





Determination of the potential of pickle wastewater as feedstock for biopolymer production

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ABSTRACT

Food industry wastewater (FIWW) streams with high organic content are among the most suitable and inexpensive candidates for polyhydroxyalkanoate (PHA) biopolymer production. Due to its high organic acid content, pickle industry wastewater (PIWW), can be considered as one of the prospective alternatives to petroleum-based polymers for PHA production. In this context, this study aimed to investigate the production of PHA with enriched microbial culture using PIWW. Two laboratory scale sequencing batch reactors (SBRs) were operated under aerobic dynamic feeding conditions at a sludge retention time of 8 days, with a total cycle duration of 24 hours. SBRs were fed with peptone mixture and PIWW. In-cycle analysis and batch respirometric tests were performed to evaluate PHA storage together with biodegradation kinetics. In-cycle analysis showed that maximum PHA content was 1,820 mgCOD/L, corresponding to 44% in the biomass (ratio of chemical oxygen demand (COD) to volatile suspended solids) for PIWW. Experimental results were also confirmed with activated sludge model simulations. As for the PHA composition, hydroxybutyrate was the major fraction. Model simulations proposed a unique conversion–degradation–storage pathway for the organic acid mixture. This paper presents a novel insight for better understanding of PHA biopolymer production using high saline FIWW.

Key words | biodegradation characteristics, biopolymers, kinetics, PHA, pickle industry, saline wastewater

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INTRODUCTION

Polyhydroxyalkanoates (PHAs) are biologically produced biopolymers that are accumulated as inclusions in the cytoplasm of cells for carbon and energy source. They can be formed by a variety of microorganisms (Van Loosdrecht *et al.* 1997; Verlinden *et al.* 2007). These PHAs are biodegradable and biocompatible, have similar mechanical properties to conventionally produced polymers, and can be produced from renewable resources (Valentino *et al.* 2014). Therefore, they are alternatives to petroleum-based polymers (Rehm 2010). The commercial PHA production is based on the pure cultures of PHA accumulating microorganisms, such

as *Cupriavidus necator* or recombinant *Escherichia coli* that require sterile growth conditions and well-defined nutrient-deficient synthetic media (Martino *et al.* 2014). The production cost is mainly related to the high price of the high purity substrates, which can account for 40–50% of the total cost. PHA production processes have been therefore constrained by high production costs compared to conventional plastics due to the need for sterilization and supply of media (Salehizadeh & Van Loosdrecht 2004; Kourmentza *et al.* 2017). In order to decrease the PHA production cost, the use of a mixed culture coupled with high

organic content waste streams appears promising (Duque *et al.* 2014). The advantages of using mixed culture, including elimination of sterilization and the possibility to use a wide variety of complex substrates, have been overviewed by many researchers (Reis *et al.* 2003; Salehizadeh & Van Loosdrecht 2004; Serafim *et al.* 2008).

The high and relatively pure organic content of food industry wastewater (FIWW) streams makes food wastes a perfect candidate for the production of different value-added bioproducts, such as PHA, biofuels, and a variety of additive feedstock chemicals. Food waste/wastewater is one of the most suitable and inexpensive carbon sources for PHA production while solving the pollution problem related to the food industry (Nielsen *et al.* 2017). Among the food processing industries, the pickle industry deserves specific attention, because it generates brine wastewater with a salt concentration of about 2–10% that results in challenges for biological treatment (Wan *et al.* 2019). A significant amount of brine wastewater is produced during the production process and decantation of fermentation broth, as well as during maintenance and equipment cleaning (König *et al.* 2012). Due to its high organic acid content, originating from the original pickling method, pickle industry wastewater (PIWW) can be considered as one of the prospective alternatives for PHA production. In the literature, research on brine wastewater, specifically on PIWW, is very limited, and there is no published work so far on the PHA production. Moreover, some salt-tolerant organisms have been reported to produce PHAs, suggesting the need for further studies on the possible use of PIWW with a high salinity content as an inherent stress condition on the production of PHA (Oren 2008).

Beside the experimental systems designed to enhance polymer storage, mathematical models could help to interpret alterations of process components, kinetics and stoichiometry. Multi-component models developed for the activated sludge process have become a standard tool for deriving degradation fingerprints of organic matter in wastewaters (Ekama *et al.* 1986). In that way, respirometry provides very rapid and reliable results for exploring biodegradation characteristics of organics developed for this purpose (Insel *et al.* 2003). The interpretation of the oxygen utilization rate (OUR) profile with multi-component models allows the estimation of critical kinetic and stoichiometric parameters that can be used in the design and process selection.

