The effect of loading rate on biopolymer production potential from pickle fermentation broth by mixed microbial cultures

D. Ozturk<sup>1</sup>, E. Ayisigi<sup>1</sup>, G.E. Zengin<sup>1</sup>, D. Guven<sup>2</sup>, D. Okutman Tas<sup>1</sup>, G. Ozyildiz<sup>1</sup>, G. Insel<sup>1</sup>, E. Cokgor<sup>1\*\*</sup> <sup>1</sup>Istanbul Technical University, Faculty of Civil Engineering, Environmental Engineering Department, 34469 Maslak, Istanbul, Turkey

<sup>2</sup>Istanbul Technical University, Faculty of Civil Engineering, Applied Biopolymer and Bioplastics Production Technologies Research Centre, Istanbul, Turkey

Presenting author email: inselhay@itu.edu.tr

Introduction



high production of cost The polyhydroxyalkanoate (PHA) biopolymers compared to petroleumbased synthetic counterparts limits its production and wide use (Dias et al., 2006). Therefore, use of low-cost substrates such as food industry wastes or waste streams with high carbon content is considered as one of the potential alternative for PHA production (Bishai et al., 2015). The aim of this study is to evaluate the PHA production potential of pickle fermentation brine as the feedstock.

Pickle fermentation broth was collected from a pickle industry facility located in Bursa, Turkey. A laboratory scale sequencing batch reactor (SBR) was operated under aerobic dynamic feeding conditions (feast-famine) at a sludge retention time (SRT) of 6 days. Reactor was initiated with the inoculum that was taken from the aeration tank of the pickle industry wastewater treatment plant (WWTP). SBR was fed with fermented broth effluents at a volumetric loading rate (VLR) of 1650 mg COD/L.day (Period 1) for 151 days and 2850 mg COD/L.day between 151st-193th days (Period 2).

## Table 1. Operational parameters of the SBRs

Parameter	Symbol	Value	Unit
Cycle time	T <sub>C</sub>	24	h
Duration of filling period	$T_{\rm F}$	30	min
Duration of aeration	$T_{AE}$	1320	min
Duration of settling	Ts	90	min
Duration of draw phase	T <sub>D</sub>	20	min
Initial volume	$V_0$	1.5	L
Total volume	$V_{\mathrm{T}}$	3	L
Duration of idle phase	TI	10	min
$V_0/V_F$	-	0.5	-

Organic acids were determined HPLC (Shimadzu Prominence LC-20A) equipped with an organic acid analysis column SCR-101H and a UV detector (at a wavelength of 210 nm). PHA measurements were performed using alkaline digestion HPLC method modified by Satoh et al. (2016).



For the evaluation of the effect of volumetric loading rate (VLR) on PHA storage, a lab-scale reactor was operated with two different VLR with a sludge age of 6 days and operated totally

## **Results & Discussion**

for 191 days. Pickle fermentation broth was used as feed in required dilutions to provide the desired VLR ratios (1.65 and 2.85 kgCOD/m<sup>3</sup>.day) and chloride concentrations were in the range of 3500 – 8000 mg/L. Within the first 151 days (Period 1) reactor was run with an VLR value of 1.65 kgCOD/m<sup>3</sup>.day ensuring an initial COD concentration of 1645 mg/L and a chlorine concentration of 3665 mg/L, in the reactor. The average steady state concentrations of SS and VSS were obtained as 4030 mg/L and 2765 mg/L, with a VSS/SS ratio of 0.71. The average COD removal was obtained as 86% indicating that a stable bioconversion of organics was achieved in the reactor.

Figure 1. Organic acids and PHA profile of SBR in-cycle analysis - lower volumetric loading rates of the operation

PHA content of the cells was also measured to evaluate the fate of COD and the microbial carbon storage capacity. The average PHA content of the cell was measured as 958 mg COD/L during Period 1. As of 153 day of operation, VLR was increased to 2.85 kgCOD/m<sup>3</sup>.day (Period 2) where the initial COD and chlorine concentrations in the reactor were 2845 mg/L and 8180 mg/L, respectively. Steady state concentrations of SS and VSS in this period were measured as 8760 mg/L and 4755 mg/L, with a VSS/SS ratio of 0.54. Obviously, VSS concentration was increased by a factor about 1.72 by doubling the COD load. Furthermore, higher cell PHA content was measured (1685 mg COD/L) in Period 2 as a result of higher COD load.



Figure 2. Organic acids and PHA profile of SBR in-cycle analysis - higher volumetric loading rates of the operation

The COD of fermentation broth was mainly composed of lactic, propionic, acetic and succinic acid and their initial concentrations as COD equivalent were 81 mg CODHAc/L, 764 mg CODHLa/L, 747 mg CODHPa /L and 54 mg CODHSa/L in Period 1. Organic acids were entirely consumed within the first 60 min. of the cycle and PHA profile reached its maximum at 60th min., containing HB and HV monomers as 1442 mg COD/L and 21 mg COD/, respectively, resulting a total PHA content of 1463 mg COD/L. The maximum PHA content of the cell was calculated as 0.31 g COD/g VSS.

The organic acids and PHA profiles of Period 2 in-cycle analysis are shown in Figure 1b. The COD equivalent of initial organic acid concentrations were measured as 116 mg CODHAc/L, 1409 mg CODHLa/L, 1242 mg CODHPa/L and 77 mg CODHSa/L. Organic acids were completely consumed within the first 45 min. of the cycle and the maximum PHA storage was observed at 60th min. The measured PHA was composed of 3167 mg COD/L equivalent of 3HB and 65 mg COD equivalent of 3HV monomers, revealing a total PHA concentration of 3232 mg COD/L. The maximum PHA content of the cell was calculated as 0.66 g COD/g VSS. For both periods, hydroxybuyrate was the major monomer due to the composition of the pickle fermentation brine as the feed was mainly composed of lactate and acetate. The current study presents the very first results in the literature so far that pickle fermentation brine is a promising alternative as cheap and sustainable feedstock for microbial PHA production.

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