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Impact of oxygen limitation on glycerol-based biopolymer production by bacterial enrichments

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ABSTRACT

The increasing production of bioethanol and biodiesel has resulted in the generation of a massive amount of crude glycerol, inducing the need for effective valorization of these waste streams. One of the valorization options could be through conversion of crude glycerol into a biopolymer using microbial community engineering in a feast–famine process. A complicating factor in the production of biopolymers from glycerol encountered in previous works is that two different types of polymers can be formed; polyhydroxyalkanoate (PHA) and polyglucose. Here we describe the effect of limiting the oxygen supply rate on the polymer distribution with the aim of defining the conditions that favour the conversion of glycerol in one single polymer. The decrease of oxygen supply rate during the biopolymer maximization step did not influence glycerol partitioning among PHA and polyglucose, but oxygen limitation during the community enrichment step favoured polyglucose storage over PHA.

1. Introduction

Polyhydroxyalkanoates (PHA) are polyesters of various hydroxyalkanoates that can be accumulated by numerous bacteria as carbon and energy reserve. The monomeric units of PHA are enantiomerically pure and can be used as chiral starting materials in fine chemical, pharmaceutical and medical industries (Ren et al., 2005). Moreover, PHAs have properties similar to those of some polyolefins. Combined with the fact that upon disposal they are fully biodegradable, PHAs have generated industrial interest for application as substitutes to petroleum-based polymers in many fields (Mergaert et al., 1992). The prevailing technology for PHA production is through the use of pure bacterial cultures utilizing a pure concentrated substrate such as glucose or propionate. Although currently being produced commercially,

Abbreviations: ADP, adenosine diphosphate; ATU, allylthiourea; ATP, adenosine triphosphate; CO_2, carbon dioxide; DHAP, dihydroxyacetone phosphate; DO, dissolved oxygen; F/F, feast/famine; FIA, flow injection analysis; FISH, fluorescence in situ hybridization; GC, gas chromatography; G3P, glyceraldehyde 3-phosphate; k_La, volumetric oxygen transfer coefficient; HPLC, high performance liquid chromatography; NAD^+/NADH, nicotinamide adenine dinucleotide; PHA, polyhydroxyalkanoate; PHB, polyhydroxybutyrate; PG, polyglucose; SBR, sequencing batch reactor; TSS, total suspended solids; VFA, volatile fatty acids.

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the product is more expensive than fossil oil based polymers and therefore is still mostly regarded as a speciality rather than a commodity polymer (Gurieff and Lant, 2007).

PHA production by microbial community engineering has become a trending research topic in recent years obtaining very promising results. Several studies have shown that the application of a dynamic feeding regime allows for enrichment of a community with a PHA production capacity that is comparable to axenic cultures (Johnson et al., 2009a; Jiang et al., 2011). Compared to pure bacterial cultures, bacterial enrichments can make use of lower quality substrates, as well as cope with variations in substrate composition. Substrate is one of the main cost factors associated to PHA production, Moreover, by implementing the use of bacterial enrichments, the need for sterile operation is prevented resulting in a decrease of overall production costs (Reis et al., 2003). Volatile fatty acids (VFA) have been the preferred substrate for PHA production by bacterial enrichments.

In biodiesel production large amounts of glycerol containing wastewater is generated. According to the European Biodiesel Board 9570 million litres of biodiesel were produced in 2010, corresponding to a crude glycerol production of 10% of this value (Thompson and He, 2006). Crude glycerol potentially is a good substrate for PHA production. Moralejo-Gárate et al. (2011) reported that glycerol indeed can be converted into PHA although also polyglucose (PG) was produced.

The influence of different reactor operational parameters on the PHA production process by bacterial enrichments has been investigated before. Dias et al. (2006) reviewed several studies investigating effects of the substrate concentration, organic loading rate, carbon to nitrogen ratio, sludge retention time, temperature, pH and the dissolved oxygen (DO) concentration. Despite its importance in the scale-up and economy of aerobic biosynthesis systems, the number of studies dealing with impact of oxygen is scarce.

