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Crude glycerol as feedstock for polyhydroxyalkanoates production by mixed microbial cultures

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ABSTRACT

The increase in global biodiesel production makes imperative the development of sustainable processes for the use of its main by-product, crude glycerol. In this study the feasibility of polyhydroxyalkanoates (PHA) production by a mixed microbial community using crude glycerol as feedstock was investigated. The selected culture had the ability to consume both glycerol and methanol fraction present in the crude. However, glycerol seemed to be the only carbon source contributing for the two biopolymers stored: poly-3-hydroxybutyrate (PHB) and glucose biopolymer (GB). In this work the culture reached a maximum PHB content of 47% (cdw) and a productivity of 0.27 g X/L.d, with an aerobic mixed cultures and a real waste substrate with non-volatile fatty acids (VFA) organic matter. The overall PHA yield on total substrate obtained was in the middle range of those reported in literature. The fact that crude glycerol can be used to produce PHA without any pre-treatment step, makes the overall production process economically more competitive, reducing polymer final cost.

1. Introduction

Due to the prospects of replacing fossil fuels, biodiesel production has continuously grown in the last decade. As a consequence, biodiesel industries are facing a surplus of its main byproduct, glycerol, which represents 10% (v/v) of the final ester. Supported by governments to increase energy independence and meet the rising energy demand, the biodiesel market is expected to reach 37 billion gallons by 2016, an average growth of 42% per year. This will result in a production of 4 billion gallons of crude glycerol that year, saturating the glycerol market (Quispe et al., 2013).

Industrial application of crude glycerol in food, pharmaceutical and cosmetics industries, its main markets, requires a costly refining process in order to achieve a necessary high purity. In the last years many research projects have been conducted with the aim of finding a new utilization for raw glycerol. In addition to new applications in the food industry, polymer industry, glycerol has also been considered as a feedstock for new industrial fermentations (Yang et al., 2012). Particularly attractive is the microbial conversion of raw glycerol into 1,3-propanediol (Hiremath et al., 2011), H_2 and ethanol (Ito et al., 2005) and citric acid (Papanikolaou and Aggelis, 2003). Equally interesting could be the conversion of the glycerol into polyhydroxyalkanoates (PHAs).

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Abbreviation

ADF	aerobic dynamic feeding
cdw	cell dry weight
COD	chemical oxygen demand
DO	dissolved oxygen
EPS	exopolymeric substances
F/F	feast/famine
F/M	food to microorganism
FFA	free fatty acid
FAME	fatty acid methyl esters
GB	glucose biopolymer
GC	gas chromatography
HPLC	high performance liquid chromatography
HRT	hydraulic retention time
MMC	mixed microbial culture
OUR	oxygen uptake rate
PHA	polyhydroxyalkanoate
PHB	polyhydroxybutyrate
SBR	sequencing batch reactor
SRT	sludge retention time
TOC	total organic carbon
TSS	total suspended solids
VFA	volatile fatty acids
VSS	volatile suspended solids.

PHAs are biodegradable polyesters with market capacity to replace some of the more commonly used elastomeric/thermo plastics. These biopolymers are naturally synthesized and stored inside the cells by several microbial species. With the rising financial investments made into production and marketing of bioplastics, PHAs prices have been reduced in the last years. However, commercialization of bacterial PHA is still restricted to the use of pure cultures fermentations and high cost synthetic substrates making their price, in average, two times higher than conventional plastics (i.e. PVC) (Chanprateep, 2010). In recent years, research has focused on the development of alternative PHA production processes, including the use of waste/surplus based feedstocks and mixed microbial cultures (MMC). This approach permits for a lower investment and operating costs for the global process (Albuquerque et al., 2007; Bengtsson et al., 2008a,b). The main problems associated with those strategies are the lower PHA content and the lower volumetric productivities achieved when compared with the ones reported for pure culture and synthetic substrates. A critical step in this strategy is the selection of a stable culture with a high PHA storage capacity. This can be achieved by subjecting microbial cultures to alternate periods of short carbon availability followed by a long unavailability, designated as aerobic dynamic feeding (ADF, also known as feast/famine). Using this approach Jiang et al. (2012) obtained a PHA content of 77% (cdw) with MMC and fermented paper mill wastewater. These results make the gap between the PHA production using pure cultures/synthetic substrates (88% of cdw) (Lee et al., 1999) and MMC/complex feedstock considerably narrowed.

Most of the study that reported the use glycerol to produce PHAs used pure cultures and observed that only the PHA homopolymer, poly-3-hydroxybutyrate (PHB) was stored. Recent studies (Moralejo-Gárate et al., 2011; Dobroth et al., 2011) have explored the use of MMC to produce PHB from glycerol. This strategy represents an opportunity to further decrease the process environmental footprint, primarily due to reduced energy usage associated with the absence of aseptic conditions. Moralejo-Gárate et al. (2011) proved the feasibility of glycerol-based PHA production by an MMC where the enriched mixed community achieved a PHA content up to 80% of cdw (0.40 g PHB/g glycerol). Dobroth et al. (2011) was the only study until now that enriched a stable mixed culture using crude glycerol. However, although the authors reported the enrichment of an MMC with an intrinsic high PHB content (62% cdw) the selected culture uses exclusively the methanol fraction of the crude glycerol to produced PHB with a low polymer yield on substrate (0.10 g PHB/g methanol).

The aim of this work was to investigate and demonstrate the feasibility of PHB production by a mixed microbial community using crude glycerol as substrate. A two-step process was used, comprising (1) selection of a PHA-accumulating culture under ADF conditions, and (2) batch PHA accumulation using the selected culture. The impact of the synthetic substrates versus crude glycerol on the PHB storage was study. Also the storage capacity of the selected culture using crude and synthetic glycerol under different feeding strategies was investigated. To the best of our knowledge this was the first study that shows the valorisation of crude glycerol into PHAs using an aerobic mixed microbial consortium.

2. Material and methods

2.1. Crude glycerol composition

The crude glycerol used in this study was obtained from an industrial biodiesel manufacturing plant (Sovena) in Portugal. Multiple vegetable oil sources are used by this industry to produce the biodiesel. The crude glycerol was removed after the bio-diesel production and before any purification step. This fraction was mainly composed by glycerol (71.66%, g C/g TOC) and methanol (25.69%, g C/g TOC). Crude glycerol also contained a small fraction (2.58% w/w) of free fatty acids and fatty acids methyl esters (FFA/FAME).

2.2. PHA-accumulation culture enrichment

The PHA-accumulating culture enrichment on crude glycerol was conducted in a sequencing batch reactor (SBR) with a working volume of 1500 ml. The reactor was inoculated with a PHA-accumulating mixed culture acclimatized to bio-oil as feedstock (Moita el al., 2013). The SBR was operated under ADF conditions. Each SBR cycle (24 h) consisted of four periods: fill (15 min); aerobiosis (23 h); settling (20 min) and withdraw (15 min). HRT was kept at 2 days. A peristaltic pump was calibrated to purge mixed liquor (300 ml) at the end of the aeration period in order to keep SRT at 5 days.

At the beginning of each cycle the reactor was fed with 30 CmM of crude glycerol. A mineral nutrients solution was added separately to the reactor that included nitrogen and phosphorus source (NH_4Cl and KH_2PO_4/Na_2HPO_4) to keep the