The aim of this study is to investigate the production of PHA with enriched microbial culture using PIWW. Laboratory scale sequencing batch reactors (SBRs) were operated under aerobic dynamic feeding conditions. In-cycle analysis

and dedicated batch respirometric tests were performed to evaluate the PHA storage together with biodegradation kinetics. The experimental data reflecting the fate of storage and biodegradation characteristics of organic matter were evaluated with the aid of a tailored multi-component model. To our knowledge, this paper presents the first detailed study of the microbial PHA production potential by mixed culture from PIWW.

MATERIALS AND METHODS

Feeding source

Pickle industry wastewater

The selected FIWW was obtained from a pickle industry facility located in Bursa, Turkey. The pickling company produces a range of different pickles from different raw materials. The plant's annual production consists of 1% olive, 5% vegetable sauce, 60% pickled vegetables, and 34% canned vegetable assortments. Pickle is a product of the lactic acid fermentation process. The pickle industry process wastewater is generated during the fermentation of vegetables. After the fermentation process, the fermentation broth, which has high organic acids concentration and salinity, is conveyed to the equalization tank together with washing water and domestic wastewater of the facility, and then discharged into the wastewater treatment plant (WWTP). Due to seasonal variations in fermented vegetable types, the composition of the pickle industry composite wastewater was monitored monthly. Grab samples from the equalization tank of the WWTP were collected during the operation of the SBRs (August 2018 – April 2019). All collected samples were kept refrigerated and immediately transported to the laboratory, where upon arrival were stored at 4 °C. The composition of the wastewater was assessed through conventional parameters.

Synthetic wastewater

The synthetic substrate included acetate (20 g/L CH₃COONa) and a peptone mixture (32 g/L peptone, 22 g/L beef extract, 6 g/L urea, 1.4 g/L NaCl, 0.8 g/L CaCl₂·2H₂O, 5.6 g/L K₂HPO₄ and 0.4 g/L MgSO₄·7H₂O) (ISO 2007).

Experimental set-up

Laboratory scale SBRs were operated to study the PHA production potential of fermented FIWW with mixed culture.

In this framework, PIWW is used for the enrichment of mixed microbial culture with a high PHA storage capacity. Two laboratory scale SBRs were operated under aerobic dynamic feeding conditions (feast–famine) at a sludge retention time of 8 days. One of the SBRs was fed with PIWW (SBR-1) whereas the other reactor operated with the feed of synthetic wastewater (SBR-2) as a control for the comparison of the PHA production capacity of PIWW. Both reactors were initiated with the same inoculum that was taken from the aeration tank of the pickle industry WWTP with a conventional activated sludge configuration. SBR-1 was fed with composite PIWW at an organic loading rate of 2,850 mg COD/L·day, while SBR-2 was fed with synthetic substrate wastewater at an organic loading rate of 1,300 mg COD/L·day.

The SBRs with a working volume of 3 L were operated in an isothermal room (22 °C). The operational conditions of the SBRs were designed for one cycle per day; detailed operational conditions of the SBRs are shown in Table 1. During the aerobic phase air was supplied by an air pump through a diffuser at a constant flow and stirred continuously.

Analytical methods

SBRs were monitored weekly through measurements of mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), pH, effluent chemical oxygen demand (COD), effluent suspended solids (SS) and volatile suspended solids (VSS) parameters. The PHA content of the enriched mixed microbial culture was determined with detailed cycle measurements through

the analyses of pH, SS, VSS, soluble COD, organic acids and PHA. Conventional parameters (pH, SS, VSS, chloride and COD) were measured according to standard methods (APHA/AWWA/WEF 2017). Organic acids were determined by high-performance liquid chromatography (HPLC, Shimadzu Prominence LC-20A) equipped with an organic acid analysis column SCR-101H (Shimadzu, Japan) and a UV detector (at a wavelength of 210 nm). The column was operated at 60 °C and 0.025% (v/v) H₂SO₄ was used as mobile phase at a flow rate of 0.7 mL/min. PHA measurements were performed using the alkaline digestion HPLC method modified by Satoh *et al.* (2016). The alkali digestion was performed through the addition of 0.5 mL of 2 N NaOH to 1 mL of mixed liquor samples and then heated at 105 °C for 1 hour to convert PHA monomers to (E)-2-butenic acid (2BE) and (E)-2-pentenoic acid (2PE). The mixture was then acidified through the addition of 0.5 mL of 2 N H₂SO₄ for further analyses in HPLC. Acidified mixed liquor samples were then centrifuged and the supernatant was filtered through 0.22 µm membrane filters. HPLC analyses of the converted organic acids were conducted on the Shimadzu Prominence LC-20A equipped with an organic acid analysis column SCR-101H (Shimadzu, Japan) and a UV detector (at a wavelength of 210 nm). The column was operated at 60 °C and 0.025% (v/v) H₂SO₄ was used as mobile phase at a flow rate of 1 mL/min. PHA concentration in the samples were computed using the conversion yields of PHA monomers into 2BE and 2PE. The alkali digestion method was applied to PHA standards to calculate the conversion yields, and obtained conversion yields in this study were in accordance with Satoh *et al.* (2016). PHA standards of sodium 3-hydroxybutyrate (Sigma-Aldrich Co, USA) and copolymer of PHA with a ratio of 88:12 (w%) (Sigma-Aldrich Co, USA) were used for the quantification of hydroxybutyrate (HB) and hydroxyvalerate (HV) monomers. (E)-2-butenic acid and (E)-2-pentenoic acid were obtained from Sigma-Aldrich Co (USA).

