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Substrate versatility of polyhydroxyalkanoate producing glycerol grown bacterial enrichment culture

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ABSTRACT

Waste-based polyhydroxyalkanoate (PHA) production by bacterial enrichments generally follows a three step strategy in which first the wastewater is converted into a volatile fatty acid rich stream that is subsequently used as substrate in a selector and biopolymer production units. In this work, a bacterial community with high biopolymer production capacity was enriched using glycerol, a non-fermented substrate. The substrate versatility and PHA production capacity of this community was studied using glucose, lactate, acetate and xylitol as substrate. Except for xylitol, very high PHA producing capacities were obtained. The PHA accumulation was comparable or even higher than with glycerol as substrate. This is the first study that established a high PHA content (≈70 wt%) with glucose as substrate in a microbial enrichment culture. The results presented in this study support the development of replacing pure culture based PHA production by bacterial enrichment cultures. A process where mixtures of substrates can be easily handled and the acidification step can potentially be avoided is described.

1. Introduction

Environmental biotechnology has been conventionally aiming at treating waste streams with the goal of removing harmful pollutants and generating clean water safe to be disposed in the environment. Recently, increasing interest for the recovery of natural resources that are present in waste streams developed a new research field defined by Kleerebezem and

List of Abbreviations: DO, Dissolved Oxygen; GC, Gas Chromatography; HRT, Hydraulic Retention Time; MCB, Mixed Culture Biotechnology; PG, Polyglucose; PHA, Polyhydroxyalkanoates; PHB, Polyhydroxybutyrate; SBR, Sequencing batch reactor; SRT, Solids Retention Time; VFA, Volatile Fatty Acids.

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van Loosdrecht (2007) as mixed culture biotechnology (MCB). MCB combines traditional environmental biotechnology, in terms of removing pollutants, with industrial biotechnology, aiming at obtaining value-added products.

A promising technology based on MCB principles is the production of polyhydroxyalkanoates (PHA) from agro-industrial wastewater. PHAs are biopolymers that many microorganisms can naturally produce with plastic-like properties (Sudesh et al., 2000). The current PHA production process at industrial scale is based on the use of pure cultures and pure substrates like glucose (Shen et al., 2009), resulting in very high prices that hamper the production of bioplastics as bulk materials. Since MCB combines the use of non-sterile processes with the use of waste as substrate, PHA production costs can be significantly reduced (Reis et al., 2003).

The waste-based biopolymer production scheme proposed previously relies on a three-step process: 1) an acidogenic reactor where the wastewater is converted into a volatile fatty acids (VFA) rich stream which will be used as substrate in the subsequent units; 2) an enrichment reactor where the PHA producing bacterial enrichment culture is established, and 3) an accumulation reactor, where the biopolymer content of the selected community is maximized. Based on the described configuration, extensive research has been developed using fermented wastewater as substrate (Albuquerque et al., 2007; Carucci et al., 2001; Dionisi et al., 2005; Jiang et al., 2012; Rhu et al., 2003; Serafim et al., 2008) but very little research has been conducted on the use of non-fermented substrates. This alternative would reduce production costs since no fermentation step would be needed. Moralejo-Gárate et al. (2011) and Dobroth et al. (2011) showed that glycerol is an excellent substrate for PHA production without the need of a fermentation step.

The three stage process makes use of the fermented stream obtained in the first step, as feed in the enrichment and the accumulation step. Herewith the bacterial community is enriched on the same substrate as used in the accumulation step. The effect of significantly changing the composition or the nature of the substrate between the enrichment and the accumulation step has been little explored. Only Morgan-Sagastume et al. (2014) studied the feasibility of PHA production using municipal wastewater, during the enrichment step, and fermented sludge, during the accumulation step, as substrates. Considering the intrinsic changing nature of wastewater, the effect of changes in substrate composition is very interesting for establishing a waste-based biopolymer production process.

In the present work these two new approaches have been studied: the feasibility of using different substrates (VFA, organic acid, polyols and carbohydrates) during biopolymer maximization using a microbial community enriched on a non-fermented substrate, glycerol. Acetate and lactate have been extensively used as substrate by bacterial enrichment cultures leading to promising results (Jiang et al., 2011; Johnson et al., 2009a; Serafim et al., 2004). The use of glucose as substrate has been limited to pure culture based PHA production. Xylitol was chosen in order to study the performance of the bacterial community when the number of atoms of the supplied polyol increases from 3 carbon atoms, glycerol, to 5 carbon atoms, xylitol.

The substrate spectrum of the enriched community was evaluated and the process robustness, when changes in the substrate composition were applied, was assessed.

2. Materials and methods

2.1. Sequencing batch reactor for biomass enrichment

The bacterial enrichment culture used for the batch accumulation experiments was selected and cultivated in a double jacket glass reactor with a working volume of 2 L (Applikon, The Netherlands) using glycerol as substrate operating in 6 h cycles under carbon limiting conditions (feast-famine regime). An amount of 1.42 g of glycerol, 0.361 g of NH_4Cl, 0.34 g of KH_2PO_4, 0.14 g $MgSO_4$ 7 H_2O, 0.05 g of KCl and trace elements were fed at the beginning of each cycle. Allylthiourea was used to prevent nitrification activity. The hydraulic (HRT) and solid (SRT) retention times were fixed at 48 h. The reactor temperature was controlled at 30 °C by means of a water jacket and a thermostat bath (Lauda, Germany). The pH of the reactor liquid was maintained at 7.0 ± 0.1 using 0.5 M NaOH and 0.5 M HCl, and during the whole cycle aeration and stirring were provided. A detailed description of the start-up and operation of the reactor can be found elsewhere (Moralejo-Gárate et al., 2013b).

2.2. Batch experiments for the determination of the maximum storage capacity

The maximum storage capacity of a bacterial enrichment culture selected using glycerol as substrate was tested in different batch accumulation experiments using glycerol, glucose, acetate, lactate and xylitol as substrate. Prior to starting the batch accumulation experiments, the enrichment reactor was operated under steady state conditions for 6 months, ensuring a stable microbial community composition and reproducibility of the batch assays. Each substrate was tested in duplicate. The experimental procedure of the present work is shown in Fig. 1. The biomass used for the accumulation experiments (750 mL) was collected fresh at the end of the operational cycle of the enrichment reactor, without allowing the biomass to settle, and excess of carbon source was dosed: between 20 and 25 g of each substrate were used diluted in 250 mL of demiwater. Biomass concentration at the beginning of the accumulation experiments was 22.5 ± 2.5 Cmmol/L. Glycerol, ≥99.5 wt% (CAS 56-81-5), glucose, ≥99.5 wt% (CAS 50-99-15), sodium DL-lactate, 60w% (CAS 72-17-3), sodium acetate trihydrated, ≥99 wt% (CAS 6131-90-4) and xylitol (CAS 87-99-0) were used to prepare the feed for the accumulation assays. No ammonium was added in the feeding to prevent growth and maximize the conversion of substrate into storage polymer.

A double jacket glass reactor with a working volume of 2 L (Applikon, The Netherlands) was used. Throughout the whole biopolymer production assay, the medium was stirred and aerated. Mixing was provided by two standard geometry six-blade turbines (Applikon, The Netherlands). The air flow rate was maintained at 0.1 L/min using a mass flow controller (Brooks Instrumental, U.S.A.). The actual flow through the