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Microbial bio-based plastics from olive-mill wastewater: Generation and properties of polyhydroxyalkanoates from mixed cultures in a two-stage pilot scale system

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ABSTRACT

The operational efficiency of a two stage pilot scale system for polyhydroxyalkanoates (PHAs) production from three phase olive oil mill wastewater (OMW) was investigated in this study. A mixed anaerobic, acidogenic culture derived from a municipal wastewater treatment plant, was used in the first stage, aiming to the acidification of OMW. The effluent of the first bioreactor that was operated in continuous mode, was collected in a sedimentation tank in which partial removal of the suspended solids was taking place, and was then forwarded to an aerobic reactor, operated in sequential batch mode under nutrient limitation. In the second stage an enriched culture of *Pseudomonas* sp. was used as initial inoculum for the production of PHAs from the acidified waste. Clarification of the acidified waste, using aluminium sulphate which causes flocculation and precipitation of solids, was also performed, and its effect on the composition of the acidified waste as well as on the yields and properties of PHAs was investigated. It was shown that clarification had no significant qualitative or quantitative effect on the primary carbon sources, i.e. short chain fatty acids and residual sugars, but only on the values of total suspended solids and total chemical oxygen demand of the acidified waste. The type and thermal characteristics of the produced PHAs were also similar for both types of feed. However the clarification of the waste seemed to have a positive impact on final PHAs yield, measured as g PHAs/100 g of VSS, which reached up to 25%. Analysis of the final products via nuclear magnetic resonance spectroscopy revealed the existence of 3-hydroxybutyrate (3HB) and 3-hydroxyoctanoate (HO) units, leading to the conclusion that the polymer could be either a blend of P3HB and P3HO homopolymers or/and the 3HB-co-3HO co-polymer, an unusual polymer occurring in nature with advanced properties.

1. Introduction

The rising environmental concerns due to overproduction and accumulation of petrochemical plastics, has lately turned research efforts toward the production of biodegradable plastics from renewable sources. Among them, great interest has been shown on the production of microbial polyhydroxyalkanoates (PHAs), which have similar properties to petroleum-derived synthetic polymers such as PVC and PP, but they are also biodegradable, biocompatible, water resistant, and oxygen impermeable. Thus, PHAs can be used in a variety of disposable packaging goods and may also have high-value applications in medicine and the pharmaceutical industry (Chen, 2009). PHAs are linear polyesters that are intracellularly

Abbreviations: OMW, olive mill wastewater; PHAs, polyhydroxyalkanoates; PVC, polyvinyl chloride; PP, polypropylene; HRT, hydraulic retention time; TSS, total suspended solids; VSS, volatile suspended solids; SCFAs, short chain fatty acids; GPC, gel permeation chromatography; NMR, nuclear magnetic resonance; DSC, differential scanning calorimetry; CSTR, continuous stirred tank reactor; SBR, sequential batch reactor; d-COD, dissolved chemical oxygen demand; T-COD, total chemical oxygen demand; TGA, thermogravimetric analysis; DTG, derivative traces; M_w, weight average molecular mass; M_n, number average molecular mass; PDI, polydispersity index; SBR, sequential batch reactor; HB, hydroxybutyrate; HO, hydroxyoctanoate.

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accumulated by various microbial species under stress conditions, caused by limitation of a nutrient, electron donor or acceptor (Anderson and Dawes, 1990) and serving as carbon and energy reserves (Lee, 1996). Numerous studies have been so far published based on either pure (Munoz and Riley, 2008; Hofer et al., 2011; Muhr et al., 2013), or mixed microbial cultures (Dionisi et al., 2004; Albuquerque et al., 2011) and parameters such as nutrient limitation, type of carbon sources, C/N or C/P ratios, thermal properties, mechanical properties, molecular masses, implicated enzymatic and metabolic mechanisms, possible application, etc. have been extensively investigated (Lee et al., 1999; Dias et al., 2006; Grage et al., 2009). However, most of them, focus on the process, the final product properties and the effect of carbon source used, whereas the origin of the carbon source used for their production is less studied (Muhr et al., 2013; Nikodinovic-Runic et al., 2013).

Lately, scientists have made a great effort toward finding ways for the effective exploitation of wastes and wastewaters, i.e. zero cost carbon sources, the management of which would be necessary anyhow before their disposal to the environment. Among them olive-mill wastewater (OMW) represents a quite abundant waste (Azbar et al., 2004) rich in carbohydrates and lipids, the management of which has been puzzling olive oil producers for years. OMW is actually the liquid by-product generated during the production of olive oil by a three-phase oil extraction method. Especially in the countries of the Mediterranean basin, where above 90% of the annual olive oil world production is produced, OMW represents a large environmental risk. This is due to the fact that OMW contains insoluble, i.e. suspended particles and lipids and also soluble organic substances such as sugars, polyalcohols, aminoacids and polyphenols that contribute to the extremely high organic load of the waste which, given its toxic effect to microorganisms, plants (Azbar et al., 2004) and marine organisms (Danellakis et al., 2011) makes its safe disposal a huge environmental concern.