Respirometric analyses and modelling

Respirometric analyses were conducted to determine the degradation and storage kinetics of the process. OUR measurements were performed with an Applitek Ra-COMBO (Nazareth, Belgium) respirometer. OUR data were interpreted using Modified Activated Sludge Model No. 3 (ASM3) (Henze *et al.* 2000). AQUASIM software was used for the parameter estimation and calculation of confidence intervals of the model parameters. Lactic acid

Table 1 | Operational parameters of the SBRs

Parameter	Symbol	Value	Unit
Cycle time	T _C	24	h
Duration of filling period ^a	T _F	30	min
Duration of aeration	T _{AE}	1,320	min
Duration of settling	T _S	90	min
Duration of draw phase	T _D	20	min
Initial volume (V ₀)	V ₀	1.5	L
Total volume	V _T	3	L
Duration of idle phase	T _I	10	min
V ₀ /V _{Fill}	–	0.5	–
Hydraulic retention time	HRT	19.5	h
Sludge retention time	SRT	8	days

^aFilling was performed in the first 30 minutes of aeration phase.

(HLA), acetic acid (HAc) and propionic acid (HPr) were found to be dominant as biodegradable COD fractions in PIWW. In this respect, the model contains direct growth and storage processes by using HAc as the major substrate (Krishna & Van Loosdrecht 1999). The heterotrophic biomass was also able to grow on HPr in parallel with the utilization of HAc. In the final segment, HLa is converted to HAc and the conversion reaction is defined by a Contois type reaction, which is known as surface saturation type degradation reaction (Henze et al. 2000). The parameter estimation was carried out in accordance with the method proposed by Insel et al. (2003).

RESULTS AND DISCUSSION

Wastewater characterization

Conventional characterization of the composite PIWW samples collected at different periods showed that the PIWW had high COD and chloride content typical of this kind of wastewater, around $2,650 \pm 565$ mg COD/L and $5,400 \pm 2,238$ mg Cl⁻/L, respectively (König et al. 2012; Guventurk 2019; Wan et al. 2019). The SS concentration of 251 ± 80 mg/L was low and almost 50% of TSS was composed of organic matter. Total phosphorus and total Kjeldahl nitrogen concentrations and pH value were found to be 28 ± 26 mg/L, 101 ± 59 mg/L and 4 ± 0.6 , respectively. The detailed organic acid measurements conducted in this study also outlined the potential of PIWW as a good candidate for the production of biopolymers through the microbial processes. As can be seen from Table 2, the striking feature of the pickle wastewater samples was the high content of lactic, propionic and acetic acid.

Table 2 | Organic acid concentrations of pickle industry composite wastewaters

Sample no.	Acetic acid (mg/L)	Lactic acid (mg/L)	Propionic acid (mg/L)	Succinic acid (mg/L)
1	549	1,279	913	<5
2	569	1,390	932	<5
3	748	1,974	784	<5
4	148	606	<5	<5
5	289	1,122	<5	<5
6	365	1,082	413	53
7	892	493	286	0
8	1,600	1,240	549	117

Reactor performance

The laboratory scale SBR-1 fed with composite PIWW was operated for 238 days and the performance of the reactor was monitored weekly through the MLSS, MLVSS and effluent COD analyses. The average solids concentrations in the reactor were $6,150 \pm 1,458$ mg MLSS/L and $3,290 \pm 816$ mg MLVSS/L with a VSS/SS ratio of 0.54 ± 0.07 . In the laboratory scale reactors, effluent SS, VSS and soluble COD parameters were monitored weekly to determine the performance of the reactor. The soluble COD, SS and VSS were measured, with effluent average values of 160 ± 87 mg COD/L, 230 ± 54 mg SS/L and 120 ± 54 mg VSS/L.

