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Effect of the organic loading rate on the production of polyhydroxyalkanoates in a multi-stage process aimed at the valorization of olive oil mill wastewater

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ABSTRACT

Mixed microbial culture polyhydroxyalkanoates (PHA) production has been investigated by using olive oil mill wastewater (OMW) as no-cost feedstock in a multi-stage process, also involving phenols removal and recovery. The selection of PHA-storing microorganisms occurred in a sequencing batch reactor (SBR), fed with dephenolized and fermented OMW and operated at different organic loading rates (OLR), ranging from 2.40 to 8.40 gCOD/L d. The optimal operating condition was observed at an OLR of 4.70 gCOD/L d, which showed the highest values of storage rate and yield (339 ± 48 mgCOD/gCOD h and 0.56 ± 0.05 COD/COD, respectively). The OLR applied to the SBR largely affected the performance of the PHA-accumulating reactor, which was fed through multiple pulsed additions of pretreated OMW. From an overall mass balance, involving all the stages of the process, an abatement of about 85% of the OMW initial COD (chemical oxygen demand) was estimated whereas the conversion of the influent COD into PHA was about 10% (or 22% by taking into account only the COD contained in the pretreated OMW, which is directly fed to the PHA production stages). Overall, polymer volumetric productivity (calculated from the combination of both the SBR and the accumulation reactor) accounted for 1.50 gPHA/L d.

1. Introduction

Conventional plastics derive from limited fossil resources and represent a serious environmental concern due to their high recalcitrance to biological degradation. Polyhydroxyalkanoates (PHAs) are among the most promising candidates as substitutes for synthetic polymers. Indeed, PHAs are biologically synthesized polyesters completely biodegradable to water and carbon dioxide and can be produced from renewable resources [1,2]. At industrial scale, PHA production occurs through microbial fermentation processes of pure cultures of selected strains (e.g. *Cupriavidus necator*) grown on ad hoc designed unbalanced medium [3,4] and there is a major challenge to reduce the high cost of production. Due to the large impact of maintenance of sterile conditions and substrate formulation on production cost [5], an interesting approach to make PHA bio-plastic economically competitive with petroleum-based plastics could be the use of mixed microbial cultures (MMCs) and low-cost feedstock. Therefore, the development of mixed culture processes for PHA production from agro-industrial waste effluents has currently garnered considerable attention from researchers [6–9]. These processes, moreover, bring the huge advantage of simultaneously reducing the polluting load of the waste stream. In this context, it is particularly interesting the possibility of using olive oil mill wastewater (OMW) as no-cost substrate for the MMC-based PHA production process [10,11]. OMW is the liquid waste deriving from the extraction process of olive oil, whose production is mainly concentrated in the Mediterranean area (over 90%) [12], and its disposal represents a critical environmental problem not only due to the high levels of production but also to its specific chemical characteristics. Indeed, OMW is dark coloured, with acid pH and characteristic smell, and in spite of a large organic load (50–200 g/L measured as chemical oxygen demand, COD) it is toxic to bacteria for the presence of relevant amounts of polyphenols (up to 10 g/L) [13] and direct biological treatment is not possible [14]. To overcome these drawbacks, several physicochemical and chemical methods for OMW treatment have been proposed,

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such as lagooning or direct watering on fields, land disposal, co-composting, chemical treatment, adsorption [15]. However, a single treatment is not sufficient to meet the environmental discharge criteria and new treatment and valorization technologies are being devised [16,17]. Based on these considerations, an integrated physicochemical–biotechnological multi-stage process for the valorization of OMW towards polyphenols recovery and PHA production is hereby proposed. The process aims at reducing the intrinsic wastewater environmental toxicity while simultaneously obtaining added value compounds. Indeed, although polyphenols are known to exert acute toxicity towards plants and microorganisms when concentrated to such extent [18,19], they are natural antioxidants with a potential application in several industrial sectors (e.g. cosmetic, food preservation, and medicine) [20].

The initial stages of the proposed process focus on OMW pretreatment, which consists of polyphenols removal and recovery followed by an acidogenic fermentation in order to maximize the organic OMW content in volatile fatty acids (VFA), which are the most direct substrates for PHA production. The pretreated OMW is then fed to the PHA-producing reactors consisting of a sequencing batch reactor (SBR) operated under *feast and famine* (FF) conditions, in which an activated sludge is enriched in PHA-storing microorganisms, and a PHA accumulation reactor. Finally, after a solid liquid separation step, the liquid fraction containing the residual COD is sent to treatment and disposal and the solid fraction to PHA recovery. The operation of OMW pretreatment stages has been widely described in a previous paper [21] and this study mainly focuses on the stages directly involved in PHA production. The latter is largely affected by the selective pressure for PHA-storing microorganisms which is, in turn, established by the FF ratio which can be changed by varying the organic loading rate (OLR) applied to the SBR [7]. In general, the OLR plays a key role on process performance since an increase in the OLR results, in principle, in an increase of biomass concentration in the SBR. A main challenge is to develop operating strategies which allow to maintain a good selective pressure while operating at high OLR and short sludge retention time (SRT) (i.e., high growth rate). In order to obtain both a high OLR and a short SRT (1 d), here the SBR was operated without settling with the SRT corresponding to the hydraulic retention time. Indeed, the selection of microbial cultures with both a high growth rate and storage capacity would allow the SBR to be operated at a high cell density with a consequent more concentrated biomass inoculum to be sent to the subsequent accumulation reactor. Based on these considerations, in a previous study the effect of the applied OLR has been investigated with a synthetic mixture of volatile fatty acids in a range of values from 8.50 to 31.25 gCOD/L d [22]. In this research, a lower range of applied OLR (between 2.40 and 8.40 gCOD/L d) has been examined due to possible inhibitory effects of olive oil mill wastewater on microbial activity.