The work described in this paper was based on the hypothesis that the DO concentration can have a major impact on the type of storage polymer produced, i.e. a polymer of glucose, PG, or PHA. Analysis of the metabolic pathways involved in the synthesis of both biopolymers showed that the PG production pathway requires less oxygen respiration than PHA production. This provides a competitive advantage to PG storage in an oxygen limited environment. Based on this hypothesis we experimentally investigated the impact of the DO concentration on the product spectrum of a glycerol grown PHA producing bacterial enrichment. The effect of the DO concentration was studied on the maximal PHA storage capacity of a previously established community and during the enrichment of a PHA producing microbial community on glycerol in a feast famine regime.

2. Materials and methods

2.1. Sequencing batch reactor for biomass enrichment

Two identical double jacket glass reactors with a working volume of 2 L (Applikon, The Netherlands) were used for the enrichment of two different microbial communities. Both communities were enriched on glycerol as sole carbon and energy source in a sequencing batch reactor (SBR). Operation of the reactors was based on a 24 h cycle according to the following scheme (time in minutes): i. Feeding phase (0–12) in which 1000 mL of fresh medium was supplied to the reactor, ii. Reaction phase (12–1418), iii. Effluent withdrawal phase (1418–1430) in which 1000 mL of reactor medium was removed, iv. Idle phase (1430–1440). Throughout the entire operational cycle, mixing was achieved by a standard geometry six-blade turbines (Applikon, The Netherlands) operated at 350–400 rpm. The end of one batch was immediately followed by the start phase of the next batch, resulting in a hydraulic and solid retention times (HRT and SRT) of 48 h. The reactors were operated as an open (non sterile) system. The temperature was maintained at 30 °C and the pH was controlled at 7 by means of the addition of NaOH (0.5 M) and HCl (0.5 M).

Two different bacterial enrichments were developed by means of the feast–famine regime: named high DO and low DO. Identical operational parameters were applied in both reactors except for the air supply set up. The air flow supply was maintained at 1 L/min using a mass flow controller (Brooks Instrumental, U.S.A.). In the high DO reactor, an air diffuser was used to sparge small bubbles throughout the reactor broth. In order to achieve oxygen limiting conditions in the low DO reactor, air was supplied through the headspace of the reactor. Consequently, no bubbles were present in the liquid, the interfacial area for oxygen transfer was restricted to the gas–liquid surface contact area and mass transfer was compromised. For the high DO, the volumetric mass transfer coefficient, k_La, was 45.6 h^{-1}, and for the low DO, k_La was 9.5 h^{-1}.

The high DO reactor had been inoculated with activated sludge from the municipal wastewater treatment plant of Dokhaven (Rotterdam, The Netherlands) and continuously operated for 12 months. A detailed description of this reactor start-up and performance can be found elsewhere (Moralejo-Gárate et al., 2011). In order to ensure the continuity of the high DO enrichment and to ensure a source of microbial diversity, the low DO reactor was inoculated with a mixture composed by 10% of sludge from the municipal wastewater treatment plant of Dokhaven, (Rotterdam, The Netherlands), and 90% of biomass from the high DO reactor and operated during 3 months previously to the beginning of this research.

Feeding medium composition contained 14.19 g/L of glycerol (154 mM). The composition of the nutrients solution was NH_4Cl 3.61 g/L, KH_2PO_4 3.39 g/L, $MgSO_4 \cdot 7H_2O$ 1.37 g/L, KCl 0.53 g/L and 15 mL/L of trace element solution (Vishniac and Santer, 1957). After sterilization of the mineral medium, 1.5 mL/L of a 33 g/L allylthiourea solution (ATU) was added to prevent nitrification activity. In the influent phase of each batch cycle, 100 mL of organic carbon solution, 100 mL of nutrients solution, and 800 mL of dilution water were dosed to the reactor. The reactors were cleaned about once per week to prevent excessive biofilm formation on the glass walls, metal parts and electrodes.

2.2. Estimation of the volumetric oxygen transfer coefficient (k_La)

The volumetric oxygen transfer coefficient was used to compare the aeration efficiency between the two enrichment reactors and in the different accumulation experiments. All