The laboratory scale control reactor SBR-2, fed with synthetic mixture of peptone and acetate, was operated for 212 days. The average MLSS and MLVSS concentrations were approximately $2,590 \pm 913$ mg/L and $2,045 \pm 653$ mg/L (VSS/SS ratio of 0.8 ± 0.08), respectively. The effluent COD, SS, and VSS values were measured as 160 ± 42 mg/L, 175 ± 101 mg/L and 146 ± 88 mg/L, respectively.

PHA production performance of the reactors were determined with in-cycle analyses. The response of the mixed microbial culture of SBR-1 to salinity was compared before and after the acclimation period through effluent COD analyses (Figure 1). Composition of the influent on the 37th day of the operation was measured as 1,845 mg/L COD and 4,500 mg/L chloride concentrations. MLSS and MLVSS concentrations in the reactor were 5,650 mg/L and 2,680 mg/L with a VSS/SS ratio of 0.47. The influent COD and chloride concentrations of the PIWW fed on the 99th day of the operation were 1,325 mg/L and 2,750 mg/L,

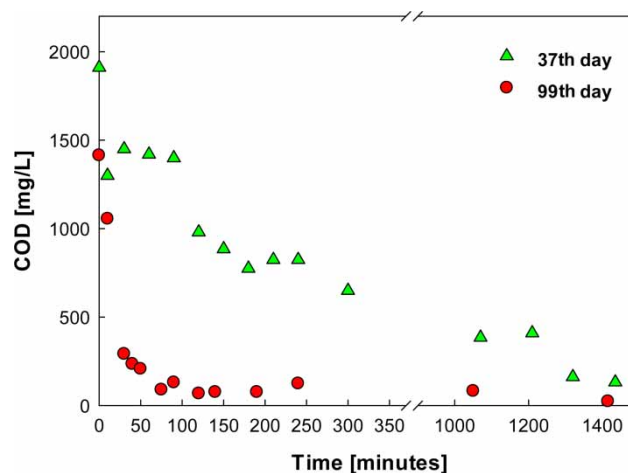


Figure 1 | COD profiles of SBR-1 in-cycle analyses.

respectively. The MLSS and MLVSS concentrations in the reactor on the 99th day were 8,420 mg/L and 4,120 mg/L with a VSS/SS ratio of 0.49. Comparative COD profiles show that the unadapted culture (37th day) can degrade only 25% of the initial COD within about 1 hour, while COD was completely consumed by the acclimated culture (99th day), within the same period (Figure 1). The COD utilization profile is a useful indicator for the PHA storage ability of the mixed culture. Since the observed COD consumption rate was too low to couple with PHA production in-cycle analyses performed on the 37th day of the operation, PHA analyses were not conducted.

As the COD consumption rate was remarkably high, organic acids as well as PHA components were monitored all through the in-cycle analyses on the 99th day of the operation (Figure 2). The initial concentrations of the major organic acids detected in SBR-1 were 984 mg/L of HLa followed by 392 mg/L of HPr and 374 mg/L of HAc. HLa was completely consumed within 30 minutes and maximum PHA content was obtained at the 90th minute of the cycle. Maximum PHA concentration (PHA_{max}) was computed as 1,820 mg COD/L corresponding to 44% in the biomass (COD/VSS). Regarding the PHA biopolymer composition, The HB fraction was the major monomer observed with a ratio of 97% (w/w) followed by the HV fraction (3%, w/w). It is well known that the polymer composition depends on substrate composition (Villano *et al.* 2014) and generally HB precursors are synthesized from volatile fatty acids

(VFA) with an even number of carbons (acetate and butyrate) and lactate, and 3HV precursors are synthesized from VFA having an odd number of carbons (propionate) (Lemos *et al.* 2006). Since the PIWW was mainly composed of lactate and acetate, the observed HB:HV composition was in accordance with the literature.

The PHA biopolymer production is mostly studied with synthetic substrate. Montiel-Jarillo *et al.* (2017) reported a PHA storage efficiency of 36% (g PHA/g VSS) without pH control and 51% (g PHA/g VSS) with nitrogen limitation in an SBR system fed with synthetic acetate mixture under aerobic dynamic feeding conditions. Valentino *et al.* (2014) reported 50% of PHA storage on COD basis with mixed microbial culture enriched with HAc and HPr mixture under nutrient deficiency. Very few studies focused on the enrichment of mixed microbial culture with FIWW. Duque *et al.* (2014) reported 56% and 65% PHA storage on COD basis for sugarcane molasses and cheese whey, respectively. Moreover, there are no studies reporting PHA production from PIWW.

