Production of PHA with mixed cultures from fermented food waste rich in ammonia

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Keywords: food waste, polyhydroxyalkanoates, mixed microbial cultures; feast and famine
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INTRODUCTION
Food waste consists of a mixture of materials that were created for human consumption and were lost or discarded unconsumed. According to an estimate of the Food and Agriculture Organisation of the United Nations, roughly 1.3 billion tonnes of food are lost or wasted every year, which accounts to one third of all food produced worldwide. As stated by the European Commission, EU-28 wastes 88 million tonnes of food annually with associated costs estimated at 143 billion euros. Even though preventive actions should be taken in order to reduce the generation of food waste, it is just as important to deal with accumulated waste. Since these wastes are mainly organic, biodegradable and frequently with high chemical oxygen demand (COD), one approach consists on the conversion of this residue into an added-value product such as polyhydroxyalkanoates (PHA). PHA is a family of polyester that can be synthesized by bacteria and it has a wide range of mechanical and thermal properties. Moreover, it is completely biodegradable and biocompatible, which makes it an environmentally friendly alternative for conventional non-biodegradable bioplastics and petroleum-based plastics.

APPROACH
This work aims at exploiting a novel feedstock for PHA production using mixed microbial cultures (MMC). In order to study the feasibility of PHA production using fermented food waste, this waste was first characterized in terms of its physicochemical properties. Afterwards, a culture selection reactor (to enrich the culture in PHA producing organisms) was set-up and operated as a sequencing batch reactor (SBR) with a nutrient-rich fermented food waste. During the study period, the organic loading rate (OLR) was increased in a stepwise manner, allowing at least 3 times the SRT before a new change in conditions. The reactor was frequently monitored, and accumulation assays were carried out after stabilizing the SBR in each step.

MATERIALS AND METHODS
The fermented food waste, that was used in the experiment, was supplied by Valorsul, and it consisted in a mixture of food from hotels, canteens and restaurants. After characterization of the feedstock, an experimental setup of 2 lab-scale reactors was assembled. The fermented food waste was fed to a 2 L SBR, which was inoculated with activated sludge and operated under a feast and famine (FF) regime for 48 days. Along reactor operation, OLR was gradually increased from 75 Cmmol L\(^{-1}\) d\(^{-1}\) to 200 Cmmol L\(^{-1}\) d\(^{-1}\). The length of the SBR cycle was 12 h, while the sludge retention time was set at 4 d. A second reactor (V = 1 L) was inoculated with the biomass from the first reactor and, in fed-batch mode, fed with fermented food waste in order to evaluate the PHA production capacity of the selected culture.

RESULTS AND DISCUSSION
Characterization of feedstock
Fermented food waste is rich in COD, both total (119 ± 0.28 gCOD L\(^{-1}\)) and soluble (37.5 ± 1.34 gCOD L\(^{-1}\)) and in fermentation products (898 ± 12.7 Cmmol L\(^{-1}\)); mixture of acetate, ethanol, propionate, lactate, butyrate, valerate and iso-valerate), which are the precursors for PHA production. Additionally, this waste is abundant in nutrients such as nitrogen (1.11 ± 0.01 g L\(^{-1}\)) and phosphorus (331 ± 3.92 mg L\(^{-1}\)) in their soluble forms. It was therefore concluded that this feedstock is likely suitable to select and grow a PHA-storing MMC, and be adequate for efficient PHA production.

First stage of PHA production: culture selection in SBR operation
The SBR was operated under feast and famine regime to obtain an enrichment of PHA accumulating culture. In order to efficiently produce PHA using a MMC, it is essential to achieve high performance and stability in the selection reactor in terms of length of feast phase with respect to cycle length. After increasing the OLR up to 130 ± 7.0 Cmmol L\(^{-1}\) d\(^{-1}\), the ratio between the feast phase and cycle length stabilized at around 0.10 ± 0.0089 h \(^{-1}\), which is considered suitable to a successful selection of PHA accumulating bacteria (Dionisi, 2006). Further characterization of the performance of the reactor was carried out and a summary is represented in Figure 1. FP
were readily consumed in the beginning of the cycle, resulting in a production of PHA. The polymer was consumed in the second part of the cycle along with nitrogen, resulting in an increase in active biomass (data not shown). Some nitrogen was consumed during the feast phase, suggesting that growth did not occur during the famine phase exclusively.

**Second stage of PHA production: accumulation of PHA in fed-batch mode.**

Biomass selected in SBR was then used in accumulation tests to assess the capacity of this culture to produce PHA. Results showed that the biomass achieved a maximum PHA production rate of $1.62 \pm 0.09 \text{ gPHA L}^{-1} \text{ h}^{-1}$ and an average PHA production rate of $0.72 \pm 0.17 \text{ gPHA L}^{-1} \text{ h}^{-1}$. Quantification of N-NH$_4$$^+$ showed that ammonium uptake rate increased as the fed-batch assay progressed, which suggests that the biomass started out producing PHA with a reasonable productivity, but the metabolism shifted towards growth during the accumulation test. Nonetheless, values of PHA content in the biomass of $43.9 \pm 3.49 \% \text{ (w/w)}$ and global productivity of $0.61 \pm 0.09 \text{ g L}^{-1} \text{ h}^{-1}$ were obtained, which are either in line or higher than other studies using a similar nutrient-rich feedstock (Morgan-Sagastume et al. 2010; Valentino et al. 2016).

**CONCLUSIONS**

These preliminary results from the SBR are promising since they have shown that the biomass responded very well to the fermented food waste, resulting in a good performance and high stability. Moreover, in the second stage, the selected biomass achieved polymer content in the biomass of over $40 \% \text{ (w/w)}$, which is of what it is estimated to be the threshold for economical recovery of the polymer (Werker et al. 2018).

**ACKNOWLEDGMENTS**

The authors are thankful for the financial support from the European project REsources from URban BlowaSte (H2020-CIRC05-2016-730-349), Fundação para a Ciência e Tecnologia (Portugal) for funding through PD/BD/126626/2016 and PD/BD/104767/2014 and UCIBIO financed by national funds from FCT/MCTES (UID/Multi/04378/2019).

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