Many researchers have investigated a number of physicochemical and biological treatment methods for reducing both the organic load and OMW valorization as well (Dionisi et al., 2005; Ena et al., 2012; Bellou et al., 2014). Although OMW is a waste of plant origin, it seems to be advantageous as a potential substrate for bioconversions over other types of biomass derived feedstocks, since it contains high amounts of readily consumable carbon sources. Moreover, OMW does not seem to require a pretreatment step prior to its use; provided of course that the microorganisms selected are not inhibited by the presence of phenolics. Regarding the bioconversion of OMW toward PHAs, there are only few studies so far published. Gonzalez-Lopez et al. (1996), Pozo et al. (2002) and Cerrone et al. (2010) have investigated the direct bioconversion of two phase olive mill effluents (olive pomace) toward PHAs using pure cultures of *Azotobacter* sp., whereas acidified olive pomace has also been tested as substrate for PHAs production via mixed cultures by Waller et al. (2012). Dionisi et al. (2005), Beccari et al. (2009) and Ntaikou et al. (2009) have focused on the exploitation of OMW for PHAs production via mixed microbial consortia. Specifically, microbial mixed cultures were previously acclimated and enriched in PHAs producers, whereas acidification of the waste had preceded either in continuous (Dionisi et al., 2005; Ntaikou et al., 2009) or sequential batch reactors (Beccari et al., 2009). The acidification step prior to PHAs production has also been proposed for other type of sugar and/or lignocellulosic based wastes (Salmiati Ujang et al., 2007; Bengtsson et al., 2010a, 2010b; Albuquerque et al., 2011), aiming mainly to the bioconversion of the available carbon sources toward mixed short chain fatty acids with both even and odd carbon numbers. Indeed, it has been suggested that when solely *n*-alkanoates are present in the culture medium, P(3HB) is generated, whereas the simultaneous uptake of *n*-alkanoates with odd carbon numbers leads to the production of copolymers with advanced properties, such as P(3HB-co-3HV) (Akiyama et al., 1992).

Although bioconversions by pure microbial cultures can lead to higher productivities and yields, the selection of mixed cultures instead of pure strains is considered by many researchers as favorable for full scale application, at least from the engineering standpoint. Indeed, when using mixed cultures control and operation of the process is facilitated since neither sterilization of the cultures media is required, nor preservation of aseptic conditions during operation of the reactor; thus overall cost of the process is reduced. Especially in the case of PHAs producing consortia, the enrichment of an initial broader consortium derived from a natural source is achieved by subjecting it to some kind of nutrient limitation under periodic feeding. By the keeping the culture conditions and carbon source stable, the consortium remains stable and high productivities can be achieved (Dias et al., 2006). Moreover, the use of such mixed/enriched cultures allows for a broader choice of feedstocks selection, since the consortium tends gradually to alter by adjusting to the carbon source provided each time. Moreover studies based on Life Cycle Analysis have revealed that PHA production using mixed cultures may be more favorable than using pure cultures in both economic and environmental terms (Gurieff and Lant, 2007).

Based on mixed cultures, Ntaikou et al. (2009) have also studied the combined biohydrogen production from OMW during the acidification phase, in order to achieve energy recovery from the waste. The overall process consisted of the acidification of the OMW via anaerobic fermentation with simultaneous hydrogen production, followed by further exploitation of the produced acids for biopolymers production using acclimated mixed consortia of *Pseudomonas* sp. (Kourmentza et al., 2009).

The aim of the present study was to investigate the operational efficiency of a two stage pilot scale system for PHAs production using OMW as substrate and also to assess the impact of the suspended solids removal method via pretreatment with aluminium sulphate (alum) on the production of final yields and properties of the final product. Alum has been widely used as coagulant for water purification (Duan and Gregory, 2003) and municipal wastewater treatment (Guida and Mattei, 2007) via coagulation–flocculation process, resulting to significant removal of total solids and total organic load. Alum was expected thus to facilitate clarification of the acidified OMW, enhancing PHAs production process and reducing the cost of PHAs recovery process. To our knowledge, it is the first time that OMW is assessed as feedstock for the production of polyhydroxyalkanoates at pilot scale, and that the extracted polymers are characterized not only in terms of final yield and composition but also in terms of purity, thermal transition temperatures and molecular masses. Thus, important information about the properties of the PHAs that can be generated from OMW is provided, and significant conclusions concerning its possible applications could be derived. Indeed, the various analyses currently performed could indicate that the produced PHA consists from both scl- and mcl-units, 3HB and 3HO, being either a blend of P3HB and P3HO homopolymers or/and the 3HB-co-3HO copolymer, an unusual polymer occurring in nature with advanced properties.

2. Materials and methods

2.1. Wastewater

Olive-mill wastewater (OMW) that was used in the present study was obtained by a three-phase continuous centrifuge olive processing plant. OMW after its collection from the olive mill was transferred to the laboratory for characterization and then it was stored at 4 °C, in batches, until used. The physicochemical characteristics of the OMW were as follows: pH 5.5; total COD,