The COD consumption of the mixed microbial culture in SBR-2 fed with synthetic peptone and acetate mixture before (37th day) and after the acclimation period (99th day) is shown in Figure 3. The influent COD value of the synthetic wastewater fed was 1,300 mg/L COD. The MLSS and MLVSS concentrations in the reactor were measured as 1,780 mg/L and 1,580 mg/L, respectively, with a VSS/SS ratio of 0.88 on the 37th day of the operation. After the

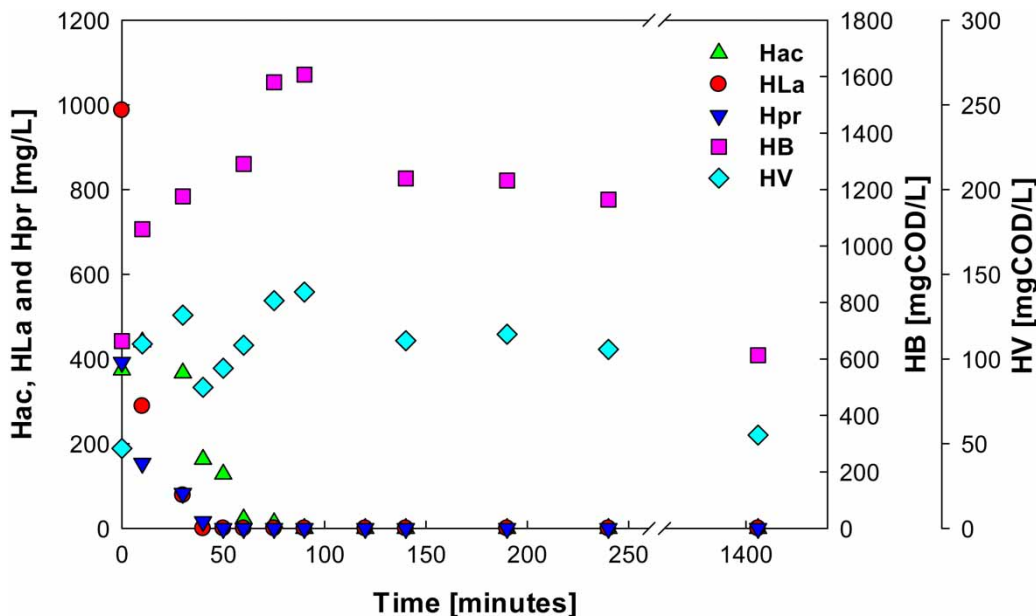


Figure 2 | Organic acids and PHA profile of SBR-1 in-cycle analysis on 99th day of the operation.

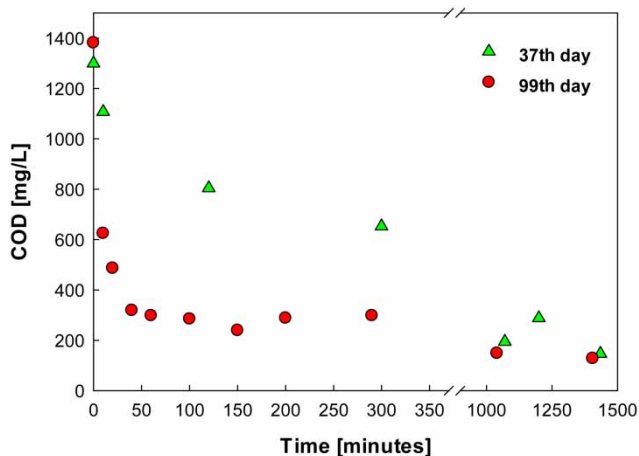


Figure 3 | COD profile of SBR-2 in-cycle analyses.

acclimation period, MLSS and MLVSS values in the reactor were observed as 1,440 mg/L and 960 mg/L, respectively. The consumption of the synthetic substrate on the 37th day was remarkably low compared to day 99. The mixed culture consumed 38% of the COD within 120 minutes at the 37th day of the operation and after the acclimation period the COD consumption increased to 82%.

The organic acid and PHA profile of SBR-2 observed on the 99th day is shown in Figure 4. Initially, HAc and HPr values were measured as 148 mg/L and 44 mg/L, respectively; within 110 minutes all of the organic acids present in the reactor were consumed. PHA_{max} content was computed as 180 mg COD/L corresponding to 19% in the biomass (COD/VSS). With respect to the PHA biopolymer composition, only the HB fraction was observed. This study focused on the PHA production from PIWW in comparison to a peptone-acetate mixture as it resembles the composition of domestic wastewater. The PHA production efficiency of PIWW (44%, g COD/gVSS) was remarkably higher compared to synthetic peptone-acetate mixture (19%, g COD/gVSS). The low content of organic acids in synthetic wastewater, which are specific to microbial PHA storage, resulted in lower PHA production.

