for PHA production are pure sources, such as pure carbohydrates (glucose, sucrose), alkanes and fatty acids. To reduce the production cost, inexpensive carbon sources such as industrial by-products including crude glycerol (Dobroth et al., 2011), cheese whey (Pais et al., 2009) or agricultural residues like sugar cane bagasse (Silva et al., 2004), sawdust or forest biomass (Keenan et al., 2006) have been used as substrates. This approach has the advantage of converting waste materials into value-added bioproducts. Optimally, the production of PHA should rely on using fewer raw materials as well as not compete with food based substrates (Queiros et al., 2014). Therefore, the use of lignocellulosic biomass would be a more suitable substrate and possibly cost effective for PHA production (Keenan et al., 2006).

Lignocellulosic materials mainly consist of cellulose, hemicellulose and lignin. Cellulose and hemicellulose are excellent carbon sources to be used in different biological processes after hydrolysis to fermentable sugars. Cellulose is a highly crystalline polymer of β-D-glucopyranose units. Hardwood hemicellulose is a branched polysaccharide that consists mainly of xylose and 4-*O*-methylglucuronic acid together with acetyl groups (Fengel and Wegener, 1984). Cellulose and hemicellulose are embedded in a complex lignin matrix which acts as a binder, impermeable and resistant to microbial attack. Therefore, a pretreatment process is needed to break down the crystalline structure and make the biomass more accessible to subsequent enzymatic hydrolysis. Hybrid poplar is a short rotation fast growing hardwood species with low lignin content and has been used as a good raw material for biofuel and other chemicals production (Kim et al., 2011). A hot-water pretreatment with controlled pH has been shown to improve enzymatic digestibility of hybrid poplar (Dai and McDonald, 2013). This approach involved an auto-catalyzed hydrothermal pretreatment process followed by a commercial cellulase treatment to afford sugars yields of 72% on original wood basis and was therefore used in this work to produce fermentable sugars for PHB production (Dai and McDonald, 2013). A recent report estimates the cost of producing cellulosic sugars at $0.26/kg (Lux Research, 2013). This price would likely decrease with the development of more efficient enzymes, improvements in enzyme recovery, use of wood residues and improved pretreatment processes.

Most of the technologies for microbial PHA production are not economically competitive with synthetic plastics production. The use of mixed microbial cultures (MMCs) has been gained a lot of interest to produce PHA with low costs due to lower sterility needs and lower equipment controls (Queiros et al., 2014). Moreover, MMCs can utilize a wide range of inexpensive substrates, including industrial and urban waste streams (Coats et al., 2011) and agro-forestry waste (Albuquerque et al., 2010). However, culture selection with a high PHA storage capacity is one of the challenges in PHA production process using mixed cultures. An aerobic dynamic feeding (ADF) regime, also called feast and famine (FF) process, is commonly designed to use mixed cultures for PHA production. ADF works under conditions in which growth is restricted by either an external nutrient or an internal factor. During this process, substrate uptake is mainly driven towards PHA accumulation, pre-empting growth. The absence of an external substrate for a considerable period of time causes a decrease in the amount of enzymes required for cell growth. Following such a starvation period, if the microbial culture is enriched with an excess of carbon source, the enzymes available in the cells is lower than that needed to reach maximum growth rate, thus the storage becomes the dominant phenomena (Salehizadeh and van Loosdrecht, 2004). Most studies have reported PHA production using MMCs in two separate stages, culture selection and PHA accumulation. Addition to ADF, nutrient depleted condition is used by both pure culture and MMCs PHA synthesis and many bacteria produce PHA under this condition (Anderson and Dawes, 1990). Therefore, it is necessary to study the appropriate substrate load, nitrogen and phosphorous concentrations and oxygen limitation of the bioreactors to understand what mechanism(s) drive PHA production under these systems.

PHA-storing populations should be evaluated by analyzing the microbial community in order to follow its evolution, identify the producer and determine their relative abundance (Queiros et al., 2014). Microbial characterization needs to be performed with different operational conditions, allowing for a better understanding of changes in the performance of MMCs that could vary with the same substrate feeding. Several studies have identified PHA-storing microorganisms. In these reports, most found organisms belong to *Alpha-proteobacteria*, *Beta-proteobacteria* and *Gamma-proteobacteria* classes and some of them were already identified at the genus level (Tanaka et al., 2011). According to these reports, the differences observed in the relative abundance of each genus could be explained based on the type of volatile fatty acids (VFAs) used (Lemos et al., 2008; Jiang et al., 2011). The genera *Amaricoccus*, *Thauera* and *Azoarcus* were found to be dominant in the bioreactors where acetic acid and propionic acid were used as substrates (Lemos et al., 2008). *Plasticicumulans acidivorans* and *Thauera selenatis* were observed in a selected MMCs system fed lactic acid (Jiang et al., 2011). Another work showed a phylogenetic profile of *Firmicutes* (71%) and *Proteobacteria* (28%) in a PHA accumulation and waste treatment reactor fed municipal waste water (Reddy and Mohan, 2012). Based on these findings, PHA-storing populations are highly selective on substrates. Therefore, it is necessary to study the microbial community in the sugar-MMC system.

To the author's knowledge, no studies have been found using both MMCs and wood hydrolysates for PHB production. The major objective for this study was to successfully synthesize PHB from wood hydrolysates by MMCs. A proposed PHB synthetic mechanism was explained through the optimization of production yield with varied operational conditions (organic loading rate and solid retention time (SRT)) achieved by a factorial design. The microbial community was identified at genus level with next-generation DNA sequencing to determine possible PHB-accumulating bacteria.

2. Methods

2.1. Culture preparation

MMC (activated sludge) was obtained from the aeration basin at a wastewater treatment plant (Moscow, ID, USA) and cultured for 20 h with aeration at room temperature in nutrient medium. The composition of the medium used for culture cultivation was (g/L): 2 g KH_2PO_4, 0.6 g Na_2HPO_4, 1 g $MgSO_4$, 0.1 g $CaCl_2$, 0.1 g $CaCO_3$, 1 g NH_4Cl, 2.5 g yeast extract, 5 g Bacto peptone, 10 g hybrid poplar enzymatic hydrolysate (30 g/L × 0.333 L), and 0.667 L Milli-Q water. Medium pH was adjusted to 7.0 before 1 L mixed seed was added. The initial mixed liquor suspended solids (MLSS) was 2.65 g/L, which was increased to 9.10 g/L after 20 h cultivation.

2.2. Carbon source

The hybrid poplar (50 g batches) hydrolysates were prepared from a hot-water pretreatment (at 200 °C, 22 min, 20% solid loading) in a 500 mL Parr Instruments model 4740 reactor followed by enzymatic hydrolysis (Novozymes Cellic CTec2, Novozymes North America Inc., NC, USA) as described by Dai and McDonald (2013). More specifically, enzymatic hydrolysis was conducted in a 2 L