2. Material and methods

2.1. OMW treatment and characterization

The wastewater employed in this research was free taken from the Sant'Agata d'Oneglia (Imperia, Italy) three phase olive mill [21]. Prior to being used as feedstock of the PHA production process, OMW was treated in order to remove and recover polyphenols. The treatment consisted of a solid phase extraction by employing Amberlite XAD16 resin as adsorbing agent and acidified ethanol (0.5% 0.1 M HCl, v/v) as desorbing solvent, as described elsewhere [23]. Thereafter, the dephenolized wastewater was fed to a mesophilic anaerobic acidogenic packed-bed biofilm reactor for the bioconversion of the organic leftover into volatile fatty acids (VFA) [21,24]. Two samples of pretreated OMW (referred to as $fOMW_1$ and $fOMW_2$, respectively) have been used throughout the experimentation. Both samples have been characterized in terms of total and soluble COD, VFA composition and amount, nutrient composition (i.e., N and P).

2.2. Sequencing batch reactor (SBR): The experimental setup

The selection and enrichment of PHA-storing mixed microbial cultures was studied in a lab-scale SBR (1 L working volume) inoculated with an activated sludge from the "Roma Nord" (Italy) full scale municipal treatment plant (780,000 p.e.; 354,000 m^3/d) and fed with dephenolized and fermented OMW at a flow rate of 1 L/d. The OMW was diluted in mineral medium in order to obtain a final applied organic load rate (OLR) of 2.40, 4.70, 6.30, and 8.40 gCOD/L d (referred to the soluble COD of the fOMW), all the other operating conditions being the same. More in detail, $fOMW_1$ was used at 2.40 and 4.70 gCOD/L d and $fOMW_2$ at the higher investigated OLRs. Each run lasted at least 30 days. The mineral medium composition was as follows (mg/L): $(NH_4)_2SO_4$ (1500), K_2HPO_4 (334), KH_2PO_4 (259), $CaCl_2{\cdot}2H_2O$ (50), $MgSO_4{\cdot}7H_2O$ (100), $FeCl_3{\cdot}6H_2O$ (2), Na_2EDTA (3), $ZnSO_4{\cdot}7H_2O$ (0.1), $MnCl_2{\cdot}4H_2O$ (0.03), H_3BO_3 (0.3), $CoCl_2{\cdot}6H_2O$ (0.2), $NiCl_2{\cdot}6H_2O$ (0.02), $CuCl_2{\cdot}2H_2O$ (0.01), $NaMoO_4{\cdot}2H_2O$ (0.03), Thiourea (20). The feed was kept in a refrigerated vessel at 4 °C. According to a previous study on synthetic feeding [25], the length of the SBR cycle was imposed at 6 h, accounting for four cycles per day. Each cycle consisted of an initial feed phase of 10 min (0.25 L/cycle, corresponding to 1 L/d), a reaction phase of 338 min, a withdrawal phase of the mixed liquor of 2 min (0.25 L/cycle corresponding to 1 L/d), and a final phase of 10 min during which the reactor was kept stirred and aerated prior to the beginning of the following cycle. The fraction of the SBR characterized by the presence of readily biodegradable COD (rbCOD) was regarded as the "feast" phase, whereas the remainder of the cycle, characterized for the presence of slowly biodegradable COD (sbCOD), was regarded as the "famine" phase. During the withdrawal phase, the mixed liquor was removed from the SBR and directly sent to the accumulation reactor. No settling phase was performed and all the excess biomass was discharged with the mixed liquor (i.e. the solid retention time, 1 day, corresponded to the hydraulic retention time). During the overall cycle, the SBR was aerated by means of membrane compressors and stirred by a mechanical impeller to obtain an oxygen transfer coefficient ($K_La \approx 0.3\ min^{-1}$) able to guarantee a non-oxygen-limiting concentration (>2 mg/L). The temperature was maintained at 25 °C by means of a thermostatic jacket and the pH was automatically controlled at 7.6 by intermittent CO_2 flushing from a compressed gas cylinder. The SBR was automatically controlled by a software which allowed to manage each phase of the reactor cycle and to continuously record and monitor the values of pH and dissolved oxygen (DO) concentration. The DO profile allowed to individuate the depletion of the rbCOD in each cycle (corresponding to the end of the feast phase), which was detected by a sharp increase of the DO level.

The SBR was daily monitored through the determination of suspended solids (SS), PHA, VFA and soluble COD in correspondence to both the end of the feast phase and the end of the cycle. Kinetic tests were also performed at each investigated OLR, in order to gain a deeper understanding of the reactor performance. During kinetic tests, the reactor was sampled at the beginning of the cycle, at the end of the feed phase, in correspondence to the end of the feast phase and at intervals of 60 min along the remainder of the cycle.

2.3. PHA accumulation reactor: The experimental setup

PHA accumulation was performed in a completely mixed reactor (0.35 L working volume) aerated through membrane compressors and thermostated at 25 °C. The pH was not controlled and it