Modelling results

The evaluation of degradation and storage kinetics was carried out with a parameter estimation study using a multi-component model as discussed in the 'Materials and methods' section. The OUR profile given in Figure 5(a) governs different stages of the biodegradation profile in the SBR system. Initial maximum OUR peak around 210 mg $\text{O}_2/\text{L}\cdot\text{h}$

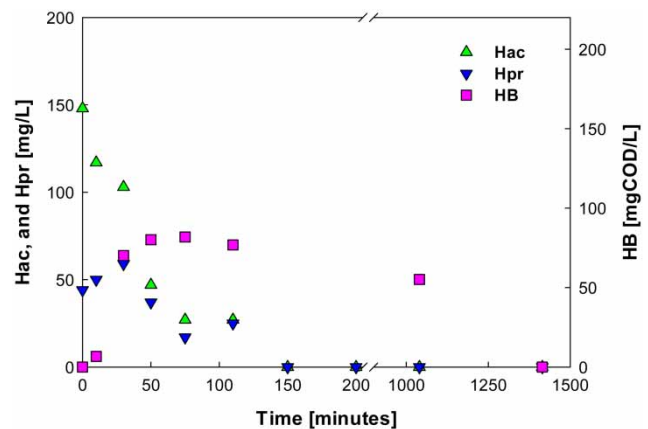


Figure 4 | Organic acid and PHA profile of SBR-2 in-cycle analyses on 99th day of the operation.

was due to HAc, HPr and HLa degradation after the addition of wastewater to the endogenous aerobic biomass. It should be noted that initial HAc, HPr and HLa fractions of the PIWW were 21%, 51% and 28%, respectively. The second OUR plateau around 130 mg $\text{O}_2/\text{L}\cdot\text{h}$ was obtained after the complete utilization of HAc; then HPr utilization lasted for 40 minutes. The last OUR plateau around 40 mg $\text{O}_2/\text{L}\cdot\text{h}$ was due to the degradation of HLa. The measured VFA concentrations provided a mass balance between biodegradable COD and area under the OUR curve at the storage and heterotrophic yields of 0.85 and 0.60 g COD/g cell COD, respectively. The heterotrophic biomass completely consumed the biodegradable COD within 100 minutes as shown in Figure 5(b). Only the soluble inert COD of 100 mg/L remained in the bulk. In addition, the HAc storage resulted in PHA increasing from 160 mg COD/L to 350 mg COD/L by yielding a net 190 mg COD/L storage capability. The maximum process yield was calculated as 41% of organic carbon converted to net PHA production within the first 200 minutes of the run. This could also be verified with the end point of exogenous OUR simulation shown in Figure 5(a). The OUR and PHA profiles obtained from in-cycle measurements were also in agreement with long-term monitoring of SBR operation as given above.

The maximum growth rates for HAc and HPr degradation were estimated to be 3.7 and 4.0 day^{-1} , respectively and the levels were compatible with the suggested range of 3.5–6.5 day^{-1} given for domestic wastewaters (Henze et al. 2000). The maximum storage rate had to be increased to 15 day^{-1} compared to its default value of 3 day^{-1} in order to fit the model on PHA data (Figure 5(b)). In addition, the maximum conversion rate and half saturation parameter

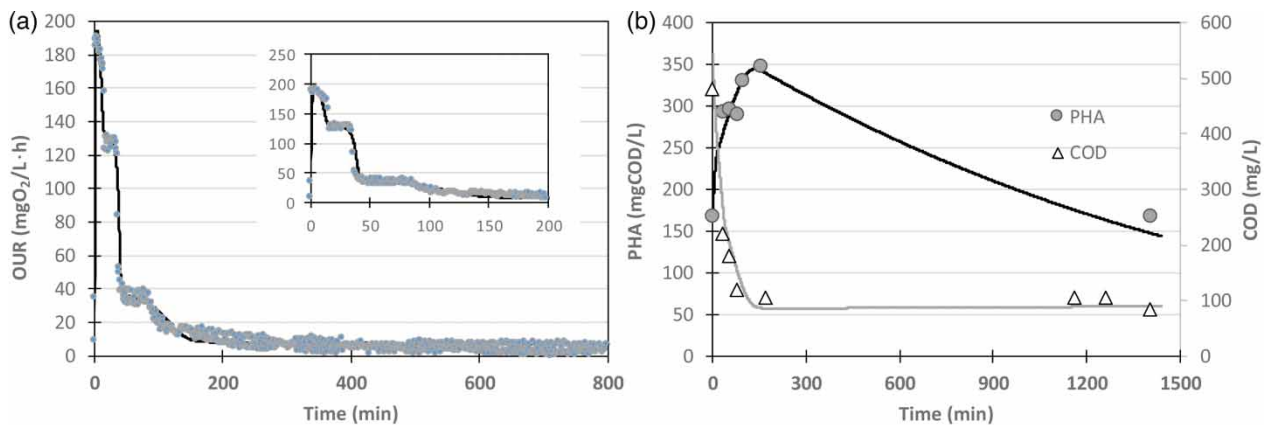


Figure 5 | Experimental data and simulation profiles for (a) OUR and (b) PHA-COD.

of HLa degradation (conversion to HAc) were estimated to be 3.5 day^{-1} and $0.03 \text{ g COD/g cell COD}$, respectively. The maximum growth rate on PHA was reduced to 0.7 day^{-1} from a default value of 2 day^{-1} suggested in ASM3 (Henze et al. 2000).

CONCLUSION

Using food waste regimes as carbon feedstock for PHA production is a promising alternative to support the circular economy and reduce environmental pollution. The main component of PIWW is the fermentation broth that contains high amount of lactate, propionate and acetate. Therefore, unlike the many other FIWWs, PIWW does not require a pre-fermentation step which comprises an additional cost. Considering also the difficulties in the treatment of PIWW due to its high salinity, our study introduced a novel waste conversion and treatment process from PIWW. This study presents the first promising results in the literature of PHA production potential of saline PIWW by enrichment of a mixed microbial culture that resists saline stress conditions. This study also explored the storage–degradation kinetics of the process, in the light of respirometric analysis and a tailored modelling approach. Model simulations proposed a unique conversion–degradation–storage pathway for the organic acid mixture, which needs more supporting experimental data for better understanding of the process. Our findings will be promising examples for the usage of different varieties of high saline food waste/wastewater (pickle industry, fish products, etc.) for PHA biopolymer production.

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REFERENCES

- APHA/AWWA/WEF 2017 *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC, USA.
- Duque, A. F., Oliveira, C. S., Carmo, I. T., Gouveia, A. R., Pardelha, F., Ramos, A. M. & Reis, M. A. 2014 [Response of a three-stage process for PHA production by mixed microbial cultures to feedstock shift: impact on polymer composition](#). *New Biotechnology* **31** (4), 276–288. doi:10.1016/j.nbt.2013.10.010.
- Ekama, G., Dold, P. & Marais, G. v. R. 1986 [Procedures for determining influent COD fractions and the maximum specific growth rate of heterotrophs in activated sludge systems](#). *Water Science and Technology* **18** (6), 91–114. doi:10.2166/wst.1986.0062.
- Guventurk, A. 2019 *Evaluation of PHA Biopolymer Production Potential of High Salinity Industrial Wastewater*. Istanbul Technical University, Istanbul, Turkey.
- Henze, M., Gujer, W., Mino, T. & van Loosdrecht, M. C. 2000 *Activated Sludge Models ASM1, ASM2, ASM2d and ASM3*. IWA Publishing, London, UK.
- Insel, G., Orhon, D. & Vanrolleghem, P. A. 2003 [Identification and modelling of aerobic hydrolysis – application of optimal](#)

- experimental design. *Journal of Chemical Technology & Biotechnology* **78** (4), 437–445. doi:10.1002/jctb.807.
- ISO 2007 *ISO8192 - Water Quality-Test for Inhibition of Oxygen Consumption by Activated Sludge for Carbonaceous and Ammonium Oxidation*. International Organization for Standardization, Geneva, Switzerland.
- König, T. N., Shulami, S. & Rytwo, G. 2012 *Brine wastewater pretreatment using clay minerals and organoclays as flocculants*. *Applied Clay Science* **67**, 119–124. doi:10.1016/j.clay.2012.05.009.
- Kourmentza, C., Plácido, J., Venetsaneas, N., Burniol-Figols, A., Varrone, C., Gavala, H. N. & Reis, M. A. 2017 *Recent advances and challenges towards sustainable polyhydroxyalkanoate (PHA) production*. *Bioengineering* **4** (2), 55. doi:10.3390/bioengineering4020055.
- Krishna, C. & Van Loosdrecht, M. C. 1999 *Substrate flux into storage and growth in relation to activated sludge modeling*. *Water Research* **33** (14), 3149–3161. doi:10.1016/S0043-1354(99)00031-7.
- Lemos, P. C., Serafim, L. S. & Reis, M. A. 2006 *Synthesis of polyhydroxyalkanoates from different short-chain fatty acids by mixed cultures submitted to aerobic dynamic feeding*. *Journal of Biotechnology* **122** (2), 226–238. doi:10.1016/j.jbiotec.2005.09.006.
- Martino, L., Cruz, M. V., Scoma, A., Freitas, F., Bertin, L., Scandola, M. & Reis, M. A. 2014 *Recovery of amorphous polyhydroxybutyrate granules from *Cupriavidus necator* cells grown on used cooking oil*. *International Journal of Biological Macromolecules* **71**, 117–123. doi:10.1016/j.ijbiomac.2014.04.016.
- Montiel-Jarillo, G., Carrera, J. & Suárez-Ojeda, M. E. 2017 *Enrichment of a mixed microbial culture for polyhydroxyalkanoates production: effect of pH and N and P concentrations*. *Science of the Total Environment* **583**, 300–307. doi:10.1016/j.scitotenv.2017.01.069.
- Nielsen, C., Rahman, A., Rehman, A. U., Walsh, M. K. & Miller, C. D. 2017 *Food waste conversion to microbial polyhydroxyalkanoates*. *Microbial Biotechnology* **10** (6), 1338–1352. doi:10.1111/1751-7915.12776.
- Oren, A. 2008 *Microbial life at high salt concentrations: phylogenetic and metabolic diversity*. *Saline Systems* **4** (1), 2. doi:10.1186/1746-1448-4-2.
- Rehm, B. H. 2010 *Bacterial polymers: biosynthesis, modifications and applications*. *Nature Reviews Microbiology* **8** (8), 578. doi:10.1038/nrmicro2354.
- Reis, M., Serafim, L., Lemos, P., Ramos, A., Aguiar, F. & Van Loosdrecht, M. 2003 *Production of polyhydroxyalkanoates by mixed microbial cultures*. *Bioprocess and Biosystems Engineering* **25** (6), 377–385. doi:10.1007/s00449-003-0322-4.
- Salehizadeh, H. & Van Loosdrecht, M. 2004 *Production of polyhydroxyalkanoates by mixed culture: recent trends and biotechnological importance*. *Biotechnology Advances* **22** (3), 261–279. doi:10.1016/j.biotechadv.2003.09.003.
- Satoh, H., Sakamoto, T., Kuroki, Y., Kudo, Y. & Mino, T. 2016 *Application of the alkaline-digestion-HPLC method to the rapid determination of polyhydroxyalkanoate in activated sludge*. *Journal of Water and Environment Technology* **14** (5), 411–421. doi:10.2965/jwet.16-027.
- Serafim, L. S., Lemos, P. C., Albuquerque, M. G. & Reis, M. A. 2008 *Strategies for PHA production by mixed cultures and renewable waste materials*. *Applied Microbiology and Biotechnology* **81** (4), 615–628. doi:10.2965/jwet.16-027.
- Valentino, F., Beccari, M., Fraraccio, S., Zanolari, G. & Majone, M. 2014 *Feed frequency in a sequencing batch reactor strongly affects the production of polyhydroxyalkanoates (PHAs) from volatile fatty acids*. *New Biotechnology* **31** (4), 264–275. doi:10.1016/j.nbt.2013.10.006.
- Van Loosdrecht, M., Pot, M. & Heijnen, J. 1997 *Importance of bacterial storage polymers in bioprocesses*. *Water Science and Technology* **35** (1), 41–47. doi:10.1016/S0273-1223(96)00877-3.
- Verlinden, R. A., Hill, D. J., Kenward, M., Williams, C. D. & Radecka, I. 2007 *Bacterial synthesis of biodegradable polyhydroxyalkanoates*. *Journal of Applied Microbiology* **102** (6), 1437–1449. doi:10.1111/j.1365-2672.2007.03335.x.
- Villano, M., Valentino, F., Barbetta, A., Martino, L., Scandola, M. & Majone, M. 2014 *Polyhydroxyalkanoates production with mixed microbial cultures: from culture selection to polymer recovery in a high-rate continuous process*. *New Biotechnology* **31** (4), 289–296. doi:10.1016/j.nbt.2013.08.001.
- Wan, L., Wu, Y., Zhang, X. & Zhang, W. 2019 *Nutrient removal from pickle industry wastewater by cultivation of *Chlorella pyrenoidosa* for lipids production*. *Water Science and Technology* **79** (11), 2166–2174. doi:10.2166/wst.2019.217.

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